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Environmental and genetic concerns on genetic gains via selection in Pequi mother tree for seeds emergence

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Genetic (populations and progenies) and environmental (planting date, germination stimulator, insecticide and fungicide) effects were evaluated on Pequi (*Caryocar brasiliense* Camb.) seeds emergence. Two Completely Randomized Block Design (E_1 and E_2, on 2005 and 2009 years, respectively) experiments were set up on field. Seeds for E_2 were harvested in mother trees whose seeds showed higher germination rate in E_1. The effects, in E_1, of planting date, insecticide, fungicide and gibberellic acid were not significant. The mother tree effects were significant for both trials. The seeds emergence rates for all and selected mother trees in E_1, and in E_2 were 17.2% (P_0), 24.2% (P_s) and 28.2% (P_m), respectively. The environment effects supposedly responded for the actual (P_m-P_0) gain being higher than the greatest expected (P_s-P_0) since the estimated correlation of common progenies between E_1 and E_2 was 36.4%. In direct sowing in the field, the emergence rate of Pequi seeds is strongly influenced by mother trees from where they were collected. Phytosanitary control and the use of germination stimulator did not change the emergence rate in Pequi seeds.

Key words: *Caryocar brasiliense*, mother tree, populations, genetic gain, correlation.

INTRODUCTION

Naturally occurring in all the Brazilian Savannah (Vera et al., 2005; Kerr et al., 2007; Correa et al., 2008), Pequi (*Caryocar brasiliense* Camb.) is considered one of the most characteristic species of this biome, mainly because of its use as a natural or processed food (Almeida and Silva, 1994; Lopes et al., 2003). Extractivism is still the main form of exploitation of this species being an important economic activity of the communities living in this biome (Almeida and Silva, 1994; Guedes et al., 2004; Fernandes et al., 2004; Silva and Medeiros Filho, 2006; Nogueira et al., 2009). In some regions, according to these authors, the harvest and commercialization of

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Pequi involves half the population, representing 55% of their incomes. It is known that extractive activity is not sustainable at levels where demand is greater than the potential supply of the product. Considering that the products derived from Pequi constitute a potential market it will be necessary, it has happened with several other species, the development of research to generate technologies and policies that aim at its domestication, contributing to its conservation and rational exploitation (Guedes et al., 2004).

The genetic improvement of a species is nothing more than its domestication in record time. Success in a breeding program depends, among other factors, on the reproductive system of the species associated with its cycle. This is a problematic aspect in Pequi, given its long cycle and the low germination rate of its seeds (Fernandes et al., 2005; Rocha et al., 2009a, b).

Several factors are indicated as determinants of the expression of this characteristic (Dombroski et al., 1998; Bilir et al., 2004; Fernandes et al., 2005; Rodrigues et al., 2007; Carvalho and Martins, 2009; Rocha et al., 2009a, b; Dombroski et al., 2010; Yazici and Bilir, 2017) in different tree species. The germination of Pequi seeds can be influenced by both the endocarp and the almond itself (Dombroski et al., 1998). Although the opening or removing of the endocarp can improve it, this practice causes damage to the seeds and makes them more susceptible to fungal attack (Lopes et al., 2003).

The effect of gibberellic acid has been widely tested by several authors both in putamens and directly on almonds (Piña Rodrigues, 1988; Souza et al., 2007; Bernardes et al., 2008; Dombroski et al., 2010).

From the information found in the literature on germination in Pequi seeds, it can be concluded that they are far from meeting two fundamental requirements: rate and speed of germination. Pequi seeds usually take more than a month to germinate and the germination rate hardly exceeds 40% (Carvalho et al., 1994; Fernandes et al., 2005; Rocha et al., 2009a, b; Moura et al., 2013).

