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Early evaluation of genetic parameters in clonal propagation of bamboo species

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Abstract

Bamboo plantation has gained great attention in Brazil due to its useful production provided to the society and the ecological, economic, social and cultural functions of these plantations. For the establishment of high productivity plantations, evaluation of germplasm and selection of superior genotypes is necessary. This work aimed at early evaluation of the genetic parameters of potential bamboo species for planting in Brasília/DF. For this purpose, a clonal test consisting of seven species was established: *Bambusa oldhamii*, *Bambusa vulgaris*, *Bambusa vulgaris* var. *vittata*, *Dendrocalamus asper*, *Dendrocalamus latiflorus*, *Guadua angustifolia* and *Guadua chacoensis*. The experiment was planted in a completely randomized block design, with three replications and fifteen plants per plot. At 360 days after planting, all individuals were evaluated for their characteristics: height of aerial part, number of sprouts, clump base area, clump coverage area and density of stalks per clump. The statistical analyses were carried out through the procedure of mixed models: Maximum Residual Likelihood/Better Non-Victimised Linear Prediction. There were significant species effects on the characteristics of the aerial part height and number of shoots, as well as high estimates of selective accuracies. Genetic parameters and genotypic values demonstrate genetic variability between species and the possibility of future gains from selection. New evaluations will be carried out annually to aim a greater knowledge of the behavior of germplasms at different ages. At the age of five (commercial court), the best genotypes will be selected for the experimental environmental conditions.

Introduction

Bamboos are remarkably robust grasses, belonging to the Poaceae Family, distributed in 115 genera, composed of approximately 1,400 species (Kelchner and Bamboo Phylogeny Group 2013). They have natural occurrence in several parts of the world, including temperate and tropical forests (Kelchner and Bamboo Phylogeny Group 2013). In South America, Brazil leads other countries in number of occurrences of bamboo species, with approximately 232 species (Drumond and Wiedman 2017; Singh et al., 2013; Pereira and Beraldo, 2007).

The history of bamboo use by society goes back to the millenary traditions of the Orient and, currently, several studies have further expanded its scope of use at a global level (Drumond and Wiedman 2017; Liese and Köhl 2015). Its stalks have been source of raw material for handicrafts, civil construction, pulp and paper, baskets, musical instruments, furniture, domestic utensils and energy use (Singh et al., 2013; Liese and Köhl, 2015; Freitas et al., 2016; Santos et al., 2016; Sette Júnior et al., 2016; Sette Júnior et al., 2017).

Some species have been used for human consumption (teas, preserves, liqueurs, pickles, wines and beers), with great nutraceutical relevance (Liese and Köhl, 2015; Pinto and Brito, 2017; Tomielis et al., 2017), and its leaves can be used for animal feed (Liese and Köhl, 2015).

Bamboo can also be used in cement mortar composites (sand and bamboo particles), plywood, composites (bamboo-PET), veneers, laminates (bamboo nanofibrils for polymer reinforcement), agglomerated panels, laminated bamboo panels, medium density fiberboard (MDF), high density fiberboard (HDF) and oriented strand board (OSB) (Liese and Köhl, 2015; Almeida, 2017; Azambuja et al., 2017; Beraldo, 2017; Guimarães Júnior et al., 2017; Lima et al., 2017; Mazzeto et al., 2017; Mendes et al., 2017; Ostapiv, 2017).

In general, bamboos have several characteristics considered relevant from a phytotechnical point of view, which favours their cultivation on a commercial scale. The main characteristics are large number of species with potential for adaptation to the most varied environmental conditions, rapid growth, productive potential, ease of management, technological properties of the thatch suitable for various uses and ease of vegetative propagation, which allows the reproduction of superior genotypes on a large scale (Liese and Köhl, 2015; Drumond and Wiedman, 2017).

