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Genetic parameters of pollen viability in guava (*Psidium guajava* L.)

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Abstract

Pollen viability is essential for the sexual reproduction in plants. Genetic and environmental factors as well as plant age can influence this characteristic. In this work, pollen viability was studied in guava (*Psidium guajava* L.), a cross-pollination species. The genetic parameters for this characteristic were estimated considering 22 genotypes, two environments and different plant ages. For that, the pollen viability of the genotypes was evaluated for three years (2013, 2014 and 2015) in two experimental orchards (installed in randomized block design, with three blocks and two plants per plot) and at two different regions of the state of Espírito Santo, Brazil. The plants were analyzed at 19, 24 and 38 months of age in the years 2013 and 2014 in Mimoso do Sul (ES) and 2015 in Linhares (ES), respectively. The flower buds, at pre-anthesis stage, were collected, fixed in ethanol:acetic acid (3:1) and stored at -20°C. Pollen viability was obtained by colorimetric methods (Alexander's, Acetic Orcein and Lugol dyes) and the genetic parameters estimated by means of mixed models. The pollen viability of the genotypes was high, with an overall mean of 93.46% in the three harvests. Mean heritability was lower in the harvests of 2013 (0.479) and 2014 (0.126) in relation to 2015 (0.583), indicating a slighter possibility of predicting genetic gains based on this characteristic. Altogether, these results provide information about pollen viability in commercial and superior genotypes of the guava crop used in this study, given that pollen-donor genotypes are reported to influence characteristics related to weight and fruit quality in this species. In addition, these genotypes showed good potential for cross-pollination, and can therefore be used as pollinators in orchards and crosses within breeding programs.

Keywords: Guava tree, pollen, plant breeding, genetic variability.

Introduction

The pollen represents an essential stage in the life cycle of plants and its viability is crucial for the sexual reproduction (Gottardini et al., 2008), as it reflects the potential of the male gamete in the fertilization efficiency (Alexander, 1980). Knowledge about pollen viability is important for taxonomy, ecology and palynology, providing information on the reproductive biology and conservation of the species (Alexander, 1980; Souza et al., 2002; Usman et al., 2013). It is also critical in genetic breeding, in the routine use of artificial pollination (Nascimento et al., 2003; Munhoz et al., 2008); in pollen monitoring during storage; in evaluation of pollen germination after exposure to stresses; in analyses of dispersion and gene flow; and in studies of genetics and pollen-stigma interactions, incompatibility and fertility (Santos et al., 2007; Bauermann et al., 2009; Almeida et al., 2011; Soares et al., 2011; Cuchiara et al., 2012).

Viability of the pollen grain can be determined by methods of cytochemical staining, *in vitro* germination, *in vivo* germination, and percentage of effective fructification (Einhardt et al., 2006; Almeida et al., 2011). The staining methods provide rapid, low-cost results, with different dyes being commonly used (Techio et al., 2006), depending on the constitution and morphology of the pollen grain and properties of the dyes.

In allogamous species, the viability of pollen grains is an essential factor, since each grain represents a distinct combination of alleles as a result of the heterozygosity of the *loci*; it is suggested that the higher the pollen viability, the stronger the possibility of forming different allele combinations and, ultimately, achieving genetic variability (Souza et al., 2002). *Psidium guajava* L. (Myrtaceae), the guava tree, is a predominantly allogamous fruit tree widely grown in tropical regions that produces fruits of high nutritional and functional value (Nishima et al., 2013). In this species, losses of up to 39.5% have been reported in the production of fruits originated via self-pollination, attributed to self-incompatibility phenomena (Alves and Freitas, 2007). In guava tree, Usman et al. (2013) reported influence of the pollen donor genotype on the traits fruit diameter and weight, total soluble solids, titratable acidity, ascorbic acid content, and total and nonreducing sugar contents. This phenomenon is known as metaxenia, where the pollen donor genotype may influence the physical and biochemical quality of the guava fruits (Usman et al., 2013). It has also been reported in apple (Bodor et al., 2008), blueberry (Silveira et al., 2011) and loquat crops (Xu et al., 2007). This observation suggests that it is possible to select pollinating genotypes in order to constitute a commercial orchard with the aim of increasing productivity and fruit quality. However, to achieve this it is

necessary to understand more broadly the characteristic of pollen viability in the species.

