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Genotypic diversity and genetic parameters of 'Tahiti' acid lime using different rootstocks

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Abstract – The objective of this work was to estimate the genetic parameters and to evaluate the genotypic diversity of 12 'Tahiti' acid lime (*Citrus latifolia*) genotypes grafted onto two rootstocks. The experiment was carried out from July 2017 to January 2019, in the municipality of São Mateus, in the state of Espírito Santo, Brazil. Vegetative (stem diameter, canopy projection diameter, and plant height), productive (yield and fruit number), and fruit quality (fruit diameter, soluble solids content, and juice yield) traits were determined. A mixed model was used to estimate heritability and repeatability coefficients, as well as to predict clonal values. Scion diversity was determined through the standardized mean difference of Euclidean distances, and genotypes were clustered by modified Tocher. Clustering quality and trait importance were evaluated using the principal component analysis (PCA). Genotypic variance was observed, which is indicative of the possibility of selection of superior genotypes. The Tocher method showed the presence of three clusters, which is in alignment with the PCA results. The multivariate analyses allows of the selection and the recommendation of superior 'Tahiti' acid lime genotypes.

Index terms: *Citrus latifolia*, multivariate analysis, plant breeding, perennial crops.

Diversidade genotípica e parâmetros genéticos de lima ácida 'Tahiti' com uso de diferentes porta-enxertos

Resumo – O objetivo deste trabalho foi estimar os parâmetros genéticos e avaliar a diversidade genética de 12 genótipos de lima ácida 'Tahiti' (*Citrus latifolia*) enxertados em dois porta-enxertos. O experimento foi conduzido de julho de 2017 a janeiro de 2019, no município de São Mateus, no estado de Espírito Santo, Brasil. Foram mensuradas características vegetativas (diâmetro de caule, diâmetro de projeção de copa e altura de planta), produtivas (rendimento e número de frutos) e de qualidade de frutos (diâmetros de fruto, teor de sólidos solúveis e rendimento de suco). Utilizou-se modelo misto para estimar os coeficientes de herdabilidade e repetibilidade, bem como predizer os valores genéticos. A diversidade entre os enxertos foi obtida por meio da diferença da distância euclidiana média padronizada, e os genótipos foram agrupados pelo método de Tocher modificado. A qualidade do agrupamento e a importância dos caracteres foi avaliada pela análise de componentes principais (ACP). Observou-se variabilidade genética, o que indica a possibilidade de seleção de genótipos superiores. O método de Tocher mostrou a presença de três grupos de genótipos, o que foi congruente com os resultados da ACP. O uso de técnicas multivariadas permite a seleção e a recomendação de genótipos superiores de lima ácida 'Tahiti'.

Termos para indexação: *Citrus latifolia*, análise multivariada, melhoramento de plantas, culturas perenes.

Introduction

Although the genus Citrus is composed of subtropical crops with worldwide economic relevance, the genotypes used in commercial orchards have a narrow genetic base (Shrestha et al., 2012; Morais et al., 2020). 'Tahiti' acid lime (*Citrus latifolia* Tanaka) has been identified as a potential alternative for diversifying citrus cultivation, providing off-season income (Santos et al., 2013) and promoting sustainable crop production (Rouiss et al., 2018). However, the species is a triploid, with 2n = 3x = 27, which represents a barrier for the implementation of conventional cross-breeding due to high rates of infertility and the occurrence of incompatibility (Nakano et al., 2019), contributing to the small number of cultivated 'Tahiti' genotypes.

'Tahiti' acid lime grafts have been combined with rootstocks of several genotypes, since the use of one or only a few may cause susceptibility to soil pathogens (Carvalho et al., 2019). To date, in Brazil, the most used rootstock for citrus is Rangpur lime (*Citrus limonia* Osbeck). However, this species is susceptible to gummosis, caused by *Phytophthora* spp., which explains the decrease in its use (Carvalho et al., 2016). In addition, the rootstock may also affect several fruit characteristics such as plant quality and size (Raddatz-Mota et al., 2019). Therefore, the use of a single rootstock for all graft varieties does not meet all the inherent characteristics of each cultivar and becomes a problem under endemic diseases, which is why rootstock variability has shown promising results (Carvalho et al., 2016).

In fruit breeding, the initial step for developing a new variety is to determine genotypic variability among accessions, either by obtaining genetic or genotypic values, allowing of the prediction of additive and the non-additive effects when there is available information on kinship relationships among individuals (Viana & Resende, 2014). However, the total genotypic value of an individual can be predicted when the additive and non-additive (dominance) effects are combined (Resende et al., 2014), which makes it possible to predict the total genetic value and total genotypic value of an individual. For the estimation of genotypic diversity, the quantification of genetic dissimilarity among individuals (Yamamoto, 2014) can be determined by using biometric models and multivariate statistical methodologies.