In recent years, efforts have been made by various institutions to create a better basis for bamboo cultivation in Brazil (Bonilla et al., 2010; Greco et al., 2011; Drumond and Wiedman, 2017). One of the most important is the National Policy to Encourage Sustainable Management and

Cultivation of Bamboo (Brazil, 2011).
This law aims to encourage the sustainable management and cultivation of bamboo species, especially among family farmers, recognizing their ecological, economic, social and cultural functions (Afonso and Silva, 2017; Lorenzetti et al., 2017). At the regional level, multidisciplinary actions have been developed by the Bambu Goiás Network. Some important advances have been made in the scientific and technical areas, but there is still a need for further research in several areas.
One of the main areas of research is related the genetic improvement of bamboo in order to improve the adaptive conditions of the species as well as increase productivity and tolerance to biotic and biotic factors and to obtain raw material in greater quantity and quality.
In this context, we aimed to perform an early evaluation of genetic parameters in clonal bamboo installed in the state of Distrito Federal

Results and Discussion

Estimates of selective accuracy
The estimates of selective accuracy were variable among the characteristics, being considered as very high for height of the aerial part and number of bamboo shoots, high for density of canes in the clump and low for base area of the clump and area of coverage of the clump (Table 1), considered and comparing with the classification proposed by Resende and Duarte (2007).
Since selective accuracy has substantial relevance in assessing the quality of experiments, and consequently, in predicting genotypic, values greater than 70% should be routinely sought (Resende and Duarte 2007).

Deviances analyses
In the analysis of deviances, significant effects of clones/bamboo species were observed by the LRT test, at 1% probability, for the characteristic's aerial height and number of bamboo shoots. From these results, it can be inferred that there are differences in the performance of the different bamboo species, with possibilities of gains with the selection for these characteristics. On the other hand, no significant effects of clones were observed for the clump base area, clump coverage area and that of the stems per clump, i.e., the behavior of the species was similar for each of the characteristics.
In this study, the characteristics with high accuracy estimates and with significant effects of clones also consist of those that are easier to measure in the field and, consequently, with a lower rate of errors embedded in the estimates, which facilitate the evaluation work. In this sense, only the results for these characteristics will be discussed.

Aerial part
For the characteristic height of the aerial part, the genotypic variance between clones/bamboo species was higher than the residual variance and the latter higher than the environmental variance between plots (Table 2). For the number of shoots, the residual variance was higher than the genotypic variance and the latter higher than the environmental variance between plots. The presence of environmental variance between plots may be associated with the occurrence of small variations present in the area such as declivity, history of use of the cultivation area, among others. As this is an evaluation at the level of a single environment, it is observed that environmental variance may be inflated by variances of dominance, epistatic and the interaction of genotypes with the culture environment (Vencovsky and Barriga 1992).
However, for both characteristics, high percentages of genotypic variation coefficients were observed, which denotes the presence of genetic variability among bamboo species. Additionally, the relative variation coefficients for the characteristic's aerial height and number of shoots were higher than 1, which confirms the presence of genetic variability among the seven tested bamboo species clones (Vencovsky and Barriga 1992).

Inheritability coefficient
The individual inheritability coefficient in the broad sense was high for the characteristic height of the aerial part and moderate for the number of shoots, both being different from zero according to the confidence interval. Inheritability in the broad sense considers all genetic variance, being important in plant improvement via asexual propagation, because all genotypic variance is transmitted to offspring (Vencovsky and Barriga 1992).
As expected, the mean clone inheritance coefficients were of greater magnitude. The selection based on heritability in the mean of clones is at a higher level of precision than the selection based on individual or plot levels according to Vencovsky and Barriga (1992). This fact results from the reduction of the influence of experimental errors with the use of means instead of individuals. In the case of the present clonal test, only the heritability in the mean of clones is of interest since the selection of clones is not made based on the parcels.

Genotypic values
Table 3 shows the genotypic values for the different bamboo species evaluated. For aerial height, the best performance was obtained by the species *Bambusa vulgaris vittata*, being 63.80% higher than the mean of the population. As for the number of shoots, the *Guadua angustifólia* species obtained the highest number of shoots, being approximately 83% higher than the population average.
The estimated genetic correlation between the characteristics of height of the aerial part and number of shoots was equal to -0.54. Although the estimate is not high, the results indicate that an increase in the height of the stems may lead to a smaller number of sprouts. The estimated genetic correlation between the characteristics of height of the aerial part and number of shoots was equal to - 0.54. Whilst the estimate is not high, the results indicate that an increase in the height of the stems may lead to a smaller number of sprouts.

