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Scientific Article

METHODS FOR KINO EVALUATION AND ESTIMATES OF GENETIC PARAMETERS IN *Corymbia*

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ABSTRACT

Species within the genus *Corymbia* are regarded as potential alternatives to *Eucalyptus*. In addition to having superior wood quality, *Corymbia* spp. are tolerant to most pests, diseases, and abiotic stresses that affecting *Eucalyptus* plantations, including physiological disorders, water deficit, and wind damage. However, environmental stresses stimulate kino production, which decreases the quality of pulp and sawn wood. This study aimed to develop a method for evaluating kinoand estimate genetic parameters in *Corymbia*. For this, 16 *Corymbia* (*C. citriodora* × *C. torelliana*) hybrid clones and 5 clones of *Eucalyptus* were used. Two evaluation methods (M1 and M2) were tested for kino evaluation; M1 consisted of drilling the bark with Pilodyn and M2 consisted of drilling the heartwood with Pilodyn. The following kino parameters were evaluated: exudation incidence, exudate length which flowed over the stem, and exudate weight. Genetic parameters were estimated by a mixed model method (REML/BLUP). The significance of random effects of the statistical model was tested by the likelihood ratio test. Significant clone effects were obtained for all kino parameters, except for exudate length as assessed by M2. Kino parameters determined by M1 exhibited higher heritability and accuracy. Therefore, M1 should be preferred for kino evaluation in *Corymbia*.

Keywords: Forest improvement; wood quality; genotypic correlation; gummosis.

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METODOLOGIAS PARA AVALIAÇÃO DE KINO E ESTIMATIVAS DE PARÂMETROS GENÉTICOS EM *Corymbia*

RESUMO – Espécies do gênero *Corymbia* são consideradas potenciais alternativas ao eucalipto. Além de possuir madeira de qualidade superior, *Corymbia* spp. são tolerantes à maioria das pragas, doenças e estresses abióticos que afetam as plantações de eucalipto, incluindo distúrbios fisiológicos, déficit hídrico e danos causados pelo vento. No entanto, as tensões ambientais estimulam a produção de kino, o que diminui a qualidade da celulose e da madeira serrada. Este estudo teve como objetivo estabelecer um método para avaliação de kino e estimativa de parâmetros genéticos em *Corymbia*. Para isso foram utilizados 16 clones híbridos de *Corymbia* (*C. citriodora* × *C. torelliana*) e 5 clones de *Eucalyptus*. Dois métodos (M1 e M2) foram testados para avaliação; M1 consistiu na perfuração da casca com Pilodyn e M2 consistiu na perfuração do cerne com Pilodyn. Foram avaliados os seguintes parâmetros: incidência de exsudação, comprimento do exsudato que fluiu sobre o caule e peso do exsudato. Os parâmetros genéticos foram estimados por um método de modelo misto (REML/BLUP). A significância dos efeitos aleatórios do modelo estatístico foi testada pelo teste da razão de verossimilhança. Efeitos clones significativos foram obtidos para todos os parâmetros, exceto para o comprimento do exsudato avaliado por M2. Os parâmetros determinados por M1 apresentaram maior herdabilidade e precisão. Portanto, M1 deve ser preferido para avaliação de kino em *Corymbia*.

Palavras-Chave: Melhoramento florestal; qualidade da madeira; correlação genotípica; gomose.

1. INTRODUCTION

Brazil's forestry sector is currently one of the most competitive in the world, with a planted area of 9 million hectares, 77% of which is composed of *Eucalyptus* plantations (IBÁ, 2020). Timber production increased from about 17.0 m3 ha−1 year−1 in 1960 to 35.3 m3 ha−1 year−1 in 2019 in the country (IBÁ, 2020), with yields of up to 83.0 m3 ha−1 year−1 at given locations (Stape et al., 2010). Genetic improvement, allied to the development of cloning techniques, cultural management, and investment in research, has contributed to such an expressive increase in productivity (Castro et al., 2016; Gonçalves et al., 2008).

Eucalyptus species conquered a prominent position in the Brazilian forest-based industry owing to the technological properties of *Eucalyptus* wood and advances in silviculture. As a result, species from other genera are little used, despite exhibiting technological potential. Members of *Corymbia* genus, for instance, show promise as raw material for a variety of industrial applications. *Corymbia* species and hybrids have numerous advantages, such as tolerance to water deficit, wind, and the majority of pests, diseases, and physiological disorders affecting *Eucalyptus*; furthermore, *Corymbia* has superior wood quality (Assis, 2014). However, *Corymbia* species are highly affected by environmental stresses, including climatic adversities, insect injury, and mechanical damage. Such factors trigger the production of resin/exudate in the phloem and/or xylem, which, when in contact with air, becomes glassy and promotes bark darkening; resin may also be retained inside the wood in the form of pockets and veins (Assis, 2014; Tippet, 1986).

The occurrence of exudate, when not associated with mechanical damage or action of biological agents, is known as gummosis or blackwood and is attributed to physiological problems (Ferreira, 1989). This type of exudate should preferably be referred to as kino, because it contains more polyphenols than carbohydrates (Tippet, 1986). Kino has high polyphenol content and low water content. The polyphenols are almost entirely flavonoid in nature, mostly tannins (Martius et al., 2012). Polyphenols account for 70–80% of kino composition (Bolza, 1978); in some species, this class of compounds may represent more than 90%, as occurs in *Eucalyptus* viminalis Labill. (Watt and Breyer-Brandwijk, 1962).

