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PLANT BREEDING

Estimation of inbreeding depression in *Psidium guajava* through seed attributes using S_0, S_1, and S_2 families

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ABSTRACT. Inbreeding depression, resulting from self-pollination or mating between closely related plants, primarily impacts quantitative traits. This study aimed to assess inbreeding depression in guava seeds from two generations of self-pollination (S_1 and S_2) relative to open-pollinated (S_0) seeds, focusing on seed physical and physiological attributes. Physical seed quality was assessed by determining the weight of 1,000 seeds and analyzing seed images using the GroundEye® instrument. Physiological quality was evaluated through germination and accelerated aging tests, along with the emergence speed index, root length, and shoot length. The results revealed no inbreeding depression in the first selfing generation. However, mild levels of inbreeding depression were observed across all traits in the second selfing generation. This study underscores the importance of understanding inbreeding depression dynamics in guava seeds, particularly in subsequent generations of self-pollination, for effective breeding and seed quality management.

Keywords: guava; self-pollination; vigor.

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Introduction

Guava belongs to the family Myrtaceae and is the main representative of its genus due to its economic, social, and food-related value (Tuler et al., 2020). Guava consumption has increased in Brazil and worldwide, mainly because of its pleasant taste, excellent nutritional qualities, affordable price, high availability of supply for a long period of the year, and the diverse forms of consumption (Parmar et al., 2018; Costa et al., 2019).

The growing demand of the guava market, both for fresh consumption and industrial purposes, can only be satisfied by using cultivars that meet the requirements of both segments. Thus, studies aimed at the genetic improvement of this species tend to select genotypes that generate higher-yielding cultivars and fruits with higher ascorbic acid content and higher vitamin and mineral concentrations. However, studies on guava breeding have some particularities, as it is an early perennial species with a long reproductive cycle, fluctuating annual production, and low survival rate individuals (Silva et al., 2021).

Guava is one of many cultivated species with broad genetic diversity due to its mixed pollination system, as it is a self-pollinated plant in which allogamy (cross-pollination) can also occur (Alves & Freitas, 2007). Selfing increases homozygosity and decreases heterozygosity in the progenies and may cause inbreeding depression, which reduces the mean value of quantitative traits related to the reproductive capacity or physiological efficiency of the plant because of the homozygosity of deleterious alleles (Falconer, 1987; Falconer & Mackay, 1996).

The guava breeding program at the State University of Northern Rio de Janeiro (UENF) has been developing promising work. The research identified the possibility of obtaining inbred guava families by self-pollination for the development of cultivars or generation of hybrids (Pessanha et al., 2011; Campos et al., 2013; Oliveira et al., 2013; Campos et al., 2016; Quintal et al., 2017; Ambrósio et al., 2021; Silva et al., 2020). In this scenario, the present study proposes to cultivate and evaluate inbred populations of guava, an unprecedented achievement for this fruit crop. Currently, no known breeding programs work in this line in Brazil or worldwide.

Therefore, this study aims to examine the occurrence of inbreeding depression by investigating the physiological quality and morphological traits of guava seeds derived from two generations of selfing (S_1 and S_2) compared to progenies obtained from open pollination (S_0).

Material and methods

Plant material

The experiment was conducted in the laboratory of the Seed Production and Technology Unit at the State University of Northern Rio de Janeiro (CCTA-UENF), located in Campos dos Goytacazes, Rio de Janeiro State, Brazil.

The plants that originated the guava seeds of the S_0, S_1, and S_2 families were selected based on results from previous studies developed in the Laboratory for Plant Breeding at UENF (Pessanha et al., 2011; Quintal et al., 2017; Ambrósio et al., 2021). The progenies that originated the S_0 and S_1 genotype seeds were selected by Quintal et al. (2017) using REML/BLUP. The seeds of the S_0 genotypes were obtained from open pollination between the same plants that originated the seeds of the S_1 genotypes. The seeds of the S_2 genotype originated from the 55 most productive progenies of the S_1 families, selected by Ambrósio et al. (2021) using REML/BLUP.

Self-pollination to obtain the S_1 and S_2 genotypes was performed by protecting the flowers by covering them before anthesis. The buds were identified, and the fruits were later protected with a paper bag. After harvesting the fruits of the S_0, S_1, and S_2 genotypes, the seeds were removed and then rubbed or pressed on a steel mesh sieve under running water.