In breeding programs, the orientation and guidance of the selection strategy to obtain superior genotypes, independently of the objectives and having or not pollinating functions, for instance, require understanding the genetic basis of the attribute in question. For this, it is necessary to carry out a genotype evaluation; obtain the variance components for the estimates of genetic parameters; and subsequently predict the genotypic values related to genetic control of the characteristic (Resende et al., 2008; Basso et al., 2009). This way, parameters such as heritability (h^2) and the variation index (ratio CVg/CVe) are estimated and used to direct the selection of genotypes. The REML/BLUP methodology is a robust approach used to estimate genetic parameters that considers various sources of variation from field experiments, being useful for unbalanced data and non-orthogonal designs. It allows unfolding the phenotypic variation into genetic, environmental and genotype x environment interaction components; comparing genotypes over time (generations, years) and space (locations, blocks); simultaneously correcting for the environmental effects, the estimation of variance components and the prediction of genetic values; and studying complex data structures (repeated measures, different years, locations and designs) (Resende et al., 2008).
In this work, we proposed evaluating the pollen viability of 22 *P. guajava* genotypes as well as studying the control of this characteristic through estimation of genetic parameters, at different production cycles and environments. For this purpose, widely known staining methods were used. This setup aimed to allow the estimation of genotypic values for pollen viability in guava.

Results and Discussion

Pollen viability

The three applied staining methods allowed differentiating between viable and non-viable pollen (Fig1), and presented correlations of 0.99 considering the data on the harvests of 2013 and 2014 together. Based on these results, only Alexander's solution was used in the evaluation of the 2015 harvest, as it enables easy distinction of pollen grains owing to the simultaneous action of the malachite green and the acid fuchsin. The former has affinity for cellulose, staining the cell wall green, while the latter colors the protoplasm. Non-viable pollen grains (without protoplasm) only acquire a green coloration (Alexander, 1980; Techio et al., 2006).

The applied staining methods allowed verifying that the pollen grains of the 22 genotypes, at the different production cycles, exhibited preserved carbohydrate content, cell morphology, and chromatin and cytoplasm integrity (detected by Orcein staining) (Vargas et al., 2009). The presence of starch was verified by staining with Lugol (Ge et al., 2011), and integrity of the protoplasm and cellulose of the pollen wall by Alexander's solution (Alexander 1980).

The 22 guava tree genotypes exhibited large amount of viable pollen, with 16 of them presenting mean pollen viability of more than 84%, a value considered high in this study (Table 1); an overall mean of 93.46% was obtained in the three harvests. In wild guava trees, pollen viability values between 73% and 99% have been reported using Lugol and Acetic Orcein (Coser, et al., 2012a). However, the present study is the first account of this feature being tracked across the first harvests of an orchard in establishment process; this way, important information is provided for the crop, as cross-pollination has been related as an important factor regarding productivity, with production losses occurring due to self-fertilization (Alves and Freitas, 2007). Moreover, the higher the viability rate in allogamous species, the higher the probability of producing different allele combinations, and of increased genetic variability (Souza et al., 2002).

The pollen viability of some genotypes differed between the harvests. The genotypes C3, C16, PS and PA presented lower values in the harvest of 2013, with occurrence of at least one plant without viable pollen. On the other hand, the genotypes CLG, CBLG and CRG had reduced pollen viability in the second harvest (2014), remaining lower for CLG in the harvest of 2015 (Fig. 2). Altogether, these genotypes present differential characteristics of fruits and seeds (Coser et al., 2012b, 2014). The genotype C3 has been described as presenting smaller fruit size, and the cultivars PA and PS as producing large amount of seeds. The genotypes CLG, CBLG and CRG are characterized by presenting large fruits (MAPA, 2014), being largest in CLG (Coser et al., 2014). These results suggest that the pollen viability of guava tree may be related to characteristics of the fruits and seeds in the pollen donor genotype. For this crop, it has been reported in the literature that the pollen donor genotype may influence fruit size and quality, being possible to select pollen donor genotypes; further, it is suggested that the pollen source plays a key role in fruit development and maturity. However, causes of metaxenial effect still have to be further explored (Usman et al., 2013).