Since the breeding of perennial species is difficult due to their long productive and reproductive cycles (Negreiros et al., 2014), the generated datasets may be unbalanced, showing, for example, an unequal representation of genetic material across locations or no yield for some genotypes due to seasonal harvesting. The restricted maximum likelihood/best linear unbiased prediction (REML/BLUP) methodology (Henderson, 1975; Patterson & Thompson, 1971) is a standard procedure for dealing with genetic and statistical unbalanced data (Alves et al., 2019). Compound symmetry is a parsimonious (co) variance structure widely used in the REML/BLUP context to predict genetic values and estimate variance components through repeated measures, assuming that all variances and covariances are equal, given the respective effect under study, also allowing of the prediction of genotypic values for all environments simultaneously and for each environment individually (Viana & Resende, 2014).

The objective of this work was to estimate the genetic parameters and to evaluate the genotypic diversity of 12 'Tahiti' acid lime genotypes grafted onto two rootstocks.

Materials and Methods

The experiment was conducted from July 2017 to January 2019, at Fazenda José Guarete, in the municipality of São Mateus, in the state of Espírito Santo, Brazil (18°48'21"S, 39°53'30"W, at 35 m above sea level). According to Köppen's classification, the climate is Aw, with rainy summers and dry winters (Alvares et al., 2013). Throughout the experimental period, the average temperature was 25ºC and average precipitation was 140 mm. The climate data were obtained from Instituto Nacional de Meteorologia (INMET, 2022).

Twenty-four 'Tahiti' acid lime scion/rootstock combinations (SRC) were grown in plots with 6.0 m between plants and 3.0 m between lines, in a completely randomized design with four replicates, in split-plots. The first level of randomization consisted of two hybrids used as rootstocks: Swingle citrumelo [*Citrus paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.] and Riverside citrandarin (*Citrus sunki* hort. Ex Tanaka x *P. trifoliata*). The second level consisted of the following scions: 'Bello Fruit', 'Éledio', 'Iconha',

'Itarana', 'Santa Rosa', 'Bearss Lime', 'CNPMF 01', 'CNPMF 02', 'CNPMF 2001', 'CNPMF 5059', 'BRS Passos', and 'Persian 58'. The experimental unit was three plants per SRC, totaling 288 individuals.

Vegetative, productive, and fruit quality traits were determined. To evaluate plant development, the measured vegetative traits were: stem diameter at the grafting site, in millimeters; stem diameter 5.0 cm above the graft, in millimeters; stem diameter 5.0 cm below the graft, in millimeters; diameter of canopy projection on the planting line, in meters; diameter between plant spacing, in meters; and plant height from the ground up to the region of the crown with most branches, also in meters. All traits were measured semiannually from July 2017 to January 2019 using a graduated tape, except the different stem diameters, with a digital caliper.

Based on the performance of eight harvests, the two following productive traits were evaluated in July, October, and November 2017 and in January, March, July, October, and December 2018: fruit yield per plant, in kilograms, by weighing total mass on a digital scale; and number of fruits per plant. To be harvested, all fruits had to be ripe, that is, have a minimum diameter of 47 mm, rough skin, and a dark-green to light-green color (Morais et al., 2020).

For fruit quality evaluation, three physicochemical attributes were analyzed in March, July, and October 2018 using ten fruits randomly separated from each experimental unit in each harvest. A digital caliper was used to measure: longitudinal diameter, equatorial diameter, and, after the fruits were cut in half, peel thickness, all in millimeters. The juice was extracted using the ESCV-I industrial juicer (Vitalex, Catanduva, SP, Brazil). Juice yield, as percentage, was calculated based on the relationship between the mass of the whole fruit and the mass of the bagasse. The PR-32 Palette series portable digital refractometer (Atago Brasil Ltda., Ribeirão Preto, SP, Brazil), with an automatic temperature compensation, was used to measure the content of soluble solids, in °Brix.