Genetic variability
The results show the relevance of the best use of natural genetic variability available in genetic improvement activities focused on the selection and vegetative propagation of superior genotypes. It is worth mentioning that there are some worrisome points regarding the improvement of bamboo. The seed production of bamboo is limited due to the long flowering cycle, which can reach up to 120 years; occurrence of monocarpal flowering, i.e., a single event of massive and synchronous flowering and fruiting; occurrence of seed sterility, low seed viability and extensive polyploidization of the genome (Singh et al. 2013).
In an extensive literature review on research related to genetics and improvement of bamboos, Singh et al. (2013) reported several articles using molecular markers in studies

Table 1. Deviances analyses in test of multispecies clones of bamboo, at 360 days after planting.

Sources of variation	Characteristics									
	Height of the aerial part (cm)		Number of shoots (unity)		Base of the stalks area (m^2)		Cover area of the stalks (m^2)		Density of stalks (unity)	
	Deviance	LRT[1]	Deviance	LRT[1]	Deviance	LRT[1]	Deviance	LRT[1]	Deviance	LRT[1]
Clones[+]	2.809,98	25,75**	1.465,80	9,93**	-1.363,46	0,00^{ns}	681,85	0,00^{ns}	1.204,13	0,95^{ns}
Proportion[+]	2.805,38	21,75**	1.479,65	23,78**	-1.187,71	175,75**	865,69	183,84**	1.264,60	61,42**
Model	2.784,23	-	1.455,87	-	-1.363,46	-	681,85	-	1.203,18	-
Accuracy	0,98		0,94		0,13		0,07		0,70	

[1]Reason test of verisimilitude, with a distribution of 1 degree of freedom; [+] deviance of ajusted model; ** significant at 1% probability, by the test of Chi-square, ns - not significant at 1% probability, by the test of Chi-square.

Table 2. Estimates of variance components for the different traits in multispecies clones of bamboo, at 360 days after planting.

Variance	Characteristics				
	Height of the aerial part (cm)	Number of shoots (unity)	Base of the stalks area (m^2)	Cover area of the stalks (m^2)	Density of stalks (unity)
σ^2_g	9.061,6251	27,0471	0,0000	0,0123	2,9345
σ^2_{parc}	523,2996	8,3247	0,0059	3,6890	8,2200
σ^2_e	2.255,1469	32,7602	0,0037	2,6166	14,4177
σ^2_f	11.840,0717	68,1320	0,0096	6,3179	25,5722
h^2_g	0,7653	0,3969	0,0037	0,0019	0,1147
IC	0,6259-0,9047	0,2965-0,4973	-0,0060-0,0013	-0,0051-0,0089	0,0607-0,1687
h^2_{mc}	0,9758	0,8853	0,0172	0,0094	0,4895
c^2_{parc}	0,0442	0,1222	0,6116	0,5839	0,3214
CV_{gi} (%)	65,2083	43,0542	6,7512	5,7274	20,7620
CV_e (%)	17,7793	26,8368	88,4740	101,6785	36,7244
CV_r	3,6676	1,6043	0,0763	0,0563	0,5653

σ^2_g: genotypic variance; σ^2_{parc}: environmental variance between plots; σ^2_e: residual variance; σ^2_f: individual phenotypic variance; h^2_g: heritability in the broad sense; *IC*: confidence interval; h^2_{mc}: heritability in the mean of clones; c^2_{parc}: coefficient of determination of the effects of plots; CV_{gi} (%): coefficient of genotype variation; CV_e (%): coefficient of residual variation; $CV_{r=}$ CV_{gi}/ CV_e (%): coefficient of relative variation.

Table 3. Genotypic values in clones of bamboo, at 360 days after planting.