The extracted seeds were placed to dry at room temperature for 48 hours. The seeds were turned over 24 hours after the start of the drying process to dry evenly. Once dried, the following physical and physiological tests were carried out:

Thousand-seed weight (TSW): following the Rules for Seed Testing (Brasil, 2009), eight portions of 100 seeds were taken from the *pure seeds* sample and weighed on a 0.0001-g precision analytical scale. The sample weight was then used in the following equation:

TSW = (Sample weight × 1000)/(Total n of seeds)

The variance, standard deviation, and coefficient of variation were calculated after determining TSW. The thousand-seed weight was calculated by multiplying the average weight obtained from replicates of 100 seeds by 10 when the coefficient of variation did not exceed 4%. Another eight replicates of 100 seeds were weighed when the coefficient of variation exceeded the limit of 4%, and the standard deviation was calculated using the 16 replicates.

Germination test: four replicates of 50 seeds of each of the genotypes under study were used on the paper roll substrate. Germination chambers were set at an alternating temperature of 35-25°C, with a photoperiod of 8h/light and 16h/dark, respectively. Evaluations were carried out every other day until the 28th day, which consisted of determining the germination speed index and the percentages of normal seedlings, abnormal seedlings, and non-germinated seeds (Maitan et al., 2020).

Germination speed index: determined when the germinating seed and its shoot had at least 1 cm in length (measured on alternate days), using the formula proposed by Maguire (1962).

Shoot and root lengths: four paper rolls were mounted with ten seeds. The seedlings were measured at the end of the 28th day using a graduated ruler.

Accelerated aging: the seeds were placed uniformly on an aluminum screen inside a germination box with 40 mL of water at the bottom. Subsequently, the germination boxes were subjected to a temperature of 41°C for 48h and then placed in a germination chamber for 28 days. After this procedure, the test was performed to evaluate germination as described above (Brasil, 2009).

Digital phenotyping of the seeds: four replicates of 50 seeds of each genotype were used. The GroundEye®Mini instrument was used to capture and analyze the seed images. The following geometric traits were evaluated: area, in square millimeters; maximum and minimum diameters, in millimeters; and circularity, a dimensionless variable whose values range from 0 to 1 (where values closer to one indicate more circular seeds).

Genetic evaluation: estimation of inbreeding effect and inbreeding depression

The following model was used to estimate the effects of inbreeding on the evaluated families (Demidenko, 2013):

$$Y_{(ij)}=\beta_{0(j)}+\beta_1 P_{(i)} + \beta_2 P{:}G_{(ij)} + \varepsilon_{(ij)},$$

where: $Y_{(ij)}$ is the vector of observed phenotypic data of each variable in progeny i of genotype j; β_0 s a parameter associated with the model (intercept), modeled for each genotype j; $P_{[i= \mathrm{S0,\ S1,\ and\ S2}]}$ is the fixed-effect parametric vector of progeny i associated with vector Y by known incidence matrix β_1; $G_{[j=1,\ldots,75]}$ is the random-effect parametric vector of genotype j, modeling both the intercept and the slope of the lines for the genotypes within each progeny, associated with vector Y by the known incidence matrix β_2; and ε is the vector of uncontrolled random errors, assuming $\varepsilon \sim N(0, I \otimes \Sigma_\epsilon)$.

The $Y \sim N(\mu, \sigma_\varepsilon^2)$ assumption was adopted for the variables of 1000-seed weight, shoot length, root length, circularity, maximum diameter, and minimum diameter, with identity link function and Restricted Maximum Likelihood (REML) estimation.

$Y \sim Poisson(\lambda)$ was assumed for the variables germination and accelerated aging, in which the expected distribution describes the number of discrete occurrences and the percentage of germination in a given number of seeds, with identity link function and Maximum Likelihood (ML) estimation with Laplace approximation.

Finally, the $Y \sim \Gamma(\alpha, \beta)$ assumption was adopted for the germination speed index and seed area, with identity link function and Maximum Likelihood (ML) estimation with Laplace approximation.

Subsequently, the post-hoc test was performed to generate the estimated means for the levels of progeny effect. Significance was considered as the absence of overlapping confidence intervals at the 95% probability level. Inbreeding depression and mean components were estimated.