For the statistical analysis associated with the evaluation of repeated measures (each harvest through time), a mixed model was used. A univariate compound symmetry model with four replicates was given as follows: $y = X_f + Z_g + Q_{gr} + T_{gm} + W_{gmr} + S_p + \varepsilon$, where y is the vector of phenotypic data (average of each experimental unit); f is the vector of the effects (assumed to be fixed) of the replicate/rootstock/measure combination added to the overall mean; g is the vector of the scion genotypic effects (assumed to be random), with $g \sim N(0, I\hat{\sigma}^2_g)$, where $\hat{\sigma}^2_g$ is the genotypic variance; gr is the vector of the effect (random) of the genotype × rootstock (G×R) interaction, with $gr \sim N(0, I\hat{\sigma}^2_{gr})$, where $\hat{\sigma}^2_{gr}$ is the variance of the G×R interaction; gm is the vector of the effects (random) of the genotypes × measures (G×M) interaction (random), with $gm \sim N(0, I\hat{\sigma}^2_{gm})$, where $\hat{\sigma}^2_{gm}$ is the variance of the G×M interaction; gmr is the vector of the effects (random) of the genotypes × rootstock × measures (G×R×M) interaction, with $grm \sim N(0, I\hat{\sigma}^2_{grm})$, where $\hat{\sigma}^2_{grm}$ is the variance of the G×R×M interaction; p is the vector of the permanent environment tree effect (random), with $p \sim N(0, I\hat{\sigma}^2_{perm})$, where $\hat{\sigma}^2_{perm}$ is the permanent environment variance; and ε is the residuals vector of each experimental unit (random), with $e \sim N(0, I\hat{\sigma}^2_e)$, where $\hat{\sigma}^2_e$ is the residual variance. The incidence matrices for those effects are represented by the capital letters X, Z, Q, T, W, and S, respectively (Resende, 2002).

The assumptions of normality, homoscedasticity, and independence of errors for each trait were verified by Shapiro-Wilk's normality test, Levene's test for equality of variances, and the Durbin-Watson statistic, respectively, using the R software (R Core Team, 2020). Only the significant variances used in the diversity analysis were checked for collinearity assumption by the Montgomery & Peck (1981) criterion; when < 100, collinearity was classified as weak.

The significance of the random effects of the statistical model was tested using the likelihood ratio test (LRT), as in Rao (1973), defined as follows: $LRT = -2[\log_e L_{p+1} - \log_e \log_p]$, where L_{p+1} and L_p represent the maximum points of the restricted likelihood function associated with the complete model and the reduced model, respectively. Significance was tested using the chi-square statistic, with 0.5 degree of freedom for traits with estimates inside the parameter space and 1.0 degree of freedom for the other traits (Stram & Lee, 1994). The probability threshold used for Type I error was equal to 5%.

As previously mentioned, the REML procedure was used to obtain the six following variance components: $\hat{\sigma}^2_g, \hat{\sigma}^2_{gr}, \hat{\sigma}^2_{gm}, \hat{\sigma}^2_{grm}, \hat{\sigma}^2_{perm}$, and $\hat{\sigma}^2_e$, from which the genotypic parameters were estimated. Phenotypic variance ($\hat{\sigma}^2_{phen}$) was calculated as

$\hat{\sigma}^2_{phen} = \hat{\sigma}^2_g + \hat{\sigma}^2_{gr} + \hat{\sigma}^2_{gm} + \hat{\sigma}^2_{grm} + \hat{\sigma}^2_{perm} + \hat{\sigma}^2_e$,
and mean phenotypic variance, as $\hat{\sigma}^2_{\overline{phen}} = \hat{\sigma}^2_g + \hat{\sigma}^2_{gr}/r + \hat{\sigma}^2_{gm}/r + \hat{\sigma}^2_{perm}/r + \hat{\sigma}^2_e/mrb$.
The heritability of the clonal mean ($\hat{H}^2_{mg}$) and clonal individual heritability ($\hat{H}^2_g$) were estimated using $\hat{H}^2_{mg} = \hat{\sigma}^2_g/\hat{\sigma}^2_{\overline{phen}}$ and $\hat{H}^2_g = \hat{\sigma}^2_g/\hat{\sigma}^2_{phen}$, respectively. Selective accuracy was obtained by $r_{\hat{g}g} = \sqrt{\hat{H}^2_{mg}}$.

Four coefficients of determination were estimated for the effects of the G×M interaction $\left(c^2_{gm} = \hat{\sigma}^2_{gm}/\hat{\sigma}^2_{phen}\right)$, G×R interaction $\left(c^2_{gr} = \hat{\sigma}^2_{gr}/\hat{\sigma}^2_{phen}\right)$, G×R×M interaction $\left(c^2_{grm} = \hat{\sigma}^2_{grm}/\hat{\sigma}^2_{phen}\right)$, and permanent tree environment $\left(c^2_{perm} = \hat{\sigma}^2_{perm}/\hat{\sigma}^2_{phen}\right)$. The repeatability coefficient was obtained using $p = \hat{\sigma}^2_g + \hat{\sigma}^2_{gr} + \hat{\sigma}^2_{perm}/\hat{\sigma}^2_{phen}$. The following seven genotypic correlation coefficients were also estimated: genotypic correlation across rootstocks valid for any repeated measure $(r_{gr} = \hat{\sigma}^2_g/\hat{\sigma}^2_g + \hat{\sigma}^2_{gr})$, genotypic correlation across measures valid for any rootstock $(r_{gm} = \hat{\sigma}^2_g/\hat{\sigma}^2_g + \hat{\sigma}^2_{gm})$, genotypic correlation across rootstocks in a given measure $(r_{grm} = \hat{\sigma}^2_g + \hat{\sigma}^2_{gm}/(\hat{\sigma}^2_g + \hat{\sigma}^2_{gm}) + \hat{\sigma}^2_{gr})$, genotypic correlation across measures in a given rootstock $(r_{gmr} = \hat{\sigma}^2_g + \hat{\sigma}^2_{gr}/(\hat{\sigma}^2_g + \hat{\sigma}^2_{gr}) + \hat{\sigma}^2_{gm})$, genotypic correlation across rootstocks for the average of all measures