Some previously characterized Cortibel genotypes present molecular and morphological divergence of fruits in commercial cultivars, including Paluma (PA) and Pedro Sato (PS) (Gomes Filho et al., 2010; Coser et al., 2012b; 2014). In the present study, it was observed that these commercial genotypes (PS and PA, along with C3 and C16) showed greater variation in the first harvest with regards to pollen viability. The genotype C3 was also reported to have molecular similarity to PA and PS (Coser et al., 2012b). The Cortibel genotypes of large fruit demonstrated greater variation in the second (CLG, CBLG and CRG) and third (CLG and CSLG) harvests (Fig. 2). However, significant variation between genotypes was observed only in the harvest of 2015, in which all genotypes with 38-month-old plants presented high viability (above 84%), with the lowest values being found in some Cortibel genotypes. No significant variation was detected in the two harvests with younger genotypes. However, the five genotypes with highest values for viable pollen in the Mulamba and Mock rank were CRG, C13, C9, C7 and C17 in the harvest of 2013; and SXXI, C17, CRM, CLM and C9 in the harvest of 2014. The genotypes C17 and C9 were classified among the best five in both harvests at the city of Mimoso do Sul. In the harvest of 2015, the five highest values for viable pollen were found in the genotypes C5, C11, C3, C12 and CLM. It was further observed that the genotype CLM was among the five highest values both in the harvest of 2014, in Mimoso do Sul, and in that of 2015 in Linhares. In joint analysis of the environments (Mimoso do Sul and Linhares) including the three harvests, only the genotype PS had less than 84% of pollen viability (Fig. 2).

Genetic parameters

The effects of the interaction genotype x environment (Table 3) were significant at a p-value of 0.063, very close to the standard of 0.05. Moreover, observing the parameter of correlation between the genotypes' performances ($\hat{r}_{gloc}$) across the environments, a p-value of 0.068 is verified (Table 4). This further indicates that the influence of the complex

Table 1. Mean phenotype percentage of the pollen viability in 22 guava tree genotypes in three harvests, carried out by staining methods using Alexander's, Lugol or Orcein solutions.

	Alexander's			Lugol		Orcein	
Genotype	2013	2014	2015	2013	2014	2013	2014
CLG	98	82	84	99	84	99	81
CBLG	98	65	97	99	66	94	66
C5	98	98	99	99	99	99	99
CRM	98	99	98	99	99	100	99
C7	99	99	97	99	99	99	99
C9	99	99	96	99	99	99	99
C10	98	97	96	100	98	99	99
C11	98	99	99	98	99	99	100
C13	99	99	98	99	99	98	99
CRG	99	87	98	99	92	99	91
CSLG	98	98	95	97	99	99	98
C17	99	99	98	98	99	99	99
SXXI	98	99	98	99	99	99	99
C12	98	99	99	98	100	99	99
CLM	99	99	99	99	99	99	98
RO	96	99	97	98	98	97	98
CBRM	98	98	99	99	100	99	99
C3	49	97	99	50	99	49	99
C16	50	97	94	49	98	50	98
PST	32	95	96	32	96	32	97
PE	98	---	98	99	---	98	---
PA	66	---	98	66	---	66	---

Note: Phenotypic means not estimated due to loss of observations.