Another aspect that can be observed in most of the studies on germination in Pequi seeds is that the factors considered has been on environmental factors only. Studies involving genetic factors such as mother trees and populations effects, as well as the interaction between these and those of environmental nature are more recent (Fernandes et al., 2005; Rodrigues et al., 2007; Souza et al., 2007; Rocha et al., 2009a, b). It was also reported that many genetically (that is, population) and environmental effects (that is, growth practices, year) in reproductive characteristics included seed quality and their growth performances in different tree species (Yazici, 2010; Dilaver et al., 2015; Yilmazer and Bilir, 2016; Yazici and Bilir, 2017).

Studies on the germination of Pequi seeds planted directly in the field are another major gap. It was not found in the literature any study involving environmental and genetic factors, as well as interactions among them, in the germination of seeds of the species, planted under these conditions. Knowledge of this nature is important because they can subsidize breeding programs for the species. Thus, the aim of this paper was to evaluate the genetic and environmental effects on the emergence of Pequi seeds planted directly in the field.

MATERIALS AND METHODS

This work composed of two experiments. For the first, seeds were collected from 133 mother trees originating from seven populations (geographically isolated populations), all from the state of Minas Gerais: Caetanópolis; Curvelo; Diamantina (district of Mendanha); São Gonçalo do Rio Preto; Carbonita; Bocaiúva (district of Terra Branca); and Montes Claros.

These seeds, harvested in the months of January and February 2005, were sown directly in the field in a farm near Carbonita, MG. The area, logistical support and financial expenses were sponsored by SADA Bio-energy Agricultura Ltda, a private company, in a cooperation agreement with Universidade Federal dos Vales do Jequitinhonha e Mucuri.

The experimental design was a randomized complete block design (DBC) with 133 treatments (mother trees), ten replications, five hills and five seeds per hill. Five blocks were planted in September 2005 and, due to some technical concerns, the other five were planted at the end of December 2005.

As some mother trees did not produce enough fruits, some blocks were incomplete (with less than 133 progenies). Considering this restriction and to assure a balanced and complete block design, evaluations were assessed in 108 progenies in six blocks (blocks 1 and 2, sown in September 2005 and blocks 6, 7, 8 and 10, sown in December 2005). The seeds of block 1 were treated with fungicide (Tecto®) and insecticide (Tuity®) and those of block 6 with these two products plus 500 mg L^{-1} gibberellic acid.

The second experiment was installed in February of 2009, with 20 mother trees, 11 from Curvelo, MG, and nine from São Gonçalo do Rio Preto, MG. Among these 20 mother trees, 18 (ten from Curvelo and eight from Rio Preto) were selected from mother trees that presented the highest emergence rates in the Carbonita experiment, installed in 2005.

Seeds were also sown directly in the field in an area of Plantar Reflorestamentos SA (PLANTAR), in Curvelo, MG, in a complete block design with 20 treatments (mother trees) with 50 replications, one hill per plot (Single Tree Plot) and five seeds per hill. The emergence rate was assessed eleven months after planting.

The data were analyzed considering the model: $Y_{ij} = m + t_i + b_j + e_{ij}$, where Y_{ij} is the record of the i^{th} treatment in the j^{th} block; t_i is the effect of the i^{th} treatment (mother tree, including the effect of populations); b_j is the effect of the j^{th} block; e_{ij} is the experimental error; and m is the overall mean. The ANOVA with the sources of variation, degrees of freedom, and expected values of the mean squares, is shown in Table 1. The effects of the model, except the overall mean, were considered random.

Data were transformed to the scale $(x+0.5)^{0.5}$ following recommendations for variables evaluated by counting and expressed in percentage.

RESULTS AND DISCUSSION

The coefficients of variation of the ANOVA's for the original data were 60.48 and 16.65% (data not shown) whereas for the transformed data they were 7.45 and 14.52% (Table 2), for E1 and E2, respectively. The

Table 1. Appropriated analysis of variance for two experiments carried out in Carbonita, MG, in the year of 2005 and Curvelo, MG, in the year of 2009, both in a complete block design.