$$(r_{gr_{am}} = \frac{\hat{\sigma}^2_g + \hat{\sigma}^2_{gm}/m + \hat{\sigma}^2_{grm}/m}{\hat{\sigma}^2_g + \hat{\sigma}^2_{gr} + \hat{\sigma}^2_{gm}/m + \hat{\sigma}^2_{grm}/m}),$$

genotypic correlation across measures for the average of all rootstocks

$$(r_{gm_{ar}} = \frac{\hat{\sigma}^2_g + \hat{\sigma}^2_{gr}/r + \hat{\sigma}^2_{grm}/r}{\hat{\sigma}^2_g + \hat{\sigma}^2_{gm} + \hat{\sigma}^2_{gr}/r + \hat{\sigma}^2_{grm}/r}),$$

and genotypic correlation across rootstocks and measures

$$(r_{grm} = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2_g + \hat{\sigma}^2_{gr} + \hat{\sigma}^2_{gm} + \hat{\sigma}^2_{grm}}).$$

In the correlation coefficients, r, m, and b refer, respectively, to the number of rootstocks (two), measures (eight), and replicates (four). All used expressions are according to Resende (2002).

The standardized mean difference of the Euclidean distances was used as a measure of dissimilarity, being estimated based on the predicted clonal values of each trait statistically significant for genotypic effects. The genotypes were clustered using the modified Tocher optimization method (Vasconcelos et al., 2007). In addition, diversity was plotted on graphs generated through the principal component analysis (PCA) using the predicted clonal values (Resende et al., 2014). The dispersion of the scores of each genotype were shown in a Cartesian system and associated with the results obtained in the clustering technique. The importance of the traits was determined through the interpretation of the eigenvectors of the PCA associated with the eigenvalues. The PCA indicates the traits that contribute the most and the least to the variation observed in the traits, an information that can be used to exclude some traits in future studies (Cruz et al., 2020).

All genetic-statistical analyses were performed using the Selegen REML/BLUP (Resende, 2016) and Genes (Cruz, 2016) software.

Results and Discussion

Genotypic variance is essential for the selection process. According to the LRT, scion genotypic effects were significant for plant height (PH), number of fruits (NF), fruit yield (FYP), longitudinal and transversal fruit diameter (LFD), shell thickness (ST), and juice yield (JY) (Figure 1). PH, LFD, equatorial fruit diameter (EFD), and ST showed variance components near the boundary of the parameter space for variance components (zero), and, therefore, were analyzed with a 0.5 degree of freedom, as proposed by Stram & Lee (1994). Since a different degree of freedom was adopted for these estimations, the new significance value of the chi-square statistic was 2.79, used as the new threshold for the LRT.

The G×M interaction effects were significant for NF, FYP, LFD, and EFD. The presence of this interaction changed genotype ranking throughout the harvests, which is common for perennial plants (Viana & Resende, 2014), as also reported in another study on 'Tahiti' acid lime grafting (Machado et al., 2017). Contrastingly, the G×R interaction effects were significant for the following traits: stem diameter 5.0 cm below the grafting site, stem diameter 5.0 cm above the grafting site, stem diameter at the grafting site, canopy diameter towards the planting line, canopy diameter perpendicular to the planting line, NF, and FYP. The significant G×R interaction reflects the different responses of the 12 genotypes regarding the used rootstock – Swingle citrumelo or Riverside citrandarin.

Only two traits – NF and FYP – presented significant G×R×M interaction effects. Moreover, the

permanent environment effect was not significant for SDB, LFD, ST, and TSS, which is indicative that rootstocks and measure times affect simultaneously certain genotypes.