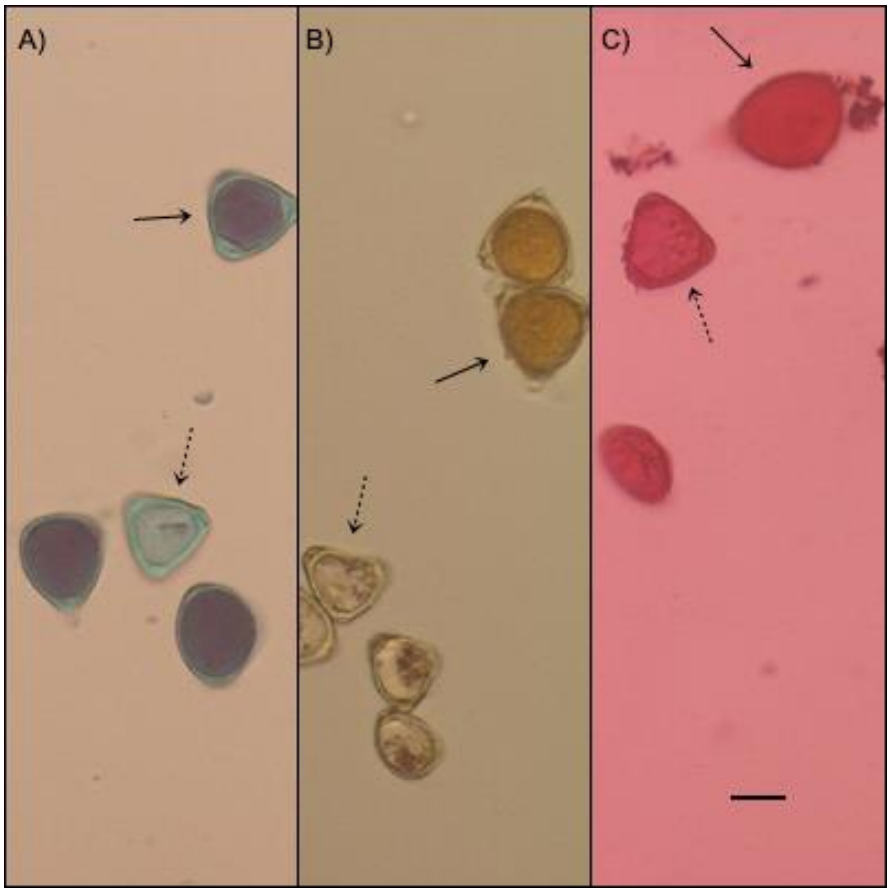

Fig 1. Viable (black arrows) and non-viable (dotted arrows) pollen of *Psidium guajava* L. by three staining methods: Alexander's (A), Lugol (B) and Orcein (C). Bar = 20 µm.

Table 2. Likelihood ratio test for pollen viability in guava trees performed by staining methods using Alexander's, Lugol ad Orcein solutions.

Analysis	Effects	Deviance	LRT	p-value (χ^2)
Alexander's Harvest 2013	Blocks	756.90^{+}	0.00	0.96
	Genotypes	759.39^{+}	2.49	0.12
	Complete model	756.90^{++}	---	---
Alexander's Harvest 2014	Blocks	566.763^{+}	0.44	0.51
	Genotypes	566.432^{+}	0.11	0.74
	Complete model	566.326^{++}	---	---
Lugol Harvest 2013	Blocks	757.75^{+}	0.00	1.00
	Genotypes	760.16^{+}	2.41	0.12
	Complete model	757.75^{++}	---	---
Lugol Harvest 2014	Blocks	563.27^{+}	0.40	0.53
	Genotypes	562.91^{+}	0.04	0.84
	Complete model	562.87^{++}	---	---
Orcein Harvest 2013	Blocks	757.71^{+}	0.00	1.00
	Genotypes	760.10^{+}	2.39	0.12
	Complete model	757.71^{++}	---	---
Orcein Harvest 2014	Blocks	564.07^{+}	0.23	0.63
	Genotypes	564.01^{+}	0.17	0.68
	Complete model	563.84^{++}	---	---

LRT – Likelihood ratio test; $^{+}$Deviance of the adjusted model without the referred effect; $^{++}$Deviance of the complete adjusted model; p-value (χ^2) – value p estimated based on the probability distribution χ^2 with one degree of freedom.

Table 3. Likelihood ratio test for the pollen viability of guava trees by staining method using Alexander's solution.

Analysis	Effects	Deviance	LRT	p-value (χ^2)
Mimoso – ES 2013	Blocks	756.900^{+}	0.002	0.963
	Genotypes	759.386^{+}	2.488	0.115
	Complete model	756.898^{++}	---	---
Mimoso – ES 2014	Blocks	566.763^{+}	0.438	0.508
	Genotypes	566.432^{+}	0.107	0.744
	Complete model	566.326^{++}	---	---
Linhares – ES 2015	Genotypes	545.857^{+}	5.741	0.017
	Complete model	540.116^{++}	---	---
Joint	Locations/Blocks	1462.199^{+}	0.000	1.000
	Genotypes	1462.218^{+}	0.019	0.890
	Genotypes x Locations	1465.656^{+}	3.457	0.063
	Complete model	1462.199^{++}	---	---

LRT – Likelihood ratio test; $^{+}$Deviance of the adjusted model without the referred effect; $^{++}$Deviance of the complete adjusted model; p-value (χ^2) – value p estimated based on the probability distribution χ^2 with one degree of freedom

Table 4. Estimates of genetic parameters for the pollen viability in guava trees.

