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Genetic variability during *in vitro* establishment of bacurizeiro (*Platonia insignis* Mart.): an Amazon species

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Abstract

As a Brazilian Amazon fruit, bacuri tree (*Platonia insignis* Mart.) has been pointed out as an important species to the sustainable management and development of the Amazon region. However, since it is not yet domesticated, techniques regarding its propagation are still incipient. This study aimed to evaluate the genetic control of traits related to *in vitro* establishment of *Platonia insignis* accessions from different regions of the Maranhão state, Brazil. Immature fruits were collected over different sites for explants obtention. The *in vitro* responsiveness of the genotypes was evaluated by assessing the percentage of explant oxidation (OXI), pinkish-colored defense structures (PCDS), callogenesis (CAL), radicle (ROOT) and shoot forming (SHOOT). Callus was classified according to its callogenic potential, namely as: globular-friable, with higher embryogenic potential; undefined structure [cell mass], with lower embryogenic potential; and white spongy-like callus, absent of embryogenic potential. The estimates of genetic parameters were accomplished via REML (Restricted Maximum Likelihood)/BLUP (Best Unbiased Linear Prediction) mixed model. The outputs showed high genetic variability within the studied population. Additionally, accessions AC.7, AC.2, AC.1, AC.6, and AC.8 showed as more efficient by considering characteristics related to the *in vitro* regeneration, thereby presenting a superior callogenesis ability; lower susceptibility to oxidation; and higher capacity for root and shoot formation. This was the first study to investigate the relationship among genetic parameters and selecting accessions of *P. insignis* for *in vitro* regeneration, thus providing support for studies related to micropropagation and domestication of this species.

Keywords: callogenesis; Clusiaceae; somatic embryogenesis; genetic diversity; micropropagation.
Abbreviations: AC_accession; BLUP_Best Unbiased Linear Prediction; CAL_callogenesis; CVr_coefficient of relative variation; DAI_days after inoculation;h^2_g_broad-sense heritability of individual plants;Kpal_kilopascal;LCT UEMA_Tissue Culture Laboratory of the Universidade Estadual do Maranhão;M_overall average of the experiment; MS_Murashige and Skoog;OXI_percentage of oxidation; PCDS_pinkish-colored defense structures; REML_Restricted Maximum Likelihood;ROOT_radicle; SENEGEN_Statistical System and Computerized Genetic Selection by Mixed Linear Models);SHOOT_shoot forming;Ve_residual variance; Vf_individual phenotypic variance; Vg_genotypic variance between individuals; (v/v)_volume by volume.

Introduction

As a fruit tree native to the Brazilian eastern Amazon, bacuri (*Platonia insignis* Mart.) stands out due to its socio-economic importance and high ability to contribute to the management and sustainable development of this region (Cavalcante, 1996; Alvarez et al., 2013). Belonging to the subfamily Clusioideae and family Clusiaceae, bacuri is the only species of the genus *Platonia* that occurs in areas that cover Maranhão, Pará, and Piauí states (Do Nascimento et al., 2007). It grows and develops easily both in upland forests and open vegetation transition zones, either in open areas or low vegetation (Cavalcante, 1996; Souza et al., 2013), within regions that present humid and sub-humid climates, as in Maranhão, in which they form dense agglomerates or settlements, mostly in plateau areas (Menezes et al., 2010). Bacuri tree is still under domestication process and its production is mainly conducted in an extractive way (Menezes et al., 2012). This species is facing a risk of genetic erosion due to the pressure of land use by agriculture and the lack of efficient techniques for its propagation. The current propagation techniques for bacuri are quite limited – e.g., sexual method, which leads to slow germination speed due to the low viability and apical bud dormancy (Carvalho and Nascimento, 2018; Menezes et al., 2012). Moreover, issues concerning genetic self-incompatibility have been also observed, a common event in Amazon flora species (Saraiva et al., 2013). Therefore, the development of alternative methods to sexual propagation can be decisive for both domestication and genetic breeding of bacuri. In this context, tissue culture technique emerges as an excellent biotechnological approach since it uses small sections with high multiplication capacity. This procedure is able to boost