The values obtained for $\hat{H}_g^2$ ranged from 0.11 to 0.30 (Table 1), which were lower than those found for $\hat{H}_{mg}^2$, classified as having a high magnitude (Resende & Alves, 2020). For $\hat{H}_g^2$, LFD, EFD, ST, and JY presented low magnitudes, whereas PH, NF, and FYP had moderate magnitudes (Resende & Alves, 2020). Several small-effect genes and environmental conditions seem to control NF and FYP, which leads to low heritability estimates (Laviola et al., 2013). For $\hat{H}_{mg}^2$, of the evaluated traits, JY showed the lowest value (0.72) and PH, the highest (0.83). The values obtained for r_{gg} were above 0.80 for all traits. In the context

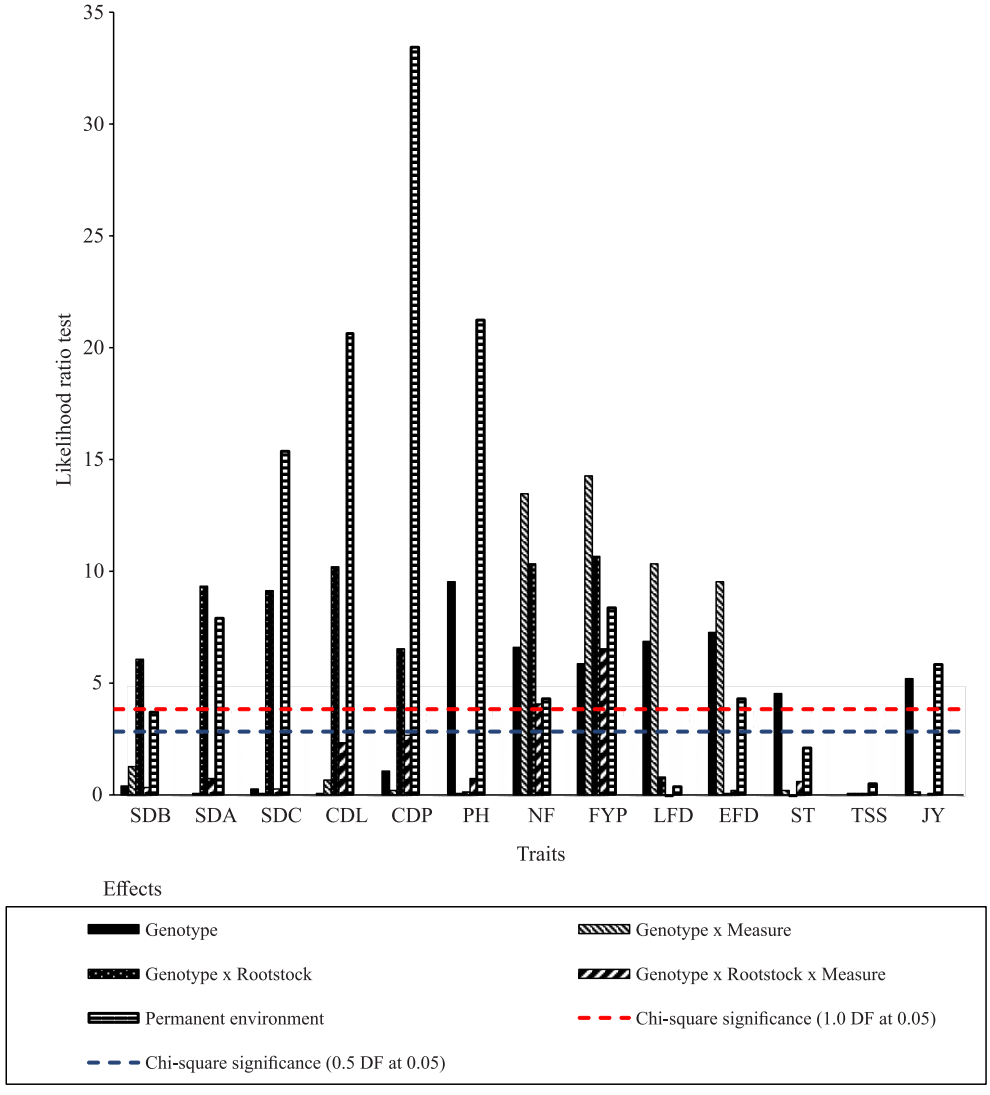

Figure 1. Likelihood ratio test (bar plots) for 13 traits evaluated in 12 'Tahiti' acid lime (*Citrus latifolia*) genotypes. SDB, stem diameter 5.0 cm above the grafting site; SDA, stem diameter 5.0 cm below the grafting site; SDC, stem diameter at the grafting site; CDL, canopy diameter towards the planting line; CDP, canopy diameter perpendicular to the planting line; PH, plant height; NF, number of fruits per plant; FYP, fruit yield per plant; LFD, longitudinal fruit diameter; EFD, equatorial fruit diameter; ST, shell thickness; TSS, total soluble solids content; and JY, juice yield. All bars above the dashed red line indicate statistical significance, considering the chi-square test, with 1.0 (= 3.84) and 0.5 (= 2.79) degree of freedom (DF), at a 0.05 significance level.

of genetic assessment, this is the most important statistical parameter (Resende & Alves, 2020), since it refers to the correlation between the true and predicted clonal values.