Parameter Estimations	Mimoso 2013	Mimoso 2014	Linhares 2015	Joint
$\hat{\sigma}^{2*}_b$	107.789	1026.824	---	14.842
$\hat{\sigma}^2_g$	17058.809	1018.044	585.102	601.553
$\hat{\sigma}^2_{g\,xl}$	---	---	---	8298.148
$\hat{\sigma}^2_e$	55693.641	21155.984	1254.291	27681.708
$\hat{\sigma}^2_f$	72860.238	23200.852	1839.392	36596.252
$\hat{h}^2_g$	0.234	0.044	0.318	0.016
$\hat{h}^2_{\acute{g}}$	0.479	0.126	0.583	0.052
$\hat{r}_{gg}$	0.692	0.355	0.764	0.227
CVg (%)	14.507	3.359	2.496	2.624
CVe (%)	26.212	15.313	3.655	17.802
CVr	0.553	0.219	0.683	0.147
$\hat{r}_{gloc}$	---	---	---	0.068
$\hat{\mu}_{overall}$	900.317	949.859	969.030	934.593

$\hat{\sigma}^{2*}_b$ – Variance between the blocks in the individual analyses or variance between blocks across environments in the joint analysis; $\hat{\sigma}^2_g$ – Genetic variance; $\hat{\sigma}^2_{g\,xl}$ – Variance of the interaction genotypes x locations; $\hat{\sigma}^2_e$ – Residual variance; $\hat{\sigma}^2_f$ – Phenotypic variance; $\hat{h}^2_g$ – Broad-sense genetic heritability; $\hat{h}^2_{\acute{g}}$ – Mean genetic heritability; $\hat{r}_{gg}$ – Selective accuracy of genotypes; CVg (%) – Genetic coefficient of variation; CVe (%) – Experimental coefficient of variation; CVr – Relative coefficient of variation; $\hat{r}_{gloc}$– Correlation of the genotypes across environments; $\hat{\mu}_{overall}$ – Estimate of the overall mean.