Inbreeding depression (ID) was estimated in percentage terms using the following equation proposed by Gardner (1965):

$$ID_{S0/S1} = [(\bar{y}S_0 - \bar{y}S_1)/\bar{y}S_0].100$$

$$ID_{S1/S2} = [(\bar{y}S_1 - \bar{y}S_2)/\bar{y}S_1].100,$$

where: $\bar{Y}S_0$ is the mean of open-pollinated genotypes; $\bar{Y}S_1$ is the mean of genotypes selfed once; and $\bar{Y}\ S_2$ is the mean of genotypes of the second generation of selfing.

The contribution of homozygous loci (m+a) and the contribution of heterozygous loci (d) were estimated using a procedure similar to that described by Vencovsky (1987), as follows:

$$m+a_{(S0/S1)} = 2\bar{y}S_1 - \bar{y}S_0 \quad \text{and} \quad m+a_{(S1/S2)} = 2\bar{y}S_2 - \bar{y}S_1$$

$$d_{(S0/S1)} = 2(\bar{y}S_0 - \bar{y}S_1) \quad \text{and} \quad d_{(S1/S2)} = 2(\bar{y}S_1 - \bar{y}S_2),$$

where: $\bar{Y}\ S_0$ is the mean of open-pollinated genotypes; $\bar{Y}\ S_1$ is the mean of genotypes selfed once; and $\bar{Y}\ S_2$ is the mean of genotypes of the second generation of selfing.

Results and discussion

The variables presented lower values in S_1 and S_2 than in S_0, but this difference was not considered significant. The means of the S_2 genotypes were lower for practically all evaluated variables, except for circularity, whose overall mean was the same in the three generations (Table 1). The variable germination speed showed an increase from 2.73 (S0) to 3.04 (S1), indicating an improvement of −11.35% (a negative value indicates an increase). The index decreased to 2.66 from S_1 to S_2, resulting in an inbreeding depression of 12.5%. The accelerated aging rate increased slightly from 92.60% (S_0) to 93.10% (S_1), with an inbreeding depression of −0.54%. The rate decreased to 85.40% from S_1 to S_2, representing an inbreeding depression of 8.27%.

Table 1. Estimation of the percentage of inbreeding depression in genotypes from open pollination (S_0), relative to the first generation of selfing (S_1), and in S_1, relative to the second generation of selfing (S_2).

Variable	Mean			Inbreeding depression (%)	
	S_0	S_1	S_2	$ID_{S0/S1}$	$ID_{S1/S2}$
Germination (%)	98.00	97.20	90.10	0.82	7.30
Germination speed index	2.73	3.04	2.66	-11.35	12.5
Accelerated aging (%)	92.60	93.10	85.40	-0.54	8.27
1000-seed weight (g)	16.50	17.60	13.21	-6.67	24.94
Shoot length (cm)	3.35	3.58	2.85	-6.87	20.39
Root length (cm)	2.71	3.39	2.09	-25.09	38.34
Seed area (mm^2)	0.10	0.10	0.09	0.00	10.00
Circularity	0.75	0.75	0.75	0.00	0.00
Maximum diameter (mm)	0.41	0.42	0.38	-3.17	9.45
Minimum diameter (mm)	0.30	0.30	0.27	0.00	10.00

IDS_0/S_1 was negative or zero for most variables, except for germination (0.82%) (Table 1). The negative values of IDS_0/S_1 occurred because the selfed genotypes (S_1) have higher means than those of open-pollinated genotypes (S_0). The germination rate showed a slight inbreeding depression of 0.82%, while the germination speed index showed a negative depression of -11.35%, suggesting that S_1 seeds germinated faster than S_0. Positive IDS_0/S_1 values, on the other hand, indicate that S_0 genotypes are superior to S_1 genotypes (Silva et al., 2019).

Table 2 shows the upper and lower confidence intervals for the traits evaluated in the generations of open pollination (S_0), first generation of self-fertilization (S_1), and second generation of self-fertilization (S_2), highlighting the variability in measures of vigor and development of guava seeds over generations of self-fertilization. The CIS0/S1 values were not significant, with no difference between the means of the two populations. The CIS_0/S_1 values were not significant, with no difference between the means of the two populations.