Table 1. Estimates of variance components and genetic parameters obtained for 12 'Tahiti' acid lime (*Citrus latifolia*) genotypes evaluated for plant height (PH), number of fruits per plant (NF), fruit yield per plant (FYP), longitudinal fruit diameter (LFD), equatorial fruit diameter (EFD), shell thickness (ST), and juice yield (JY).

Component/ parameter[1]	Trait[2]						
	PH	NF	FYP	LFD	EFD	ST	JY
$\tilde{\sigma}^2_g$	0.02	260.27	2.14	0.85	0.50	0.01	4.23
$\tilde{\sigma}^2_{gm}$	0.00	141.71	1.28	0.67	0.49	0.00	0.22
$\tilde{\sigma}^2_{gr}$	0.00	86.23	0.81	0.11	0.01	0.00	0.08
$\tilde{\sigma}^2_{grm}$	0.00	59.77	0.62	0.03	0.06	0.01	0.21
$\tilde{\sigma}^2_p$	0.01	31.92	0.37	0.10	0.21	0.01	4.79
$\tilde{\sigma}^2_e$	0.02	553.39	4.38	5.86	3.31	0.09	23.15
$\tilde{\sigma}^2_{phen}$	0.05	1133.30	9.59	7.63	4.59	0.12	32.69
$\tilde{\sigma}^2_{\overline{phen}}$	0.01	341.46	2.90	1.11	0.67	0.02	5.87
$\tilde{H}^2_g$	0.29	0.22	0.22	0.11	0.10	0.10	0.12
$\tilde{H}^2_{mg}$	0.83	0.76	0.73	0.76	0.73	0.73	0.72
r_{gg}	0.91	0.87	0.86	0.88	0.86	0.86	0.85
c^2_{gr}	0.01	0.08	0.08	0.01	0.00	0.00	0.00
c^2_{gm}	0.01	0.13	0.13	0.09	0.11	0.02	0.01
c^2_{grm}	0.03	0.05	0.06	0.00	0.01	0.04	0.01
c^2_p	0.17	0.03	0.04	0.01	0.05	0.08	0.15
p	0.48	0.33	0.34	0.13	0.15	0.19	0.28
r_{gr}	0.95	0.7511	0.73	0.89	0.98	0.98	0.98
r_{gm}	0.98	0.65	0.63	0.56	0.50	0.83	0.95
r_{grm}	0.95	0.82	0.81	0.93	0.99	0.98	0.98
r_{gmr}	0.98	0.71	0.70	0.59	0.51	0.84	0.95

[1] $\tilde{\sigma}^2_g$, genotypic variance; $\tilde{\sigma}^2_{gm}$, variance of the genotype x measure interaction; $\tilde{\sigma}^2_{gr}$, variance of the genotype x rootstock interaction; $\tilde{\sigma}^2_{grm}$, variance of the genotype x rootstock x measure interaction; $\tilde{\sigma}^2_p$, permanent environmental variance; $\tilde{\sigma}^2_e$, residual variance; $\tilde{\sigma}^2_{phen}$, individual phenotypic variance; $\tilde{\sigma}^2_{\overline{phen}}$, mean phenotypic variance; $\tilde{H}^2_g$, clonal individual heritability; $\tilde{H}^2_{mg}$, heritability of the clonal mean; r_{gg}, selective accuracy; c^2_{gr}, coefficient of determination of the effects of the genotype x rootstock interaction; c^2_{gm}, coefficient of determination of the effects of the genotype x measurement interaction; c^2_{grm}, coefficient of determination of the effects of the genotype x rootstock x measurement interaction; c^2_p, coefficient of determination of the permanent environment tree effects; p, individual repeatability; r_{gr}, genotypic correlation across rootstocks valid for any repeated measure; r_{gm}, genotypic correlation across measures valid for any rootstock; r_{grm}, genotypic correlation across rootstocks in a given measure; r_{gmr}, genotypic correlation across measures in a given rootstock. [2]Traits that showed statistical significance for genotypic variance.

The repeatability coefficient refers to the ability of a genotype to repeat a target performance across measures, being a function of genetic and common-environment effects (Malikouski et al., 2021). Repeatability was of a low magnitude ($\rho \leq 0.3$) for LFD, EFD, ST, and JY, but of a moderate magnitude ($0.3 < \rho < 0.6$) for PH, NF, and FYP (Resende & Alves, 2020). For other species of the genus Citrus, similar estimates of repeatability were found: 0.35 for production traits in *Citrus* x *sinensis* (L.) Osbeck and 0.26 for number of fruits per plant in *Citrus* x *aurantium* L. (Negreiros et al., 2014).