The presence of kino is the most serious form of defect in *Eucalyptus* and *Corymbia* wood, as it significantly reduces the quality and quantity of cellulose pulp and increases the consumption of chemicals during pulping (Hillis, 1964, 1972). Kino also decreases the economic value of lumber boards (Assis, 2014). The influence of the defect in charcoal and bioenergy production is still unknown; further studies are needed to investigate the impact of kino pockets.

Kino also affects some stages of tree improvement programs, as it makes it difficult to obtain breeding stocks in the field. High resin production may occur as a response to girdling, preventing the sprouting of cuttings for cloning. Thus, it is necessary to cut the affected individuals, making it impossible to carry out early (at age 3) or late (at age 7) selection.

Studies on the chemical composition of kino have demonstrated its medicinal potential, mainly associated with the presence of flavonoids with antimicrobial properties (Nobakht et al, 2014; Nobakht et al, 2017). Kinotanic acid (which accounts for up to 45% of kino gum), kinoin, red kino, and catechol are among the major components of kino from different species, such as *Acacia nilotica* (L.) Delile (Ali et al., 2012), *Eucalyptus camaldulensis* Dehnh. (Watt and Breyer-Brandwijk, 1962), and *Pterocarpus marsupium* Roxb. (Badkhane et al., 2010). These components are of high value to the pharmaceutical industry.

The most economically valued kinos for use in the medical and food industries are extracted from *Acacia nilotica* (L.) Willd. ex Delile, *Acacia senegal* (L.) Willd., *Acacia seyal* Delile, *Butea monosperma* (Lam.) Taub., *Coccoloba uvifera* (L.) L., *Moringa oleifera* Lam., *Pterocarpus erinaceus* Poir., and *P. marsupium*. Kino can also be obtained from tree species belonging to the genera *Angophora* and *Corymbia* (Locher and Currie, 2010).

It is interesting to note that kino production may be desirable for some purposes as medical and food applications but undesirable for others such cellulose and sawn wood production. Genetic selection serves as an important tool in this regard, allowing the production of specific genotypes for different applications. There are, however, no studies on the genetic selection of kino traits. This study aimed to establish a method for kino evaluation and estimate genetic parameters in *Corymbia*.

2. MATERIAL AND METHODS

2.1 Experimental design and genetic materials

The experiment was installed in Martinho Campos (19°19′22″S 45°14′46″W, 663 m a.s.l.), Minas Gerais State, Brazil. The climate is of the Aw type in Köppen's classification, with average annual temperature and rainfall of 22.6 °C and 1.323 mm, respectively.

The genetic materials used in this study are part of the genetic improvement program of ArcelorMittal Bioflorestas and consisted of 16 hybrid clones of *Corymbia* (*C. citriodora* × *C. torelliana*) and 5 commercial clones of *Eucalyptus* spp. (Table 1).

Table 1 – Genetic materials used in the study
Tabela 1 – Materiais genéticos utilizados no estudo

Clone	Genetic material	Origin
AM01	*C. torelliana* × *C. citriodora*	AMBIO
AM02	*C. torelliana* × *C. citriodora*	AMBIO
AM03	*C. torelliana* × *C. citriodora*	AMBIO
AM04	*C. torelliana* × *C. citriodora*	AMBIO
AM05	*C. torelliana* × *C. citriodora*	AMBIO
AM06	*C. torelliana* × *C. citriodora*	AMBIO
AM07	*C. torelliana* × *C. citriodora*	AMBIO
AM08	*C. torelliana* × *C. citriodora*	AMBIO
AM09	*C. torelliana* × *C. citriodora*	AMBIO
AM10	*C. torelliana* × *C. citriodora*	AMBIO
AEC0001	*C. citriodora* × *C. torelliana*	APERAM
AEC0004	*C. torelliana* × *C. citriodora*	APERAM

cont...

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Clone	Genetic material	Origin
AEC0007	*C. torelliana* × *C. citriodora*	APERAM
AEC0022	*C. citriodora* × *C. torelliana*	APERAM
AEC0043	*C. citriodora* × *C. torelliana*	APERAM
AEC0044	*C. citriodora* × *C. torelliana*	APERAM
AEC144	*E. urophylla*	APERAM
AEC1528	*E. urophylla* × *E. grandis*	APERAM
AEC2233	*E. urophylla* × *(E. camaldulensis* × *E. grandis)*	APERAM
AEC2475	*E. urophylla* × *E. pellita*	APERAM
VM04	*E. urophylla* × *E. grandis*	VALLOUREC