Table 2. Estimation of the upper and lower confidence intervals of genotypes from open pollination (S_0), first generation of selfing (S_1), and second generation of selfing (S_2).

Variable	Confidence interval		
	S_0	S_1	S_2
Germination (%)	95.00-100.00	94.10-100.00	84.90-95.40
Germination speed index	2.58-2.87	2.85-3.22	2.64-2.65*
Accelerated aging (%)	89.40-95.80	87.40-98.70	80.40-90.30
1,000-seed weight (g)	15.10-17.90	16.40-18.70	12.50-13.90*
Shoot length (cm)	3.03-3.66	3.25-3.90	2.69-3.01*
Root length (cm)	2.26-3.15	3.02-3.76	1.90-2.28*
Seed area (mm^2)	0.09-0.11	0.09-0.11	0.08-0.09
Circularity	0.74-0.76	0.74-0.76	0.74-0.76
Maximum diameter (mm)	0.40-0.42	0.41-0.43	0.37-0.40*
Minimum diameter (mm)	0.29-0.31	0.29-0.31	0.27-0.28*

95% confidence level. *Significant when comparing $CI_{S1/S2}$. There was no significant difference in the comparison of means between S_0 and S_1. S_0 and S_2 were not compared.

The low levels of inbreeding found in S_1 guava seeds are attributed to its mixed breeding system. It means that guava has a higher self-pollination rate than cross-pollination, ranging from 25.7% to 41.3%, with an average of 33.5% (Medina et al., 1991). This mixed system allows gene recombination to occur in guava, which helps to partially reduce the negative genetic load associated with the species' natural self-pollination. Thus, individuals that show little or no inbreeding depression can be selected, as discussed by Vencovsky and Barriga (1992) and Bernini et al. (2013). Nevertheless, genetic evaluations of the S_1 families performed in field trials already allowed the identification of individuals with different aspects of growth habits, such as a more open canopy and a smaller plant size. Some families displayed a prostrate growth habit, which constitutes evidence of possible impacts of inbreeding on guava, which was not yet pronounced in the seed variables in this generation.

However, $ID_{S1/S2}$ showed positive values, which ranged from 0 to 38.34%. Thus, the S_1 genotypes had higher means than the S_2 genotypes (Table 1. The variables with the highest $ID_{S1/S2}$ were shoot length (20.39%), 1,000-seed weight (24.94%), and root length (38.34%) (Table 1). In addition to showing a higher percentage of $ID_{S1/S2}$, these variables also exhibited a significant $CI_{S1/S2}$ (Table 2). Other variables, such as the germination speed index, maximum diameter, and minimum diameter, also showed a significant $CI_{S1/S2}$ (Table 2).

Seed area and circularity did not differ significantly for CIS1/S2. However, S_2 seeds exhibited a wide variety of shapes and sizes, indicating that the homogeneity of the genotypes and the fixation of alleles in the next generations of self-pollination must continue to be evaluated. As regards the percentage of germinated seeds, the level of inbreeding depression from the first (S_1) to the second generation of selfing (S_2) ranged from 0.82 to 7.30%, respectively. Despite the increase in inbreeding depression, the rate of germination of guava seeds remained above 90%, demonstrating their high vigor (Table 1).

The presence of inbreeding during the germination process of the S_2 genotypes was evidenced through genotypes that showed abnormal germination, resulting from some anomaly such as lack of radicle emission, which reduced their percentage of germination (Figure 1B) when compared to seeds that considered normal germination (Figure 1A) and normal seeds used in the growth assessment after 28 days (Figure 1C). Charlesworth and Charlesworth (1987) stated that self-pollination exposes these mutations to selection, thereby reducing the magnitude of inbreeding depression. In other words, genotypes with abnormal germination exposed to the environment do not complete their development cycle and their progenies are not taken to the subsequent generations.

Figure 1. A – normal germination of guava seeds; B - abnormal germination; C - normal seedlings used in the evaluation of root and shoot growth 28 days after the germination test.

The ratio between the germination rate and the germination speed index is an indirect indicator of seed vigor. However, the $ID_{S1/S2}$ values for the germination speed index were slightly higher and showed a significant $CI_{S1/S2}$ compared to the germination variable. It indicates that seeds of the S_1 genotypes germinated more quickly than seeds of the S_2 genotypes although both groups showed satisfactory germination rates at the end of the test (above 90%), i.e., the seeds of the S_1 genotypes germinated in less time than the S_2 seeds but both groups exhibited satisfactory germination at the end of the test (above 90%).