The interaction between non-genetic and genetic effects makes it difficult to select superior individuals, since it contributes to phenotypic values. The coefficient of determination of the effects of the GxM interaction (c^2_{gm}) showed the highest estimates for NF, FYP, LFD, and EFD, which is indicative that the values of the evaluated productive traits vary among genotypes throughout the harvests. Regarding the coefficient of determination of the permanent environment tree effects (c^2_{perm}), the highest values were found for PH, ST, and JY. The significance of the effect associated with high c^2_{perm} values shows the existence of correlation between the measures of those traits. Furthermore, the coefficients of determination of the G×R and G×R×M interactions (c^2_{gr} and c^2_{grm}, respectively) presented moderate values for all studied traits.

All estimated correlation coefficients showed different values, considering rootstocks and harvests (Table 1). For r_{gr}, the obtained values ranged from 0.72 to 0.98. A correlation higher than 0.9 was observed for PH, EFD, and ST, but lower than 0.75 for NF and FYP. For r_{gm}, the highest values were 0.97 and 0.95, respectively, for PH and JY, and the lowest ones were 0.50 and 0.55, respectively, for LFD and EFD. The values of r_{grm} ranged from 0.44 to 0.89 for FYP and JY, respectively.

The higher the correlation coefficients, the smaller the effects of the G×R, G×M, and G×R×M interactions on phenotypic variance. It should be noted that correlation coefficients higher than 0.7 are not indicative of selection hassle (Viana & Resende, 2014), whereas low ones show that scion performance is not similar across measures and across rootstocks. In this case, the recommendation of superior individuals may consider each rootstock and/or measure. Therefore, r_{gr} and r_{gm} are indicative that their respective interactions

are not of great magnitude, with genotype performance remaining somewhat stable over the harvests.

The magnitudes of the correlation coefficients were classified as: low, between 0 and 0.33; moderate, between 0.34 and 0.66; and high, above 0.67 (Resende & Alves, 2020). Therefore, r_{grm} was considered of high magnitude for all traits when genotypic variance was significant. This is indicative that the ranking of single-trait genotypes remained similar throughout the evaluations. However, r_{gmr} was classified as having a moderate magnitude for LFD and EFD, but a high magnitude for the other traits. Therefore, it seems that the genotypes with the highest clonal values for LFD and EFD were not the same when a different rootstock was used. Similar results were observed regarding the classification of the genotypic correlation coefficient across measures for the average of all rootstocks. Other studies also reported the impact of the correlation between sites/genotypes and between measures/genotypes (Bastos et al., 2007; Colombari Filho et al., 2013), showing the direct effects of these estimates on genetic selection under complex G×E or G×M interaction. Higher correlations make selection easier because genotype performance is similar across repeated measures and rootstocks. However, if correlations are low, it is necessary to consider the scion effect in each rootstock and measure.

The modified Tocher method grouped the scions into three clusters (Figure 2). The first was composed of: 'Bello Fruit', 'Elédio', 'Iconha', 'Itarana', 'Bearss Lime', 'CNPMF 01', and 'CNPMF 5059'. The second consisted of 'Santa Rosa', 'CNPMF 02', 'CNPMF 2001', and 'Persian 58', whereas the third included only 'BRS Passos'.

Although the Tocher method provides the number and the composition (by means of genotypes) of each cluster, it does not provide information on the distance between pairs of clusters as does the PCA. Therefore, in a genetic diversity study, the use of different methodologies is recommended (Cruz et al., 2020).

The PCA resulted in three eigenvalues – PCA1, PCA2, and PCA3 – that explain 47.3, 26.1, and 13.2% of total variation (Table 2), accounting for 86.6% of the