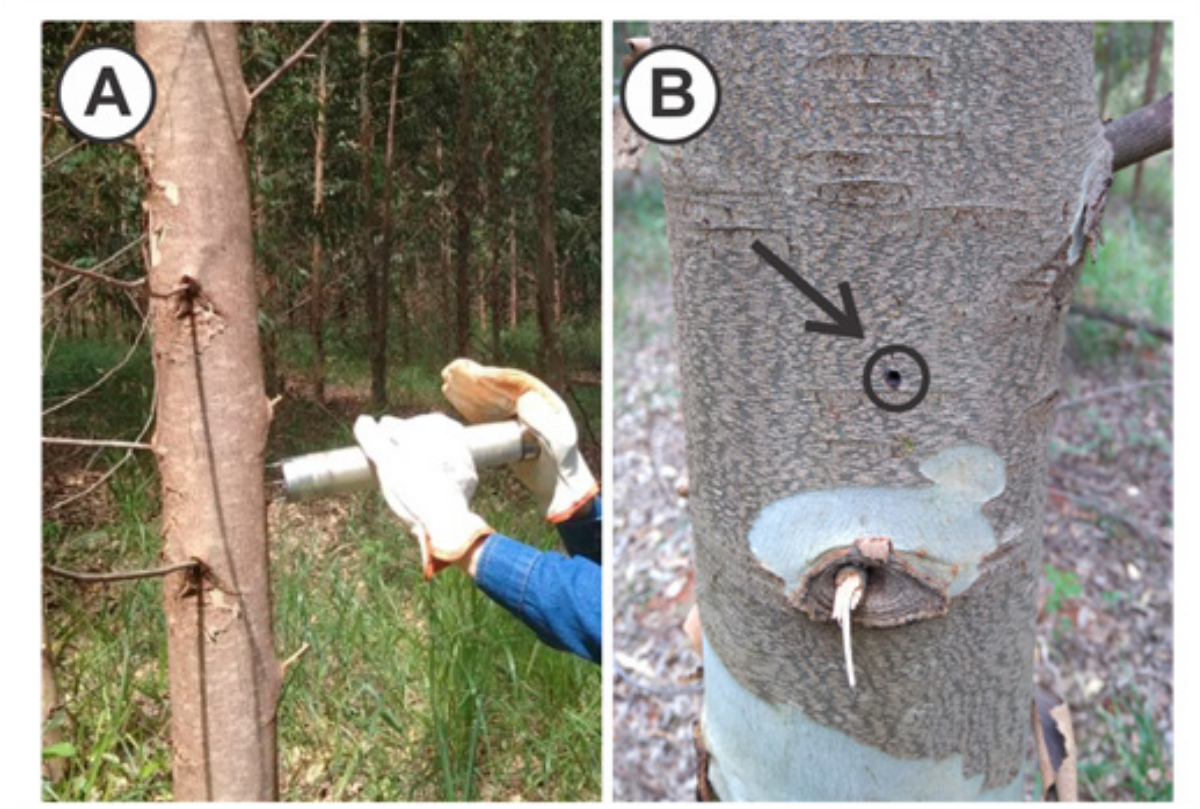

Figure 1 – Pilodyn perforation of tree bark (Method 1). (a) Pilodyn being inserted into a tree trunk to simulate an insect piercing injury. (b) Perforation caused by Pilodyn (indicated by an arrow).

Figura 1 – Perfuração da casca de árvore (Método 1) por meio do Pilodyn. (a) Pilodyn sendo inserido em um tronco de árvore para simular uma lesão perfurante de inseto. (b) Perfuração causada por Pilodyn (indicada por uma seta).

The experimental design used for the clonal test was randomized blocks, with 21 treatments, 10 blocks, and 6 plants per plot. Trees were planted at a spacing of 4.5 × 2.0 m. Planting was carried out on April 20, 2016. When the trees were 32 months old, two methods were used to induce the formation of exudates, simulating stress conditions that could activate the kino production system.

2.2 Methods used for induction of kino production

Two methods were used to stimulate kino production in trees. In both cases, injuries

Figure 2 – Pilodyn penetration after bark removal (Method 2). (a) Chisel used for removing a circular section of the bark. (b) Tree stem without bark. (c) Pilodyn piercing the stem without the bark, simulating an insect attack. (d) Heartwood perforation caused by Pilodyn

Figura 2 – Penetração do Pilodyn após remoção da casca (Método 2). (a) Cinzel usado para remover uma seção circular da casca. (b) Caule de árvore sem casca. (c) Pilodyn perfurando o caule sem a casca, simulando ataque de inseto. (d) Perfuração do cerne causada por Pilodyn.