the efficiency of seedlings production since *in vitro* plant cells can regenerate and originate many new plants via "cellular totipotency" (Canhoto, 2009; Kumar, 2011; Hussain et al., 2012; Guerra, 2016). However, it is well-known that heterogeneous genotypes do not respond homogeneously to the same stimuli; namely genotype-specificity or genotype-dependent explants. For this reason, knowledge on the genetic control of *in vitro* regeneration related-traits is essential for a further selection of more responsive genotypes. For instance, by estimating genetic parameters, it is possible to identify the action nature of genes related to overall characters and assess the efficiency of different breeding strategies to obtain genetic gains and/or desirable genetic bases within populations (Cruz and Carneiro, 2006; Oliveira et al., 2015). Estimations of *in vitro* heritability, for instance, have been used to determine *in vitro* genetically dependent characteristics and viability of early selection of responsive progenies in increasing final production (Bergmann and Stomp, 1994). For this, the optimal estimation/prediction REML/BLUP (restricted maximum likelihood/best linear unbiased prediction) method has been widely recommended. Currently, there are no reports about *in vitro* propagation protocols or studies concerning genetic variability of varied materials with desirable characteristics to micropropagation methods for bacuri species. It is worth mentioning that an established protocol would be extremely useful to overcome general issues, such as slow germination speed and genetic self-incompatibility, which may provide *in vitro* propagation of superior genetic materials on a large scale. Furthermore, it may provide a theoretical foundation for the development of other techniques such as micrografting, which may pave the way for the implementation of more sustainable production systems in the Amazon. Therefore, this study evaluated the genetic control of traits related to *in vitro* establishment of *Platonia insignis* accessions from different regions of Maranhão state/Brazil, seeking to select responsive genotypes for further cloning programs.

Results

Bacuri accessions present high variability for in vitro responses

Based on the decontamination protocol, no significant differences were observed in the percentage of contamination. At 60 DAI, explants of all accessions showed *in vitro* germinal and/or callogenic responses.

Embryogenic callus (CAL) classified as friable, globular, and clustered were frequently observed in both AC.1 and AC.10 accessions (Figure 3A and 3J, respectively). There were also formations of mass of callus with undefined shape (Figure 3E) in accessions AC.3, AC.2 and AC.4, thereby presenting a lower embryogenic potential. Additionally, a proliferation of white spongy-like callus without embryogenic potential was also observed (Figure 3I) in all other evaluated explants.

The highest values of percentage of explants oxidation (OXI) were observed in AC.1 and AC.3 explants (Figure 3C). However, all evaluated accessions showed a certain occurrence of OXI, but with no relevance. Pinkish-colored defense structures (PCDS) also occurred in bacuri explants, mainly in AC.7 (Figure 3E) and AC.6 accessions. Radicle formation (ROOT) occurred majorly in AC.4 (Figure 3F) and AC.7 accessions. On the other hand, both AC.7 and AC.2 presented a higher incidence of shoot growth and development (SHOOT) (Figure 3B and 3H, respectively).

Among parameters that contribute to the detection of genetic variability within a population, it is important to assess the coefficient of relative variation (CVr). CVr is obtained by the relationship between coefficients of genetic and experimental variation, and it is not influenced by character average. Based on some evaluated characteristics, it was observed a presence of genetic variability among bacuri genotypes. Regarding CAL and OXI, CVr values were higher than the unity (1.09 and 1.24, respectively), demonstrating the presence of a higher genetic variation of bacuri genotypes available for *in vitro* cultivation. However, ROOT, SHOOT, and PCDS showed lower values of relative coefficient (0.59, 0.41 and 0.31, respectively) (Table 3).

CAL, OXI, ROOT and SHOOT showed broad-sense heritability values higher than 18% and significant accuracy by over 70%. Furthermore, CAL and OXI presented accuracies considered as very high (above 90%) (Table 3). This result indicates higher reliability for estimated values related to these traits and, therefore, certain accuracy by selecting bacuri genotypes based on them.

The heritability of individual plants in the sense-broad (h^2g) was considered moderate for CAL, ROOT, and SHOOT, while high for OXI (Table 3). Altogether, it characterizes considerable genetic control of these characteristics and, thus, a high heritability for future generations to be developed in genetic breeding programs.

Selective accuracy is an important parameter to be estimated, as it shows a correlation between true and predicted genetic values. In other words, the higher value, the more is the confidence of individuals' evaluation. According to the classification of heritability and accuracy in terms of magnitude and their associations, proposed by Resende (1998), the values observed in the present study were of high magnitude for CAL, OXI, ROOT, and SHOOT (above 70 %), which indicates that selection based on such characteristics hold an advantage.