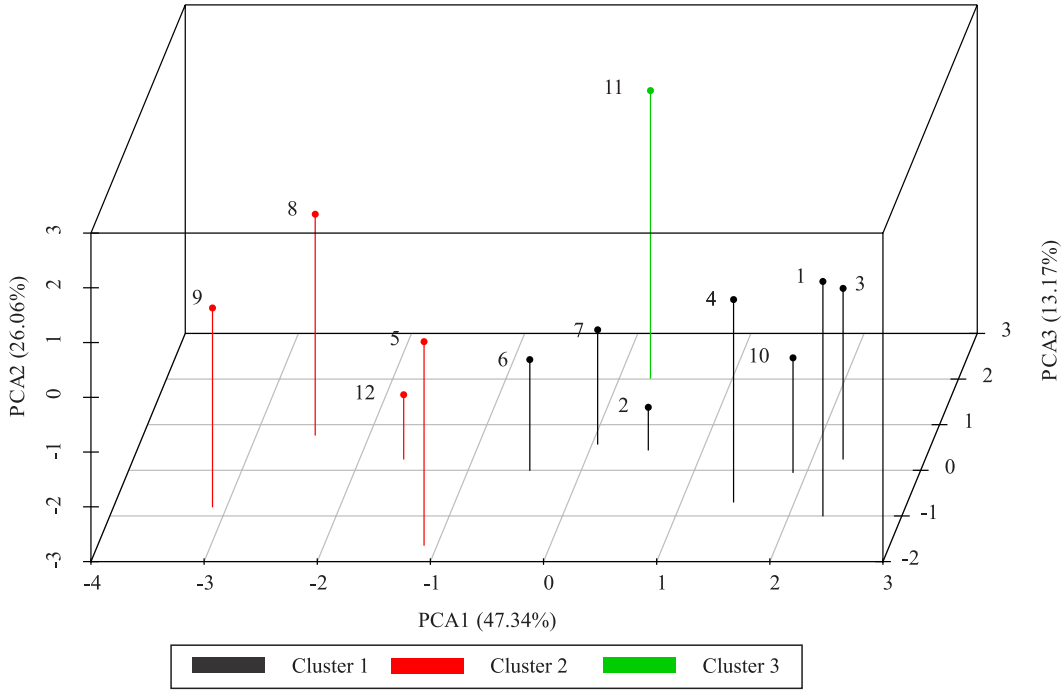

Figure 2. Representation of the principal component analyses and modified Tocher method, showing: the graphical dispersion of the 12 'Tahiti' acid lime (*Citrus latifolia*) genotypes explained by 86.58% of the variation in a three-dimensional plane with the three main components of the principal component analysis (PCA1, PCA2, and PCA3); and the clusters (clusters 1, 2, and 3) generated by the modified Tocher method using the standardized mean for Euclidean distances. Evaluated genotypes: 1, 'Bello Fruit'; 2, 'Éledio'; 3, 'Iconha'; 4, 'Itarana'; 5, 'Santa Rosa'; 6, 'Bearss Lime'; 7, 'CNPMF 01'; 8, 'CNPMF 02'; 9, 'CNPMF 2001'; 10, 'CNPMF 5059'; 11, 'BRS Passos'; and 12, 'Persian 58'.

Table 2. Principal component analysis for seven traits evaluated in 12 'Tahiti' acid lime (*Citrus latifolia*) genotypes[1].

Trait[2]	Principal component						
	1	2	3	4	5	6	7
PH	0.1526	0.5115	0.5036	0.2551	0.1050	-0.3677	0.5001
NF	0.6035	0.0061	-0.0408	-0.3674	**-0.6720**	-0.2171	0.0196
FYP	-0.0133	0.3578	0.3757	**-0.6359**	0.1363	**0.5535**	-0.0375
LFD	**0.6496**	0.0539	0.0748	0.5085	0.1447	0.4597	-0.2805
EFD	-0.2865	-0.0233	-0.0363	0.3067	-0.5800	0.5285	0.4542
ST	0.3282	-0.2737	-0.3675	-0.2069	0.4012	0.1435	**0.6767**
JY	0.0212	**-0.7294**	**0.6794**	-0.0111	-0.0235	0.0088	0.0724
Eigenvalue	3.3139	1.8249	0.9221	0.5196	0.2511	0.1672	0.0010
Variation (%)	47.341	26.069	13.1724	7.4232	3.5885	2.3896	0.0163
Cumulative (%)	47.341	73.411	86.5824	94.0056	97.5941	99.9837	100.0

[1]In bold, eigenvector that presented the highest value and associated eigenvalue. [2]PH, plant height; NF, number of fruits per plant; FYP, fruit yield per plant; LFD, longitudinal fruit diameter; EFD, equatorial fruit diameter; ST, shell thickness; and JY, juice yield.

variation of the studied *C. latifolia* genotypes. These components were represented by axes X, Y, and Z, respectively (Figure 2). The PCA showed the distance between 'BRS Passos' and the other scions, and the 'Santa Rosa' scion highly diverged from the others.

LFD and NF presented the two highest eigenvectors in PCA1, with values of 0.64 and 0.60, respectively. JY showed the highest coefficients in PCA2 and PCA3, which were -0.72 and 0.67, respectively. The other traits presented the lowest eigenvalues in the analysis, that is: 0.40 in PCA7 for ST; 0.55 and -0.63, respectively, in PCA6 and PCA4 for FYP; and -0.67 in PCA5 for NF. Since eigenvalues below 0.7 should be disregarded (Cruz et al., 2020), the ST trait does not need to be evaluated in future experiments with *C. latifolia* genotypes.

The genotypic variability observed in the present study is probably due to the different origins of the selected genotypes and to the recurrent mutations that modify the plant genome (Santos et al., 2013). The obtained results are relevant, since information regarding 'Tahiti' acid lime cultivation is scarce. When considering repeated measures, rootstocks, and unbalanced data, the REML/BLUP method represents an alternative widely applied in plant breeding.

Conclusions

1. The compound symmetry model predicts clonal values and precisely estimates variance components.

2. The principal components analysis and modified Tocher method indicate genotypic diversity.

3. The modified Tocher method groups the 'Tahiti' acid lime (*Citrus latifolia*) scions into three clusters.

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