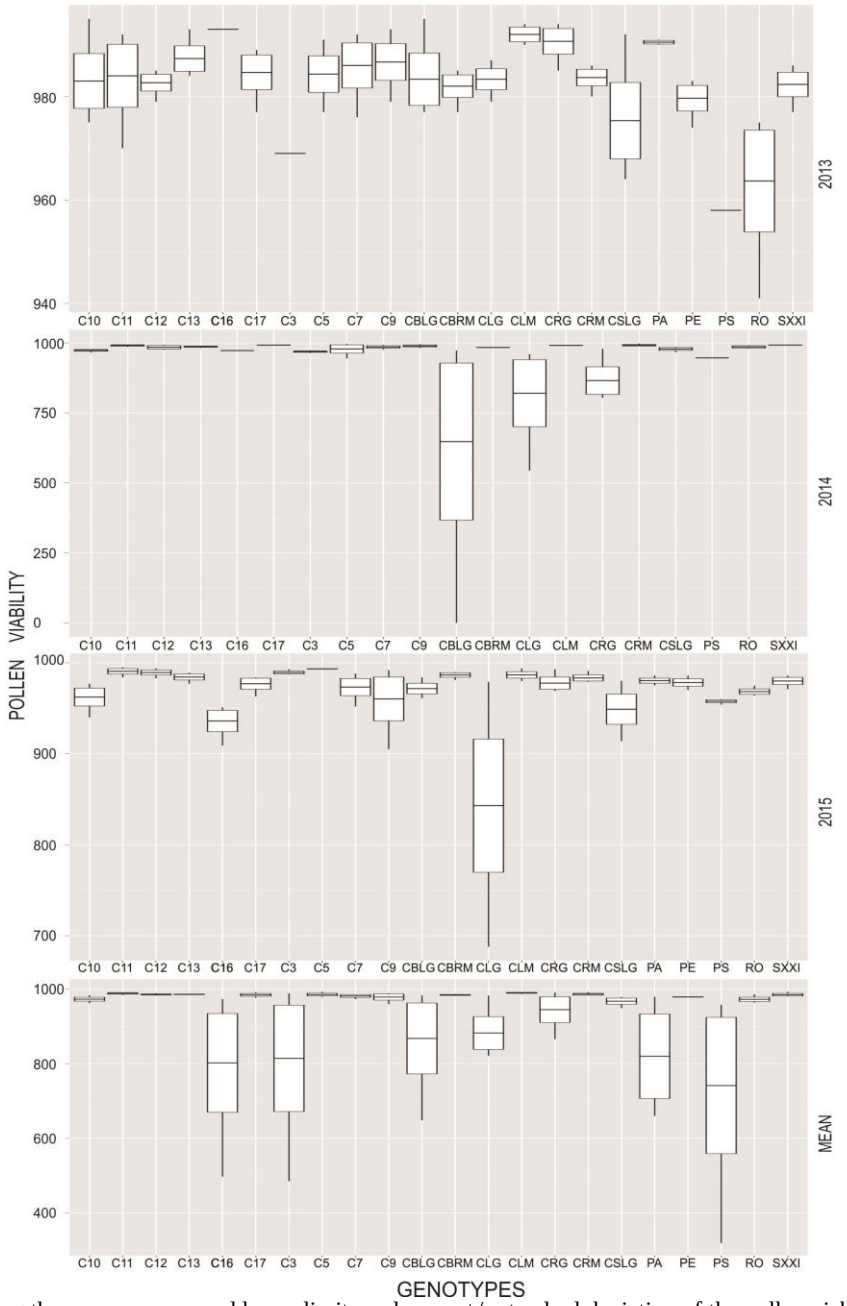

Fig 2. Graphic presenting the means, upper and lower limits and mean +/- standard deviation of the pollen viability for 22 guava tree genotypes, harvests of 2013, 2014 and 2015.

part of the interaction is only effective starting at this value, which was also very close to the standard of 0.05; therefore, the classification of the genotypes was not significantly altered in the different environments (Linhares and Mimoso do Sul). This fact is interesting because, in this work, the particularity exists that the genotypes were at different ages at the two locations, with the plants in Mimoso do Sul being nine months younger than those of Linhares.

Regarding the estimates of parameters, the smallest genetic ($\hat{\sigma}^2_g$), phenotypic and ($\hat{\sigma}^2_f$) residual ($\hat{\sigma}^2_e$), variances were found in the harvest of 2015 (38-month old plants), and the largest in the harvest of 2013 (19-month old plants) (Table 4). The mean heritability was smaller in the harvests of 2013 (0.479) and 2014 (0.126) in relation to 2015 (0.583), owing to the largest fraction of the phenotypic variation in Mimoso do Sul being associated to environmental variation. This result allowed establishing a slighter possibility of predicting genetic gains based only on this characteristic, due to the environmental changes influencing the phenotype (Resende and Duarte, 2007). In guava trees, it has been observed that commercially important characteristics, such as yield, size and quality of the fruits, as well as some types of resistance to diseases, present moderate to low heritability, in the broad and narrow sense (Pelea et al., 2012; Thaipong and Boonprakop, 2005). In one review (Nimisha et al., 2013), moderately high heritability in the narrow sense was reported for fruit length (44%) and contents of soluble solids (43%), and smaller heritability (32%) for fruit width, indicating that some characteristics present lower genetic control and higher environmental influence in the transmission of the trait compared to others. The coefficient of genetic variation, as well as the heritability, is an important parameter in the