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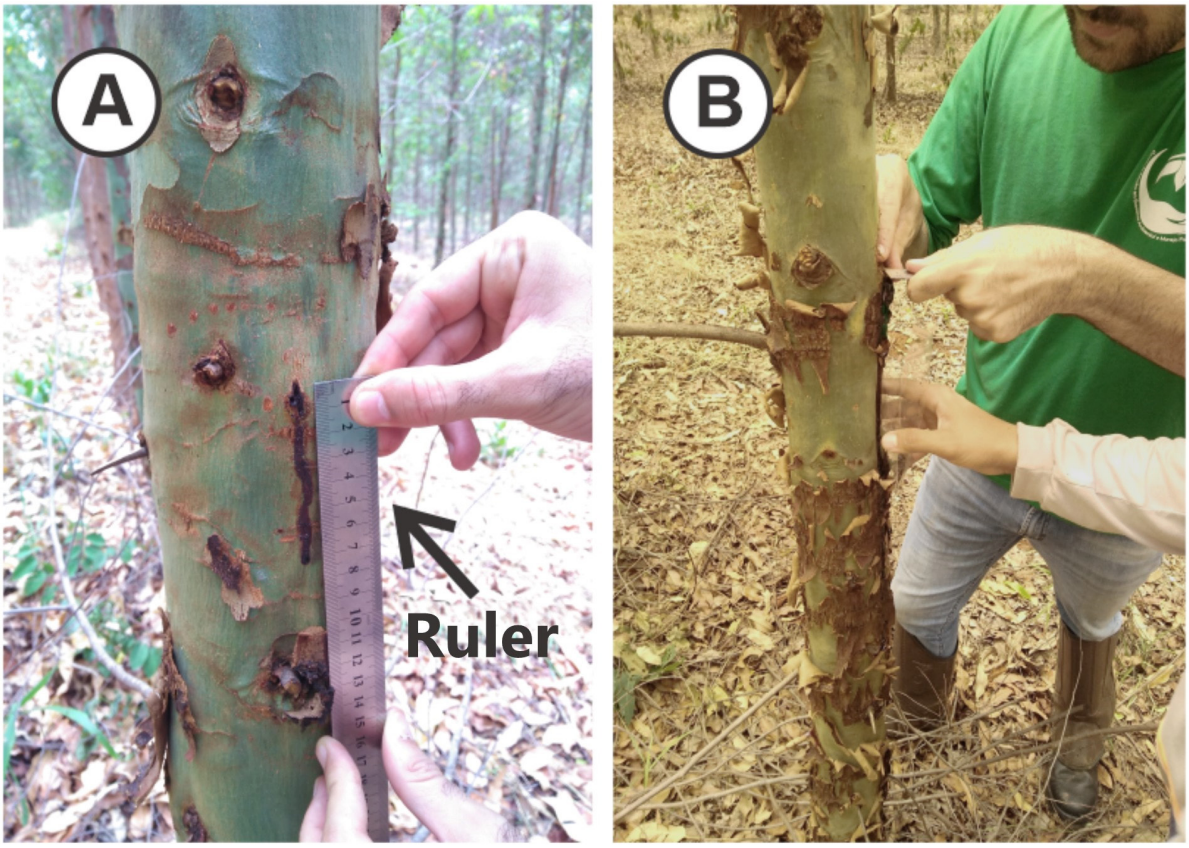

Figure 3 – Evaluation of kino production. (a) Measurement of exudate length. (b) Exudate scraped with a metal spatula and collected into a plastic bag.

Figura 3 – Avaliação da produção de kino. (a) Medição do comprimento do exsudato. (b) Exsudato raspado com espátula metálica e coletado em saco plástico.

were caused and, subsequently, the production of exudate was determined.

Method 1 consisted of piercing the bark using a Pilodyn, simulating an insect piercing injury. The Pilodyn (2.5 mm diameter steel needle) was inserted into the outer face of the trunk, driven by a spring with a constant energy of 6 J (Greaves et al., 1996). The Pilodyn was triggered twice, at a height of 1.3 m from the ground, in the same place, in order to obtain deeper penetration by the needle into the trunk, always in the planting line direction of (Figure 1).

Method 2 consisted of removing a circular section (about 2.5 cm in radius) of the trunk using a chisel at a height of 1.3 m from the ground. The Pilodyn was triggered twice directly into the heartwood, perforating in the direction of the planting line (Fig. 2).

Half of the plants of each plot were tested by method 1 and the other half by method 2. Thus, each clone was evaluated by both methods in all plots.

2.3 Kino evaluation

Kino production was assessed after 45 days of the experiment installation. Exudate incidence (presence or absence) was determined by counting the number of exuding trees, and exudate length was measured with a metal ruler (Figure 3a). After measurements were taken, the exudate was scraped, placed in a plastic bag, and taken to the laboratory for weight determination. Exudate weight was measured on a 3-digit precision scale (Figure 3b). Exudation incidence (I), exudate length (L), and exudate weight (W) were determined in trees subjected to kino induction by method 1 (I1, L1, and W1) and method 2 (I2, L2, and W2).

For statistical analysis, data were collected from three plants per plot per method.

2.4 Statistical analysis

The restricted maximum likelihood method (REML) (Patterson and Thompson, 1971) was used to estimate genetic parameters, and the best linear unbiased prediction method (BLUP) (Henderson, 1975) was used to predict genotypic values. The following statistical model was applied to assess the significance of the Method × Genotype interaction (Eq. 1):

$$y=Xm+Zr+Wg+Tp+Qi+e \qquad (Eq.1)$$

where y is the data vector; m is the vector of method effects (assumed to be fixed) added to the overall mean; r is the vector of repeat effects (assumed to be random), $r\sim N(0,\sigma_r^2)$; g is the vector of genotype effects (assumed to be random), $g\sim N(0,\sigma_g^2)$; p is the vector of plot effects (assumed to be random), $p\sim N(0,\sigma_p^2)$; i is the vector of the Method × Genotype interaction effects (random), $i\sim N(0,\sigma_i^2)$; and e is the vector of errors or residuals (random), $e\sim N(0,\sigma_e^2)$. Capital letters represent the incidence matrices of these effects.