Selection of bacuri for in vitro establishment

The lowest value in the rank indicates a more suitable combination between established characteristics; the highest, an unsuitable condition. Among selected accessions, AC.2 (Bacabeira - Santa Luzia) and AC.7 (Bacabeira), AC.1 (Codó - Bom Jesus) and AC.6 (Codó) and AC.8 (Morros) showed as promising genetic materials to *in vitro* responses, standing over the first five positions based on the rank of averages. Therefore, such accessions are indicated for selection procedures since they have demonstrated suitable characteristics for cloning via tissue culture technique (Table 4). Moreover, they can be indicated for both genetic breeding programs and future improvement of tissue culture techniques, including micrografting.

Discussion

This was the first study to investigate the relationship between genetic parameters and the selection of bacuri accessions for *in vitro* regeneration. Bacuri is a plant under the domestication process, thus there is still a lack of studies and protocols for its *in vitro* cultivation. In this context, tissue culture is an ideal technique to tackle obstacles during fruit production, such as a longer juvenile period, sporophytic self-incompatibility, and seed dormancy (Villachica, 1996).

Tissue culture is an attractive alternative to the propagation of *P. insignis* on a large scale. However, *in vitro* response

Table 1. Identification of *Platonia insignis* Mart. accessions selected over ten locations of the State of Maranhão, Brazil, and their respective geographic coordinates.

Locations	Geographic coordinates		Accessions ID
	Latitude	Longitude	
Codó (Bom Jesus)	4° 27' 18'' S	43° 52' 44'' O	AC.1
Bacabeira (Santa Luzia)	2° 58' 14'' S	44° 18' 32'' O	AC.2
São Luís - Angelin	2° 31' 51'' S	44° 18' 24'' O	AC.3
Viana	3° 12' 26'' S	44° 59' 57'' O	AC.4
Fazenda Escola	2° 31' 51'' S	44° 18' 24'' O	AC.5
Codó	4° 27' 18'' S	43° 52' 44'' O	AC.6
Bacabeira	2° 58' 14'' S	44° 18' 32'' O	AC.7
Morros	9° 27' 1'' S	46° 17' 53'' O	AC.8
Itapecuru	3° 23' 42'' S	44° 21' 36'' O	AC.9
Santa Bárbara	2° 31' 51'' S	44° 18' 24'' O	AC.10