SV	DF	MS	Expected value of MS
Carbonita, 2005			
Blocks	5	MS_b	$V_e + 108V_b$
Pop. and Mother Trees (Treatments)	107	MS_t	$V_e + 6V_t$
Populations	6	MS_p	$V_e + 6V_{m/p} + 47V_p$
Mother Trees/Pop.	101	$MS_{m/p}$	$V_e + 6V_{m/p}$
Mother Trees/Curvelo	20	$MS_{m/p1}$	$V_e + 6V_{m/p1}$
MotherTrees/Caetanópolis	3	$MS_{m/p2}$	$V_e + 6V_{m/p2}$
MotherTrees/Diamantina	28	$MS_{m/p3}$	$V_e + 6V_{m/p3}$
MotherTrees/Rio Preto	21	$MS_{m/p4}$	$V_e + 6V_{m/p4}$
MotherTrees/Carbonita	2	$MS_{m/p5}$	$V_e + 6V_{m/p5}$
MotherTrees/Bocaiúva	6	$MS_{m/p6}$	$V_e + 6V_{m/p6}$
MotherTrees/M. Claros	21	$MS_{m/p7}$	$V_e + 6V_{m/p7}$
Error	535	MS_e	V_e
Total	647	-	-
Curvelo, 2009			
Blocks	49	MS_b	$V_e + 20V_b$
Pop. and Mother Trees (Treatments)	19	MS_t	$V_e + 50V_t$
Populations	1	MS_p	$V_e + 50V_{m/p} + 495V_p$
Mother Trees/Pop.	18	$MS_{m/p}$	$V_e + 50V_{m/p}$
Mother Trees/Curvelo	10	$MS_{m/p1}$	$V_e + 50V_{m/p1}$
MotherTrees/Rio Preto	8	$MS_{m/p4}$	$V_e + 50V_{m/p4}$
Error	931	MS_e	V_e
Total	999	-	-

efficiency of the transformation was greater, therefore, in E_2. On the other hand, the F test estimates and their respective levels of significance for the various sources of variation considered almost did not change with the transformation of the data. This finding implies a greater confidence in the conclusions obtained from the ANOVA's for the transformed data.

The non-significance ($p>0.05$) of the block effects for the Carbonita experiment (Table 2) demands further discussion. The seeds of block 1 (sown in September 2005) were treated with fungicide (Tecto®) and insecticide (Tuity®) and those of block 6 (sown in December 2005) with these two products plus Giberelic Acid (GA_3) 500 mg L^{-1}. Therefore, not only the effects of blocks themselves were not significant, nor were the effects of planting season and GA_3, fungicide and insecticide treatments.

The treatment in Pequi seeds by fungicides is recommended by Lopes et al. (2003), besides the effect of GA_3 being reported by several authors (Piña Rodrigues, 1988; Souza et al., 2007; Bernardes et al., 2008; Dombroski et al., 2010), both in putamen and directly on almonds. However, the effect of these factors was not observed in this work, that is, the treatment with GA_3 and fungicide did not influence the emergence of Pequi seeds in the field. The absence of effects of GA_3 may be due to the fact that the tests were conducted directly in the field and not in shaded sand beds as done by these authors. Another possibility is that both environmental and genetic factors that influence the endogenous level of hormones and their antagonistic substances (Agusti and Almela, 1991) may have reduced or nullified the effect of GA_3. It is also likely that the time at which the seeds were treated (seven and 11 months post-harvest for blocks 1 and 6, respectively) is the main factor responsible for the lack of response to treatment with the insecticide and fungicide.

The effects of populations were not significant for Curvelo (2009) (Table 2). Essentially, under satisfactory sampling, population's effects are not significant for many other traits (Melo Júnior et al., 2004; Souza et al., 2007; Rocha, 2009a). The significant effect for Carbonita population for 2005 (Table 2) is probably due to the small number of mother trees for some populations. In such cases, the effects of populations can be confounded with those of mother trees (Rocha et al., 2009a). This is likely the main reason for the great difference between the 29.5% observed for three mother trees from Carbonita and the 12.1% observed for 29 mother trees of Diamantina (Table 3). The difference observed between

Table 2. Analysis of variance for seed emergence rate in two experiments carried out in Carbonita, MG, in the year of 2005 and Curvelo, MG, in the year of 2009, both in a complete block design.