The significance of random effects for the statistical model was verified by applying the likelihood ratio test (LRT) using chi-square statistics with a one degree of freedom and a significance level of 5% (Resende, 2016). Then, kino evaluation methods were compared by the following statistical model (Eq. 2):

$$y=Xr+Zg+e \qquad (Eq.2)$$

where y is the data vector; r is the vector of repeat effects (assumed to be fixed) added to the overall mean; g is the vector of genotypic effects (assumed to be random), $g\sim N(0,\sigma_g^2)$; and e is the vector of errors or residuals (random), $e\sim N(0,\sigma_e^2)$. Capital letters represent the incidence matrices of these effects.

The significance of genotypic effects (clones) was tested by LRT using chi-square statistics with one degree of freedom and significance level of 5% (Resende, 2016). The phenotypic variance (σ_p^2), heritability of total genotypic effects (h_g^2), heritability of the clone mean (h_{mc}^2), accuracy of clone selection ($r_{\hat{g}g}$), genotypic coefficient of variation (CV_{gi}), experimental variation coefficient (CV_e), coefficient of relative variation (CV_r), and standard error of the predicted genotypic value (SEP) for parameters I1, I2, L1, L2, W1, and W2 were obtained, respectively, by the following equations (Eqs. 3–10):

$$\sigma_p^2=\sigma_g^2+\sigma_e^2 \qquad (Eq.\ 3)$$

$$h_g^2=\sigma_g^2/\sigma_p^2 \qquad (Eq.\ 4)$$

$$h_{mc}^2=\sigma_g^2/(\sigma_g^2+(\sigma_e^2)/r) \qquad (Eq.\ 5)$$

$$r_{\hat{g}g}=\sqrt{(1-PEV/\sigma_g^2)} \qquad (Eq.\ 6)$$

$$CV_{gi}\,(\%)=(\sqrt{((\sigma_g^2)/m)})100 \qquad (Eq.\ 7)$$

$$CV_e\,(\%)=(\sqrt{((\sigma_e^2)/m)})100 \qquad (Eq.\ 8)$$

$$CV_r=CV_{gi}/CV_e \qquad (Eq.\ 9)$$

$$SEP=\sqrt{PEV} \qquad (Eq.\ 10)$$

where σ_g^2 is the genotypic variance, σ_e^2 is the residual variance, r is the number of replications (3), PEV is the variance of the prediction error extracted from the diagonal of the generalized inverse of the matrix of coefficients in the mixed model, and m is the overall mean.

Genotypic correlations between predicted genotypic values for the evaluated kino parameters (I1, I2, L1, L2, W1, and W2) were calculated using the following equation (Eq. 11):

$$\hat{\rho}=(\Sigma_i(x_i-\bar{x})(w_i-\bar{w}))/(\sqrt{(\Sigma_i(x_i-\bar{x})^2\,\Sigma_i(w_i-\bar{w})^2)} \qquad (Eq.\ 11)$$

where $\hat{\rho}$ is the Pearson correlation coefficient and x_i and w_i are the predicted genotypic values associated with I1, I2, L1, L2, W1, and W2.

Statistical analyses were performed using Selegen REML/BLUP software (Resende, 2016).

3. RESULTS

There were significant genotype effects on the incidence, length, and weight of kino ($P < 0.05$). Method × Genotype interaction effects, however, were not significant (Table 2).

Significant genotype (clone) effects were observed on I1, L1, W1, I2, and W2, as assessed by LRT (Table 3). The effect of genotype on L2 was not significant.

Table 3 lists the variance components and genetic and nongenetic parameters for all evaluated traits. Regarding kino incidence (I1 and I2), genotypic variance (σ_g^2) was higher for I2 (0.0106) than I1 (0.0058), as was residual variance (σ_e^2) and phenotypic variance (σ_p^2). I1, however, exhibited higher broad-sense heritability (h_g^2) and heritability of the clone

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Table 2 – Deviance and likelihood ratio test (LRT) for the incidence, length, and weight of kino in 21 clones of *Corymbia* spp. and *Eucalyptus* spp.

Tabela 2 – Deviance e teste da razão de verossimilhança (LRT) para incidência, comprimento e peso de kino em 21 clones de *Corymbia* spp. e *Eucalyptus* spp.

Effect	Incidence		Length (cm)		Weight (g)	
	Deviance	LRT	Deviance	LRT	Deviance	LRT
Genotype	−1436.75	−8.44*	4755.24	−10.54*	16138.31	−5.68*
Method × Genotype	−1445.00	−0.19ns	4744.73	−0.03ns	16132.73	−0.10ns
Full model	−1445.19		4744.70		16132.63	

*, significant at P < 0.05 by the chi-square test; ns, not significant at P < 0.05 by the chi-square test; H_0, null hypothesis (full model = reduced model; the reduced model does not consider Genotype or Method × Genotype effects).

*, significativo a P < 0,05 pelo teste qui-quadrado; ns, não significativo para P < 0,05 pelo teste qui-quadrado; H_0, hipótese nula (modelo completo = modelo reduzido; o modelo reduzido não considera os efeitos Genótipo ou Método × Genótipo).

Table 3 – Deviance and likelihood ratio test (LRT) for the incidence (I1 and I2), length (cm) (L1 and L2), and weight (g) (W1 and W2) of kino induced by method 1 (Pilodyn perforation of tree bark) or method 2 (Pilodyn penetration after bark removal) in 21 clones of *Corymbia* spp. and *Eucalyptus* spp.