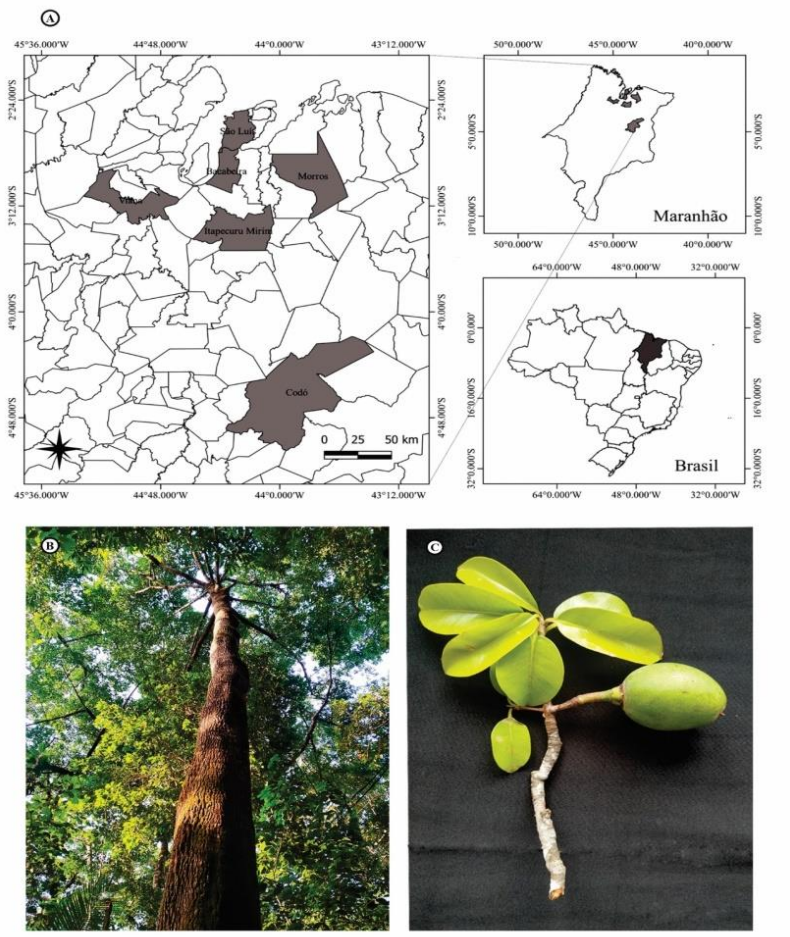

Fig 1. Distribution map of *Platonia insignis* accessions over the State of Maranhão, Brazil (A). *Platonia insignis* adult plant (B). Immature fruit of *Platonia insignis* (C).

Table 2. Classification of heritability magnitudes and selective accuracy to estimate *in vitro* genetic parameters of *Platonia insignis*.

Selective accuracy	Classification of magnitudes of individual heritability	Classification of magnitudes of accuracy for individual selection
0.51	Low	Low
0.55	$0.01 \leq h_a^2 \leq 0.150$	$0.10 \leq \text{raâ} \leq 0.40$
0.58		
0.61	Mean or	Mean or
0.66	Moderate	Moderate
0.71	$0.15 \leq h_a^2 \leq 0.50$	$0.40 \leq \text{raâ} \leq 0.70$
0.76	High	High
0.80 to 0.95	$h_a^2 \leq 0.50$	$\text{raâ} \geq 0.70$

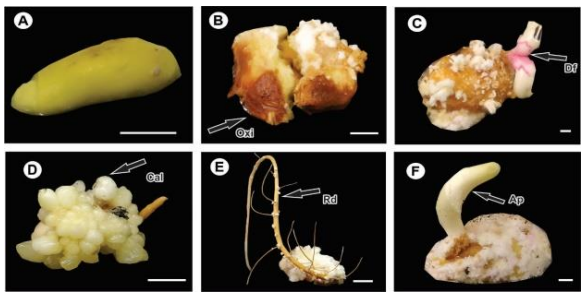

Fig 2. *In vitro* establishment of *Platonia insignis* embryos. (A) Early-stage embryo - 0 DAI coated with cortical meristem at the time of inoculation in MS medium absent of growth regulators. (B) Oxidation (OXI) of the embryo at 60 DAI. (C) formation of pinkish-colored defense structures (PCDS) in the shoot initiation region (arrowheads). (D) Callogenesis responses with the formation of globular-like embryogenic callus (CAL) (arrowheads) at 60 DAI (E) Radicle formation at 60 DAI (Rd). (F) Shoot formation at 60 DAI (Ap) Abbreviations: Oxi – the percentage of oxidation; Df – pinkish-colored defense structures; Cal – callogenesis; Rd - radicle emission; Ap – shoot emission. Bar: 3.5 cm.

Table 3. Estimates of genetic parameters based on the analysis of 10 accessions of *P. insignis in vitro*, at 60 DAI, for callogenesis (CAL), oxidation percentage (OXI), radicle emission (ROOT), shoot emission (SHOOT), and formation of pinkish-colored defense structures (PCDS).

Parameters	CAL	OXI	ROOT	SHOOT	PCDS
Vg	0.0713	993.24	0.0673	0.0275	0.013
Ve	0.1053	822.68	0.1988	0.1615	0.1485
Vf	0.1766	1815.93	0.2662	0.1890	0.1624
CVr	1.09	1.24	0.59	0.41	0.31
h^2g	0.4037	0.5469	0.2530	0.1890	0.0856
Accuracy	0.90	0.92	0.84	0.79	0.70
M	0.7634	39.49	0.5515	0.2453	0.1946

Vg: genotypic variance between individuals; Ve: residual variance; Vf: individual phenotypic variance; CVr: coefficient of relative variation; h^2_g: broad-sense heritability of individual plants; M: overall average of the experiment.