Accelerated aging behaved similarly to germination, with the S_1 genotypes showing higher means than the S_2 genotypes (93.10 and 85.40%, respectively) although there was no significance by $CI_{S1/S2}$ (Tables 1 and 2). The percentage of germinated seeds in the accelerated aging test is calculated after exposing them to high temperatures and relative humidity. In this test, seed vigor is also evaluated based on the storage potential. The decrease in the rate of germinated seeds after the accelerated aging test also led to a small increase in $ID_{S1/S2}$. This fact does not translate into great vigor loss, as the seeds of the S_2 genotypes showed high germination rates even after being subjected to changes in temperature and humidity (Table 1).

The mean values of 1,000-seed weight, shoot length, and root length decreased significantly from the S_1 to the S_2 generation, while their inbreeding depression increased (Tables 1 and 2). The negative effects of inbreeding are explained by classical genetic theories: the partial-dominance hypothesis, when deleterious recessive alleles are expressed due to increased homozygosity in related individuals; or the overdominance hypothesis, that is, a reduced frequency of superior heterozygous genotypes, which explains the increase in inbreeding with each generation of selfing (Charlesworth & Charlesworth, 1999).

The obtained results of inbreeding depression can be confirmed by estimating the contribution of homozygous (m+a) and heterozygous (d) loci to the performance of the generations (Table 3). Inbreeding depression is positively correlated with the contribution of heterozygous genes, i.e., the greater the contribution of heterozygous genes, the greater the inbreeding depression with the advancement of selfing generations, as shown in Table 3. Therefore, the probability of the genotypes having copies of the same ancestral allele is higher, thus increasing homozygosity and possibly the frequency of undesirable deleterious genes, which leads to a decrease in hybrid vigor (Gonçalves et al., 2011; Ramalho et al., 2012).

According to the results, one selfing did not generate inbreeding depression, whereas the presence of inbreeding depression was already observed in different proportions for each trait when a second self-pollination was performed. Therefore, populations that show high means and no or little inbreeding depression for the traits of interest have a great chance of generating superior lines for the later development of hybrids. Therefore, it is crucial to continue evaluations with the S_3 population, observing the levels of inbreeding at each generation of selfing and determining the possibility of later cultivation of superior lines from seeds.

S_2 families from these seeds are already in the field in the evaluation phase. Preliminary results reveal the existence of earlier, more uniform plants with greater uniformity of fruit production. The slight inbreeding depression found in this study, based on seed attributes, demonstrates the effectiveness of obtaining inbred lines of guava. Further studies on combining ability between partially inbred lines should be conducted to explore intraspecific heterosis in this crop.

Table 3. Estimation of components of the means and contribution of homozygous (m+a) and heterozygous (d) loci of the genotypes from open pollination (S_0), first generation of selfing (S_1), and second generation of selfing (S_2).

Variable	Contribution of homozygous loci		Contribution of heterozygous loci	
	$m+a_{(S0/S1)}$	$m+a_{(S1/S2)}$	$d_{(S0/S1)}$	$d_{(S1/S2)}$
Germination (%)	96.40	83.00	1.60	14.20
Germination speed index	3.35	2.28	-0.62	0.76
Accelerated aging (%)	93.60	77.77	-1.00	15.40
1,000-seed weight (g)	18.70	8.82	-2.2	8.78
Shoot length (cm)	3.81	2.12	-0.46	1.46
Root length (cm)	4.70	0.79	-1.36	2.60
Seed area (mm^2)	0.10	0.08	0.00	0.02
Circularity	0.75	0.75	0.00	0.00
Maximum diameter (mm)	0.45	0.34	0.00	0.08
Minimum diameter (mm)	0.30	0.24	0.00	0.06

Conclusion

The conclusion of this study reveals that the inbreeding process did not have a negative impact on the vigor of guava seeds in terms of inbreeding depression. This result indicates that guava can tolerate moderate levels of inbreeding without significantly compromising seed quality. Therefore, it is feasible to use controlled self-pollination techniques in guava genetic improvement programs, aiming to select and cultivate lines that maintain the desired vigor and productivity.

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