Tabela 3 – Deviance e teste de razão de verossimilhança (LRT) para incidência (I1 e I2), comprimento (cm) (L1 e L2) e peso (g) (W1 e W2) de kino induzido pelo método 1 (perfuração de Pilodyn em casca de árvore) ou método 2 (penetração de Pilodyn após remoção da casca) em 21 clones de *Corymbia* spp. e *Eucalyptus* spp.

Effect	I1	I2	L1	L2	W1	W2
Genotype	−555.97	−252.74	674.55	487.79	2407.14	2434.56
Full model	−593.51	−260.96	650.44	485.93	2392.33	2429.61
LRT	37.54*	8.22*	24.11*	1.86ns	14.81*	4.95*

*, significant at P < 0.05 by the chi-square test; ns, not significant at P < 0.05 by the chi-square test; H_0, null hypothesis (full model = reduced model; the reduced model does not consider genotype effects).

*, significativo a P < 0,05 pelo teste qui-quadrado; ns, não significativo para P < 0,05 pelo teste qui-quadrado; H_0, hipótese nula (modelo completo = modelo reduzido; o modelo reduzido não considera efeitos genotípicos).

mean (h_{mc}^2) (Table 4).

L1 showed higher genotypic variance (σ_g^2), residual variance (σ_e^2), and phenotypic variance (σ_p^2) than L2. Broad-sense heritability (h_g^2) and heritability of the clone mean (h_{mc}^2) were also higher for L1 (Table 4).

Weight-based assessment showed higher genotypic variance (σ_g^2), broad-sense heritability (h_g^2), and heritability of the clone mean (h_{mc}^2) for W1 than W2 (8.9976 and 5.4066, respectively). However, residual variance (σ_e^2) and phenotypic variance (σ_p^2) were higher for W2 (Table 4).

Accuracies (r_{gg}) were greater than 0.70, except for L2 (for which genotype effects were not significant, as indicated by LRT). CV_{gi} and CV_e varied according to the trait and method evaluated. Method 1 afforded the highest values for all kino parameters; the coefficient of relative variation (CV_r) ranged from 0.23 to 0.65, and the highest value was observed for I1 (Table 4).

Kino incidence (I1 and I2) exhibited the highest heritability and accuracy values (0.30 and 0.90, respectively) by both methods compared with length (L1 and L2) and weight (W1 and W2) (Table 3). In comparing the estimates for incidence (I1 and I2), length (L1 and L2), and weight (W1 and W2) within the same method, it was found that incidence exhibited lower PEV and SEP (Table 4).

Table 4 – Variance components and genetic and nongenetic parameters for the incidence (I1 and I2), length (L1 and L2), and weight (W1 and W2) of kino induced by method 1 (Pilodyn perforation of tree bark) or method 2 (Pilodyn penetration after bark removal) in 21 clones of *Corymbia* spp. and *Eucalyptus* spp.

Tabela 4 – Componentes de variância e parâmetros genéticos e não genéticos para a incidência (I1 e I2), comprimento (L1 e L2) e peso (W1 e W2) de kino induzido pelo método 1 (perfuração de Pilodyn na casca da árvore) ou método 2 (penetração de Pilodyn após remoção da casca) em 21 clones de *Corymbia* spp. e *Eucalyptus* spp.

Component	I1	I2	L1	L2	W1	W2
σ_g^2	0.0058	0.0106	2.0920	0.1846	8.9976	5.4066
σ_e^2	0.0138	0.0787	7.1201	3.4364	44.2904	55.7140
σ_p^2	0.0196	0.0893	9.2121	3.6210	53.2880	61.1206
h_g^2	0.30 (0.11)†	0.12 (0.07)	0.23 (0.09)	0.05 (0.04)	0.17 (0.08)	0.09 (0.06)
h_{mc}^2	0.81	0.57	0.75	0.35	0.67	0.49
$r_{\hat{g}g}$	0.90	0.76	0.86	0.59	0.82	0.70
CV_{gi} (%)	8.01	14.10	205.28	108.32	205.90	52.49
CV_e (%)	12.31	38.40	378.70	467.33	456.83	168.49
CV_r	0.65	0.37	0.54	0.23	0.45	0.31
PEV	0.00	0.01	0.53	0.12	2.97	2.75
SEP	0.03	0.07	49.60	0.35	0.68	52.38
Overall mean	0.95	0.73	0.70	0.40	46.07	140.09

σ_g^2, genotypic variance; σ_p^2, phenotypic variance; h_g^2, heritability of total genotypic effects; h_{mc}^2, heritability of the clone mean; $r_{\hat{g}g}$, accuracy of clone selection; CV_{gi} (%), genotypic coefficient of variation; CV_e (%), experimental coefficient of variation, CV_r, coefficient of relative variation; PEV, prediction error variance; SEP, standard error of predicted genotypic value; †, standard deviation.

σ_g^2, variância genotípica; σ_p^2, variância fenotípica; h_g^2, herdabilidade dos efeitos genotípicos totais; h_{mc}^2, herdabilidade média do clone; $r_{\hat{g}g}$, precisão da seleção do clonal; CV_{gi} (%), coeficiente de variação genotípico; CV_e (%), coeficiente de variação experimental, CV_r, coeficiente de variação relativa; PEV, variância do erro de predição; SEP, erro padrão do valor genotípico previsto; †, desvio padrão.