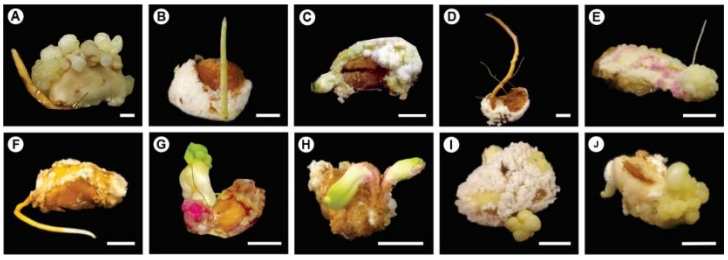

Figure 3. Embryos from 10 accessions of *Platonia insignis* at 60 DAI. AC.1 showing intense callogenesis, containing friable globular callus in the opposite region to the radicle emission (A). AC.2 displaying shoot formation and white spongy-like callus absent of embryogenic potential (B). AC.3 presenting intense oxidation and a little formation of white spongy-like callus (C). B.C. 4 showing intense radicle emission and formation of a white spongy-like callus in opposite to the root axis (D). AC.5 demonstrating a suitable friable globular callus formation and undefined callus mass shape with less embryogenic potential, additionally to the formation of pinkish colored defense structure and radicle formation (E). AC.6 displaying root formation, and white spongy-like callus and undefined callus mass shape (F). AC.7 presenting pinkish-colored defense structures in the shoot-forming region, and undefined callus mass shape (G). AC.8 showing oxidized regions, besides callus and shoot formation (H). B.C. 9 displaying intense callogenesis (I). AC.10 presenting intense callogenesis and root initiation (H) Bar: 3.5 cm.

Table 4. Classification based on sum of ranks for ten genotypes of *P. insignis* for callogenesis (CAL), percentage of oxidation (OXI), radicle emission (ROOT), shoot emission (SHOOT), and formation of pinkish-colored defense structures (PCDS) at 60 DAI *in vitro*.

Rank	Genotype	Location	CAL	OXI	RAIZ	SHOOT	PCDS	Average Rank
1°	AC.7	Bacabeira	3	7	2	1	1	2.8
2°	AC.2	Bacabeira (Santa Luzia)	7	6	3	2	3	4.2
3°	AC.1	Codó (Bom Jesus)	6	1	7	4	5	4.6
4°	AC.6	Codó	5	8	5	3	2	4.6
5°	AC.8	Morros	2	5	8	5	4	4.8
6°	AC.4	Viana	10	4	1	6	8	5.8
7°	AC.10	Santa Bárbara	1	9	6	8	6	6.0
8°	AC.9	Itapecuru	4	10	4	7	7	6.4
9°	AC.5	Fazenda Escola	8	3	9	9	9	7.6
10°	AC.3	São Luís (Angelim)	9	2	10	10	10	8.2

efficiency is strongly associated not only with growth conditions - such as growth regulators in the culture medium - but also with the genetic material used as an explant (Jiménez, 2001), which depends highly on the genotype.

A relevant aspect of *in vitro* culture is the explants oxidation, a common issue in tissues of tropical trees (Grattapaglia and Machado, 1998). However, the younger the tissue, the less susceptibility to oxidation (Paiva and Paiva, 2001). In this study, it was observed a higher influence of oxidation during *in vitro* regeneration processes of bacuri.

The results of this study suggest that genotypes with superior callogenic ability, less oxidation susceptibility, and higher capacity for root and shoot formation, present a higher probability of success in establishing *in vitro* regeneration protocols applied to bacuri. Overall, the outcomes indicate that there is a genetic variability that can be exploited by *in vitro* genetic breeding and cloning programs of this species. Therefore, the mean of ranks index by Mulamba and Mock (1978) was applied to the values to rate the genotypes in a favorable order of genetic breeding.

One of the most important genetic parameters in pre-breeding and plant breeding studies is the coefficient of relative variation (CVr). The coefficient of genetic variation makes it possible to infer genetic variability between different characters, provide a base for superior genotypes selection, and enable genetic variability levels evaluation among different genotypes, environments, and characters (Ferrão et al., 2008).

The CVr can be used as an indicative index for genotype selection based on evaluated characters. When the estimated ratio is equal to or higher than 1.0, there is a favorable condition for the selection process - since it reflects in a higher proportion of genotypic variability regarding the environment (Vencovsky and Barriga, 1992; Farias Neto, 2003). In the present study, significant CVr was observed for two important characteristics during *in vitro* regeneration process, as callogenesis and explant oxidation. This indicates that selections based on such variables may be promising for bacuri clones via tissue culture technique.

Heritability is the major genetic parameter used in plant breeding programs. Its relevance lies in how many genetic effects are in the individual's phenotypes - since the genotype values are what influence, in fact, the next generations (Falconer and Mackay, 1996). The broad-sense heritability estimates were significant for CAL, OXI, ROOT and SHOOT.