SV	DF	MS	F	P(F)
Carbonita, 2005				
Blocks	5	0.0031	0.82	52.95
Pop. and Mother Trees (Treatments)	107	0.0347	9.41	0.00
Populations	6	0.0704	2.17	5.20
Mother Trees/Pop.	101	0.0325	8.83	0.00
Mother Trees/Curvelo	20	0.0233	6.32	0.00
MotherTrees/Caetanópolis	3	0.0332	9.01	0.00
MotherTrees/Diamantina	28	0.0372	10.10	0.00
MotherTrees/Rio Preto	21	0.0215	5.83	0.00
MotherTrees/Carbonita	2	0.1216	33.01	0.00
MotherTrees/Bocaiúva	6	0.0552	14.98	0.00
MotherTrees/M. Claros	21	0.0312	8.46	0.00
Error	535	0.0037	-	-
Total	647	-	-	-
CV	7.45%	-	-	-
Curvelo, 2009				
Blocks	49	0.0142	0.89	68.31
Pop. and Mother Trees (Treatments)	19	0.1003	6.31	0.00
Populations	1	0.1516	1.55	22.91
Mother Trees/Pop.	18	0.0975	6.13	0.00
Mother Trees/Curvelo	10	0.0930	5.85	0.00
MotherTrees/Rio Preto	8	0.1031	6.49	0.00
Error	931	0.0159	-	-
Total	999	-	-	-
CV	14.52%	-	-	-

Rio Preto and Diamantina can be attributed to a higher rate of emergence of the first one (Table 3). However, even under satisfactory sampling conditions, the effect of populations is not always significant (Melo Júnior et al., 2004, Souza et al., 2007; Rocha, 2009a).

The effects of mother trees, on the other hand, were highly significant in both experiments (Table 2). It is observed that in the experiment conducted in Carbonita, there were significant differences between mother trees for all the populations, reinforcing the conclusions reported by Fernandes et al. (2005), Rodrigues et al. (2007) and Rocha et al. (2009a, b).

Table 4 shows that in most of the mother trees, emergence rates are below 30% for the Carbonita experiment, 2005 and below 40% for Curvelo, 2009. At least one mother tree in Carbonita, 2005 had an emergence rate above 60%. These results are probably related to the genetic factors of the matrices (maternal effect) or the seed itself (xenia effect) (Fernandes et al., 2005).

Other environmental factors may also be involved. Rocha et al. (2009b) observed the presence of an insect (Coleoptera Bruchidae of the genus *Amblycerus*, a species not yet identified) that has not been reported in Pequi seeds, which apparently remains for a long time within the seed without being possible to be detected only through direct observation. As the frequency of attack of this pest varies greatly among mother trees, some of which may reach 60% of its fruits attacked, the low germination rate of its seeds will of course reduce greatly (Rocha et al., 2009b). Another pest that causes serious damage to Pequi seeds is *Carmenta* species, Lepidoptera: Sesiidae (Lopes et al., 2003), making them inappropriate for consumption as well as seriously compromising the germination of its seeds. Although apparently healthy seeds have been used, these pest attacks may have occurred, altering the emergence of seeds.

A relevant comparison is that referring to the average seed emergence rates of the 18 mother trees common to the experiments of Carbonita in 2005 and Curvelo in 2009, which were 24.2 and 28.2%, respectively (for Curvelo experiment (2009) the mother trees with the highest emergence rate of Carbonita experiment (2005) were selected). The expectation, considering the correlation between the emergence rates for these two

Table 3. Seed emergence rate, by populations, in two experiments carried out in Carbonita, MG, in the year of 2005 and Curvelo, MG, in the year of 2009.