Genotypic correlations (ρ̂) between the predicted genotypic values of traits induced by method 1 were greater than 0.85, and those of traits induced by method 2 ranged from 0.57 to 0.78. I1 had the highest ρ value with L1 (0.93) and I2 with W2 (0.78) (Fig. 4). Genotypic correlations between I1 and I2, L1 and L2, and W1 and W2 were 0.68, 0.85, and 0.48, respectively (Fig. 4).

4. DISCUSSION

The tested methods allowed the evaluation of kino production in *Corymbia* and *Eucalyptus* clones. There was significant genetic variance for the evaluated traits, except L2. The observed genetic variability can be attributed to the location, synthesis, and transport of kino. The compounds that constitute kino are usually synthesized in the Golgi complex and transported by smooth vesicles mediated by the Golgi complex. Kino is deposited between the plasma membrane and the cell wall until it is exuded (Fahn, 1988b). Polyphenols are synthesized by the endoplasmic reticulum and then released and transported through the vesicle transfer system (Shahidi and Yeo, 2016). At the same time, other exudate components, such as polysaccharides, are released and begin to form kino. As a result, kino composition and exudation may vary according to the sampled individual.

Such findings agree with field observations: kino induced by method 2 was mostly retained in the circumference of the removed bark area, making it impossible to measure length. Another fact that contributes to inaccuracy in exudate length measurement is the kino viscosity. Viscosity differs according to kino composition, which varies between and within species (Martius et al., 2012). Furthermore, changes in exudate flow, as

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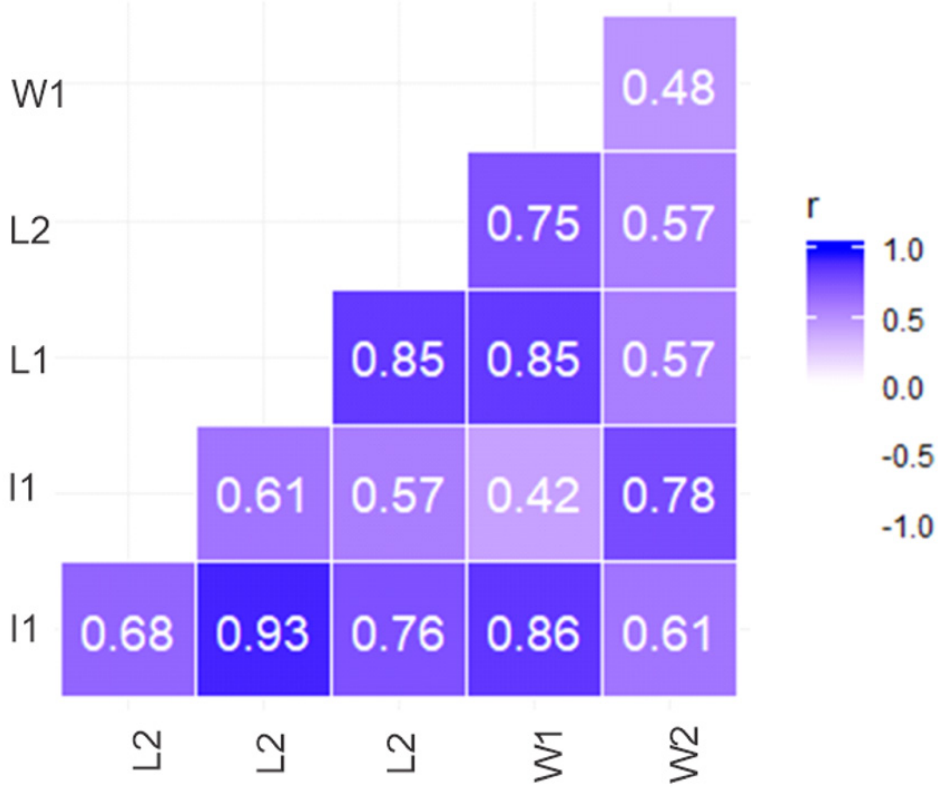

Figure 3 – Genotypic correlations (ρ̂) between predicted genotypic values for the incidence (I1 and I2), length (L1 and L2), and weight (W1 and W2) of kino induced by method 1 (Pilodyn perforation of tree bark) or method 2 (Pilodyn penetration after bark removal) in 21 clones of *Corymbia* spp. and *Eucalyptus* spp.

Figura 3 – Correlações genotípicas (ρ̂) entre valores genotípicos previstos para a incidência (I1 e I2), comprimento (L1 e L2) e peso (W1 e W2) de kino induzido pelo método 1 (perfuração de Pilodyn na casca da árvore) ou método 2 (penetração de Pilodyn após remoção da casca) em 21 clones de *Corymbia* spp. e *Eucalyptus* spp.

observed in *A. nilotica* (Kuruwanshi et al., 2017) and *A. senegal* (Vasishth and Guleria, 2017), demonstrate that crystallization may depend not only on composition but also on environmental factors, such as temperature and humidity. Such factors allow kino to drain more easily in some genotypes than in others, forming a longer albeit thinner layer of exudate.