On the other hand, scarce are the traits related to *in vitro* regeneration that present estimates of heritability and selection of responsive genotypes for cloning. In this vein, seeking to select genotypes for cloning, Corrêa et al., (2015) observed significant values in broad-sense heritability for callogenesis and formation of embryogenic lines (15 and 19%, respectively) in oil palm (*Elaeis guineensis* Jacq.). Nugroho et al., (2014) observed higher estimates of sense-broad heritability for percentages of callogenesis and embryogenic callus (49 and 77%, respectively) from leaf explants in *Elaeis guineensis*. As claimed by the authors, such studies can be used to select more responsive progenies.

Methods that estimate or predict genotypic values must provide the most accurate and realistic inference as possible. In this sense, selective accuracy is a parameter of immense importance (Henderson, 1984). For instance, the greater the accuracy, the greater the correlation between predicted breeding values and phenotypic values (Pimentel et al., 2014). In the present study, values observed in selective accuracy were high for CAL, OXI, ROOT, and SHOOT. Overall, CAL and OXI presented values higher than 90%, leading to higher confidence during evaluations, as well as an accurate inference of genotypic values. Navroski et al., (2012) have used selective accuracy to assess experimental precision of callogenesis in apical and internodal stem segments of *Satureja hortensis*, in which a moderate accuracy (66%) was demonstrated for rhizogenic callus; and high (85-93%) for friable ones. In *Elaeis guineensis*, Corrêa et al. (2015) observed high values (>90%) in selective accuracies for embryogenic callus formation. According to these authors, high accuracies for *in vitro* culture experiments are due to the high number of replications, low number of contaminations, and reliability in estimated genetic values.

The mean of ranks index postulated by Mulamba and Mock (1978) classifies the accessions for each characteristic by attributing lower absolute values to those with superior performance. Subsequently, each characteristic value is summed and the average of ranks is obtained, thereby indicating the accession classification.

In the present study, accessions with superior callogenesis ability, low oxidation susceptibility, and high capacity for root and shoot formation were classified as favorable according to the order of genetic breeding. Thus, since they have ideal characteristics for both cloning and the establishment of future micrografting protocols, the first five of the rank were selected. The success of tissue culture depends on the studied genotype (Silva et al. 2012; Thawaro and Te-chato, 2009). Herein, the outcomes demonstrated that the accessions AC.7, AC.2, AC.1, AC.6 and AC.8 are more efficient for *in vitro* regeneration and related characteristics. The sampling of bacuri accessions at different sites of collection provides high accuracy to select these accessions as progenies due to the high genetic variability (Pena et al., 2019).

Results concerning the micropropagation of this species are still incipient. Moreover, *in vitro* culture protocols are not available for seedlings production. Thus, it is worth highlighting the relevance of similar studies for bacuri, both for the prospect of somatic embryogenesis and organogenesis techniques, which can be adapted to the propagative management of this species.

Bacuri is a tree with a high capacity root branch formation (Homma et al., 2018), and new buds is able to emerge up to 1 km from the mother plant without genetic variability. This is harmful to bacuri trees since it leads to genetic self-incompatibility, commonly observed in other native species. An alternative to this event is the establishment of cultures originating from different grafted clones. Therefore, studies concerning genetic variability involving novel propagation techniques for this species can provide a theoretical foundation for the development of more advanced propagation methods, such as *in vitro* micrografting. This would make it possible to select materials responsive to the most desirable characteristics, and contribute to a more sustainable agricultural production system and biodiversity valuing in the Amazon region.

Knowledge on the genetic control of traits related to *in vitro* regeneration will be important for further selections of more responsive genotypes, essential to either obtain gains into the establishment of a clonal mini-garden or to select matrices that may be used in tissue culture techniques, including micrografting; mainly for species that present self-incompatibility such as *Platonia insignis*.

Materials and methods

Collection of plant material

Immature fruits (Figure 1C) were collected from ten matrices (accessions) of bacuri tree (*Platonia insignis* Mart.) (Figure 1 B) obtained over ten different locations of Maranhão state, Brazil (Table 1, Figure 1A). They were subsequently moved to the Tissue Culture Laboratory of the Universidade Estadual do Maranhão (LCT-UEMA) São Luís - MA, Brazil. At the laboratory, they were submitted to the pre-washing procedure under running water and neutral detergent.