prediction of genetic gains. For pollen viability, the genotypic coefficient of variation (CVg) was smaller than the environmental one, indicating that there was greater influence of the environment compared to the genetic effect (Table 4). This way, in guava trees the pollen viability may suffer strong influence from the environment. Still in the context of genotype evaluation, one of the most important statistical parameters is the selective accuracy, which refers to the correlation between the true genotypic value of the genetic material and that predicted based on information from field experiments. Therefore, the accuracy depends on the heritability and repeatability of the trait, the amount and quality of the information, and on the procedures used to predict the genetic values (Resende et al., 2008). In this work, the accuracy was calculated based on the parameters genetic CV (CVg), experimental CV (CVe) and number of repetitions. The accuracy ($\hat{r}_{gg}$) of this work reached 69.2% in the harvest of 2013, 35.5% in that of 2014, and 76.4% in the harvest of 2015 (Table 4). The accuracy is considered high for values ≥ 70%, moderate for values ≥ 50%, and low if < 50% (Resende, 2008). It is demonstrated that the accuracy was high in the harvest of 2015 in Linhares; it was low in the harvest of 2014 and moderate in that of 2013 in Mimoso do Sul, showing that the environmental variation within an experiment affected the prediction of genetic values for a certain characteristic.

The low correlation value of the genotypes between the environments ($\hat{r}_{gloc}$ = 6.8%) indicates that the alteration in pollen viability occurring in one environment will not necessarily occur in other environments for the same genotypes. The highest CVr (relation between CVg and CVe) was obtained for the data from Linhares (0.683), below 1.0 (Table 4), which indicates large environmental effect. The low magnitude of r_{gloc} (6.8%) demonstrates high influence of the interaction genotype x environment, which causes lack of correlation between the performance of the genotypes in the tested environments. In these cases, greater influence of the complex interaction becomes evident, indicating that the classification of the genetic materials in the environments will not necessarily be the same (Bastos et al., 2007). The CVr estimated for the individual and joint analyses were below 1.0(Table 4), meaning that the environmental variability outstripped the genetic variability, which constituted in these cases the greatest difficulty for identifying the superior materials (Vencovsky and Barriga, 1992).

The highest estimate for the overall mean ($\hat{\mu}_{overall}$) of pollen viability in guava trees was 96.9%, in the genotypes of the 2015 harvest. Yet, considering all the performed evaluations, it corresponded to an overall value of 93.46%, showing that the studied guava tree genotypes had good cross-pollination potential to be used as pollinators in orchards and in crossings within breeding programs (Table 4). Fertilization efficiency is crucial for the success of genetic breeding, being related to pollen viability (Souza et al., 2002).

Therefore, pollen viability can be considered an additional parameter when choosing genotypes in the selection of parents. Pollen viability in guava trees is high and strongly influenced by the environment in the first harvests. The heritability for pollen viability in guava tree is medium to low, leading to a slighter possibility of predicting genetic gains based on this characteristic, as environmental changes considerably influence the phenotype during initial harvests.

Materials and Methods

Plant materials and experimental design

For greater reliability regarding the pollen viability of guava, in this study the plant samples were collected in two orchards at different environments (cities of Mimoso do Sul and Linhares). Each orchard was installed, in a field experimental design, as a randomized block design with three replications and two plants per plot in each environment. Spacing of 6 m between rows and 4 m between plants was adopted. The studied genotypes included 12 commercial and ten improved ones, as described in Supl. Table 1. The commercial genotypes were: Cortibel LG, Cortibel LM, Cortibel Branca LG, Cortibel RM, Cortibel Branca RM, Cortibel RG, Cortibel SLG – described as C1, C2, C4, C6, C8, C14 and C15, respectively; Paluma – PA; Pedro Sato – PS; Petri – PE; Roxa – RO; and Século XXI – SXXI. The improved genotypes were denominated Cortibel C3, C5, C7, C9, C10, C11, C12, C13, C16 and C17, after the cross-pollination orchard of same name (Cortibel) where they were selected in the state of Espírito Santo (ES, Brazil). The plants in the two environments received the same crop conduction aiming at fruit production. One environment was located in the South of Espírito Santo (Rural Community of Palmeira – Mimoso do Sul), at latitude 21° 01' 12.99" S, longitude 41° 17' 13.48" W and elevation of 250 meters. The second environment was located in the North of Espírito Santo (Production Farm of Frucafé – Linhares), at latitude 19° 23' 27 "S, longitude 41° 04' 17" W and altitude of 30 meters.