Among the genetic parameters evaluated, heritability and genetic variability are the parameters that deserve the most emphasis, as they are fundamental to the success of genetic improvement. It is essential that the characteristics of interest are heritable, maintaining variation in the selected population (Cruz, 2005). Genetic variability, in conjunction with the value of heritability, provides the breeding program with a good indication of the potential for progress to be achieved in generations (Garrido, 1997). Genetic variance is established by additive variance, dominance variance, and epistatic effects (Cruz, 2005).

Heritability is expressed as the proportion of phenotypic variance that has a genetic origin. It can be broken down into heritability in the broad sense, which considers the total genetic variance and is used to define vegetative propagation, and heritability in the narrow sense, which considers only additive genetic variance and is used to define sexual reproduction (Pires et al., 2011; Borém et al., 2017).

Low heritability is found in quantitative traits because, in addition to being controlled by a large number of genes, they are greatly influenced by the environment. This requires more elaborate selection methods than those with high heritability (Pires et al., 2011).

Heritability may vary according to several

factors, such as trait, estimation method, population diversity, level of inbreeding, sample size, number and type of environment, experimental unit, and precision in experimentation and data collection (Resende, 2002). In this study, the only factor that varied was the method used to induce kino production, allowing comparison of methods by heritability and accuracy estimates.

Individual heritability values can be classified as low (0.01 to 0.15), medium/moderate (0.15 to 0.50), or high (>0.50) (Resende, 2002). On the basis of this classification, kino incidence can be said to have moderate genetic control, indicating the possibility of obtaining significant genetic gains with selection. When a large number of genes of small effect control a given trait, it is suggested that a large part of the phenotypic variability is due to environmental variation (Resende, 2002; Resende, 2015).

When promoting selection, in addition to having a clear idea of what is expected from the process; meaning predicting the selection result; it is crucial to estimate the reliability achieved when adopting a particular procedure. This reliability is defined by the accuracy of the selection. With the exception of L2 (which was not significant by LRT), all kino parameters showed high accuracy (70–90%). Accuracy values between 15% and 50% are considered moderate for perennial crops (Resende, 2007), whereas values between 70% and 90% are classified as high and those greater than 90% as very high (Resende and Duarte, 2007). Accuracies of 70% or greater are desirable in genetic improvement programs (Viana, 2014). This parameter refers to the correlation between predicted genotypic values and true genotypic values; the greater the accuracy, the greater the confidence in selection and the efficiency of the improvement program (Resende, 2002).

To achieve 90% accuracy, it is necessary to obtain CV_r values of 0.70 (10 replications) to 1.50 (2 replications) (Resende and Duarte, 2007). Of the traits evaluated here, kino incidence induced by method 1 (I1) provided the best results. In addition to having higher heritability (0.30), I1 had a relative coefficient of variation (CV_r) of 0.65. Thus, as supported by the high number of replications (n = 10), accuracy (0.90) and heritability of the clone mean (0.81) were high, resulting in high selective reliability, which is corroborated by the low SEP value.

As previously discussed, differences in kino viscosity were identified in field observations. More viscous kino travels less, forming a thicker layer of exudate, whereas less viscous kino forms a thinner layer, influencing exudate length and weight. It is more difficult to scrape thin exudate, as it becomes vitreous in contact with air, also leading to bark removal, even when great care is taken during collection. This is because the act of scraping may shatter the exudate, generating considerable losses, which increase experimental error. Such observations explain the high PEV of W1 and W2.

L1 showed a high correlation (0.93) with I1 and good accuracy and heritability (0.86 and 0.23, respectively). The use of method 1 proved to be the best alternative for quantifying kino production in breeding programs. It is noteworthy that, operationally, method 1 is practical, fast, and easy to accomplish compared with method 2. It consists of fewer steps for phenotypic evaluation. The results of the current study are very significant for forest improvement programs. This is the first study on methods for assessing kino production. Evaluations based on the incidence of kino may positively contribute to genetic selection.

Future studies on the quantitative evaluation of kino production in *Corymbia* and *Eucalyptus* should be carried out for the proposal of new evaluation methods. It is of paramount importance the quantitative measurement of kino in improvement programs for greater (higher) accuracy in genotype ranking.

5. CONCLUSION

The tested kino induction methods allowed evaluating kino production in *Eucalyptus* and *Corymbia* clones. Assessment of kino incidence by method 1 (Pilodyn perforation of tree bark) proved to be the most adequate.

AUTHOR CONTRIBUTIONS

Michele Brandão Damacena: literature review, data acquisition, data analysis and interpretation, and manuscript preparation. Rodrigo Alves: conception and design of the study, literature review, data analysis and interpretation, preparation of the manuscript. Gleison Augusto dos Santos: conception and design of the study, supervision of the

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experiment, and preparation of the manuscript. Leonardo Lopes Bhering: conception and design of the study, supervision of the experiment, and preparation of the manuscript. Genaina Aparecida de Souza: conception and design of the study, literature review, data analysis and interpretation, preparation of the manuscript. Karine Fernandes Caiafa: intellectual review of the manuscript, final approval of the version submitted to the journal. Caio Varonill de Almada Oliveira: data acquisition. Ana Luiza Machado Gouvêa: data acquisition.

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