Disinfestation and *in vitro* establishment of *Platonia insignis* explants

In a laminar flow chamber, immature fruits were immersed in 70% alcohol (v/v) for five min and subsequently soaked in commercial sodium hypochlorite solution (Jesus Ltda®) (with 2% active chlorine), containing 1 drop of Tween® (Isofar Ltda®, Duque de Caxias - RJ) by 100 mL of solution, in which fruits remaining for 20 min. Afterward, they were rinsed three times for 2 min each with autoclaved distilled water. Both peel and pulp of the fruits were removed with a knife, being then submitted to seed disinfestation by immersion in 70% alcohol for 1 min, followed by sodium hypochlorite (with 2% active chlorine) containing 1 drop of Tween® in each 100 mL of solution, during 2 min. Finally, they were rinsed three times with autoclaved distilled water for two min each.

The seeds were excised until cortical meristem obtention (Figure 2A), which were inoculated in 350 mL flasks containing 30 mL MS medium (Murashige and Skoog, 1962) (PhytoTech Lab® Kansas - USA), supplemented with 30 g L^{-1} of sucrose (Isofar® Ltda, Duque de Caxias - RJ), 100 mg L^{-1} of myo-inositol (Sigma-Aldrich®, St.Louis, Missouri - USA), 2.0 g L^{-1} of Phytagel® (Sigma-Aldrich®, St.Louis, Missouri - USA) and 3.0 g L^{-1} activated charcoal (Sigma-Aldrich®, St.Louis, Missouri - USA). The pH of the medium was set up to 5.8+0.1 before autoclaving at 121°C and 108 kPa for 15 minutes.

Characterization of in vitro responses and estimates of genetic parameters

The explants were kept in growth room conditions, under dark, for 60 d, at a temperature of 24±2ºC and. The *in vitro* genotypes responsiveness was evaluated at 60 DAI (days after inoculation) by assessing the percentage of explant oxidation (OXI) (Figure 2B), pinkish-colored defense structures (PCDS) (Figure 2C), callogenesis (CAL) (Figure 2D), radicle (ROOT) (Figure 2E) and shoot forming (SHOOT) (Figure 2F). Callus was classified according to its callogenic potential, such as: globular-friable, with higher embryogenic potential; undefined structure [cell mass], with lower embryogenic potential; and white spongy-like callus, absent of embryogenic potential. The occurrence of a pinkish color, like anthocyanins, was considered as a defense structure. The percentage of explant oxidation was expressed as 0, for non-oxidized explants; 25%, for explants oxidized by up to 1/4; 50%, for explants oxidized by up to ½; 75%, for explants oxidized by up to ¾; and 100%, for fully oxidized explants.

Estimates of genetic parameters were accomplished by the REML procedure (Restricted Maximum Likelihood) / BLUP (Best Unbiased Linear Prediction) mixed model and deviation analysis (Anadev) (Resende et al. 2014), as follows:

$$y = Xu + Zg + e_2$$

where: y is the observed vector, b is the fixed-effects vector (overall mean), g is the random-effect vector of the total genotypic effects.

To classify the magnitudes of heritability and selective accuracy, parameters postulated by Resende 1998 were then used (Table 2).

Selection index

The predicted genotypic values were used to calculate the selection index, which is based on the sum of ranks to classify the relationship between genotypes and characters (Mulamba and Mock, 1978). Based on this classification, values of each character were summed, thus resulting in a general value considered as a selection index (Cruz et al., 2004).

Statistical analysis

The statistical analysis was accomplished by the SELEGEN-Reml/Blup (Statistical System and Computerized Genetic Selection by Mixed Linear Models) software v. 2016, model number 83 (Resende, 2016).

Conclusion

We have shown that there is an available genetic variability among bacuri accessions from different locations of the State of Maranhão, Brazil, for *in vitro* establishment. Based on the demonstration that callogenesis, percentage of oxidation, root formation, and shoot formation are important characteristics for *in vitro* selection of *Platonia insignis*, as it presents a high genetic control, our data suggest that the selection of AC.7 (Bacabeira), AC.2 (Bacabeira Santa Luzia), AC.1 (Codó), AC.6 (Codó - Bom Jesus) and AC.8 (Morros) present a higher probability of success for *in vitro* regeneration protocols for *Platonia insignis*, thereby providing the basis for future studies concerning the micropropagation of this species.

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