Populations	Carbonita, 2005[1] All populations and mother trees		Carbonita, 2005[2] Mother trees selected from two populations		Curvelo, 2009 Mother trees selected from two populations	
	Emergence (%)	No. of mother trees	Emergence (%)	No. of mother trees	Emergence (%)	No. of mother trees
Curvelo	17.3	21	21.5	10	30.4	10
Caetanópolis	15.1	4	-	-	-	-
Diamantina	12.1	29	-	-	-	-
Rio Preto	22.5	22	27.6	8	25.6	8
Carbonita	29.5	3	-	-	-	-
Bocaiúva	14.1	7	-	-	-	-
Montes Claros	18.2	22	-	-	-	-
Average/Total	17.2	108	24.2	18	28.2	18

[1]Seeds were harvested in 108 mother trees came from seven geographically isolated populations. [2]Ten, out of 21, and eight, out of 22, mother trees from Curvelo and Rio Preto, respectively, that showed the highest emergence rate in Carbonita, 2005[1] were selected, their seeds harvested again in 2008 and their emergence rate evaluated in Curvelo, 2009.

Table 4. Distribution of mother trees with in some intervals for emergence rate. Data came from two experiments carried out in Carbonita, MG, in the year of 2005 and Curvelo, MG, in the year of 2009.

Emergence rate interval (%)	No. of mother trees in the interval		
	Carbonita, 2005	Curvelo, 2009	Total
<10	42 (38.9)	0 (0.0)	42 (32.8)
10 - 20	27 (25.0)	3 (15.0)	30 (23.4)
20 - 30	21 (19.4)	10 (50.0)	31 (24.2)
30 - 40	13 (12.0)	6 (30.0)	19 (14.8)
40 - 50	4 (3.7)	1 (5.0)	5 (3.9)
50 - 60	0 (0.0)	0 (0.0)	0 (0.0)
>60	1 (0.9)	0 (0.0)	1 (0.8)
Total	108	20	128

Data inside parenthesis are in %.

experiments (r = 36.4%) was that in 2009 the emergence rate was above 17.2% (Table 5) but below 24.2% (Tables 3 and 5). A possible explanation for the fact that the gain was above expected is that the seeds, planted in March 2009, were collected in January of the same year, therefore, much newer than those of the 2005 experiment that were collected in January and planted in September and December, which was observed by (Rocha et al., 2009a) who observed a great influence of the age of the seed on the rate of emergence in Pequi seeds.

Based on the estimate of the correlation between the emergence rates for these two experiments (r = 36.4%, Table 5), a plausible inference is that a mother tree whose seeds has a high rate of emergence in a given year may not repeat it in another year. That is, the expectation of genetic gains from the selection of mother trees whose seeds have the highest emergence rate should not be high.

Conclusion

In direct sowing in the field, the emergence rate of Pequi seeds is strongly influenced by mother trees from where they were collected. The genetic gains in emergence rates via selection of mother trees with high emergence rate seeds is not expected to be large.

Phytosanitary control (insecticide and fungicide) and the use of germination stimulator (gibberellic acid) did not change the emergence rate in Pequi seeds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Table 5. Seed emergence rate, by mother tree, in two experiments carried out in Carbonita, MG, in the year of 2005 and Curvelo, MG, in the year of 2009.

Population	Mother tree	Carbonita 2005	Curvelo 2009
Curvelo	2	26.67	28.00
	4	22.30	45.60
	8	16.22	24.40
	9	27.93	33.60
	18	4.19	30.40
	22	48.15	30.00
	33	13.12	21.60
	40	18.67	27.60
	42	16.16	26.40
	48	21.77	36.00
Rio Preto	160	26.22	31.20
	167	32.67	30.40
	168	17.33	20.80
	169	37.33	29.60
	170	40.67	38.00
	171	32.00	21.60
	172	8.67	12.00
	175	26.00	21.20
-	Average	24.23	28.24
-	-	r = 36.4%	P(t) = 6.9%

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