Harvest procedure and times

The collections were accomplished in two production cycles in the years 2013 and 2014, after the first and the second fructification pruning, respectively, in experimental orchard installed in the South of the state of Espírito Santo (ES), city of Mimoso do Sul – Brazil (250 m of altitude). The first collection was performed in October 2013, the second in March 2014 (19- and 24-month-old plants, respectively). After analysis of these results, a new collection was carried out in the city of Linhares, in the North of ES (38 m of altitude) in February 2015, after the third fructification pruning (38-month-old plants).

Flower collection and measurement of pollen traits

Flower buds in pre-anthesis stage were collected from 22 guava genotypes in the two orchards. The collected buds were fixed in ethanol: acetic acid (3:1) and stored at –20°C until performance of the analyses. For preparation of the slides, five anthers obtained from the flower buds were transversely sectioned and immersed in hydrochloric acid solution (5N HCl) for 3 minutes. Subsequently, the hydrochloric acid was removed with filter paper and a dye was applied, for 10 min, while the anthers were shredded. Three different dyes were used: 2% Acetic Orcein, to verify chromatin and cytoplasm integrity (Vargas et al., 2009); 2% Lugol, which indicates the presence of starch (Ge et al., 2011); and Alexander's solution, containing acid fuchsin and malachite green, which react with the protoplasm and the cellulose of the pollen wall, respectively (Alexander 1980). The pollen grains removed from the flower buds of the 2013 and 2014 harvests were stained with Alexander's, 2% Acetic Orcein and 2% Lugol solutions. Based on the results obtained with the three dyes, and after verification of the statistical

similarity between them, it was decided to use only Alexander's solution for the samples of the 2015 harvest.
The pollen grains were classified according to size, morphology and staining capacity using the screening method, until a total of 1,000 pollen grains per slide (genotype) was reached. Pollen grains with regular shape, darker coloration and larger size were considered viable. Grains with irregular shape, weak or no coloration and smaller size were classified as non-viable. For the harvests of 2013 and 2014, considering 3 dyes × 3 blocks, a total of 9,000 pollen grains were evaluated per genotype. For the harvest of 2015, as only one dye was used, a total of 3,000 pollen grains per genotype was analyzed. The analyses were carried out with an Olympus microscope and 40× objective lens.

Statistical analysis

Statistical analysis of the data was performed using linear mixed models to obtain the estimates for the variance components and genetic parameters, by the method of restricted maximum likelihood (REML) via EM algorithm (Dempsteret et al., 1977), following the model $y = Xu + Zg + Wb + \varepsilon$. The data obtained at the harvest of 2015 were analyzed according to the model $y = Xr + Zg + \varepsilon$. Joint analysis of the harvests was performed using the model $y = Xl + Zg + Wl/b + Ti + \varepsilon$. In these models, *y* is the data vector, *u* is the fixed overall mean, *r* is the vector of fixed repetition effects added to the overall mean, *l* is the vector of fixed location effects added to the overall mean, *g* is the vector of the random genotypic effects, *b* is the vector of random block effects, *l/b* is the vector of the block effects within the random locations, *i* is the vector of the random effects of the genotype x environment interaction, and ε is the vector of errors or random residues. The capital letters represent the incidence matrices for the referred effects. Based on the described models, the deviances (-2lnL) were estimated for performance of the likelihood ratio test (LRT), aiming to verify the significance of the random effects of the used models.

The comparison between dyes was accomplished by Wald's test via F statistics with the model $y = Xc + Zg + Wb + Pi + \varepsilon$ in each harvest, where: *c*: fixed effects of the dyes, *g*: random effects of the genotypes, *b*: random effects of the blocks, and *i*: random effects of the interaction between dyes and genotypes. The genetic and phenotypic correlations were obtained for the evaluated genotypes with the different staining methods. All analyses were carried out with the computational application R (R Team, 2015) and the program SELEGEN-REML/BLUP (Resende, 2002).

Conclusion

The pollen viability in orchards of guava trees is high and influenced by the environment in the first harvests. The heritability for pollen viability in guava tree is medium to low, leading to a slighter possibility of predicting genetic gains based on this characteristic, as environmental changes considerably influence the phenotype during initial harvests. These results provide information about the pollen viability in commercial and superior genotypes of the guava crops used in this study, which showed good potential for cross-pollination and which can be used as pollinators in orchards and crosses within breeding programs.

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