Species/clone	Genotypic values (m+g)				
	Height of the aerial part (cm)	Number of shoots (unity)	Base of the stalks area (m^2)	Cover area of the stalks (m^2)	Density of stalks (unity)
BO	98,5663	7,8578	0,0885	1,9322	6,7138
BV	238,4288	8,0349	0,0898	1,9451	8,1171
BVV	302,8587	9,7662	0,0880	1,9252	9,6836
DA	155,6927	12,1862	0,0882	1,9306	8,8460
DL	116,4519	10,9664	0,0879	1,9298	8,5523
GA	62,2095	22,1020	0,0877	1,9305	9,2485
GC	47,6677	13,6421	0,0889	1,9385	6,5942
Mean	145,9822	12,0794	0,0884	1,9331	8,2508

BO (*Bambusa oldhamii*), BV (*Bambusa vulgaris*), BVV (*Bambusa vulgaris* var. *vittata*), DA (*Dendrocalamus asper*), DL (*Dendrocalamus latiflorus*), GA (*Guadua angustifolia*) and GC (*Guadua chacoensis*)

of polymorphism and phylogenetic relationships (45); micropropagation using somatic embryogenesis, calogenesis and indirect organogenesis (111); in vitro flowering induction (18), genetic fidelity testing of propagated genotypes in vitro (18) and gene cloning and genetic transformation of (5). These studies have contributed to filling important gaps and generating information on the use of biotechnological techniques for several bamboo species.

However, published studies on evaluations of different genotypes, origins and species at the level of field experiments, i.e., on classical improvement, seem to be scarce or non-existent, which demonstrates the importance of the present work and the implementation of several other experiments covering as many germplasms as possible, in order to take better advantage of the available natural genetic variability.

These studies are fundamental because they allow testing and selection of superior genotypes regarding the characteristics of interest such as high biomass production, tolerant to biotic and abiotic factors of economic importance such as diseases, insect pests, water deficit, among others. The adequate balance between classical improvement activities and the use of biotechnological tools are fundamental for the generation of bamboo elites' cultivars, similarly to what has occurred routinely with other annual and perennial species.

Materials and Methods

Selection of bamboo species

The selection of bamboo species used in this study was based on criteria such as adaptation to the tropical climate, planting conditions, plant size, rusticity, sympodial growth system and availability of vegetative propagules.

Seven species were defined: *Bambusa oldhamii* Munro, *Bambusa vulgaris* Schrad. ex J.C. Wendl., *Bambusa vulgaris* var. *vittata*, *Dendrocalamus asper* (Schult. & Schult. f.) Backer ex K. Heyne), *Dendrocalamus latiflorus* Munro, *Guadua angustifolia* Kunth and *Guadua chacoensis* (Rojas) Londoño and Peterson.

Substrate and irrigation

As the main form of propagation of bamboos is vegetative, clonal seedlings of each of the seven species were obtained and implanted in a clonal test format. The clonal seedlings were produced in the forest nursery of the University of Brasilia (UnB).

A substrate composed of a 1:1 subsoil sample and Bioplant® was used, which was packed in black polyethylene bags (25 cm x 35 cm), with a volumetric capacity of 875 cm^3. The irrigation of the seedlings was performed periodically in the early morning and late afternoon, via sprinklers. The production time of the seedlings was 120 days.

Study place

The experiment was implemented in Água Limpa Farm - FAL, also belonging to UnB, located in the Federal District, in the geographic coordinates 15°94'86" South latitude and 47°93'40" West longitude and altitude of 1,100 m. The climate is tropical, with a dry winter (Aw), characterized by a rainy season in summer from November to April and a clear dry season in winter from May to October. The maximum annual average temperature is estimated at 28.5 ºC and the minimum annual average temperature is 12.0 ºC (Alvares et al., 2013).

Preparation of planting

After cleaning the planting site with a crawler tractor, the pits were demarcated with 5.0 m x 4.5 m spacing. The pits with dimensions of 30 cm x 30 cm x 30 cm were opened with the aid of a manual digger. Covering fertilization was performed with 150g of NPK (formula 20-05-20) added to the pit, as recommended by Pereira and Beraldo (2007).

The experiment was implemented in complete randomized blocks, with seven treatments (species), three repetitions and five plants per plot, totaling 15 plants per species in each block. Planting was carried out in March 2016, and replanting was carried out 30 days later.

At 360 days after the planting date, five characteristics were measured: height of the aerial part (cm), number of shoots (unit), clump base area (m^2), clump coverage area (m^2) and stem/clump density (unit). To evaluate this last characteristic, a distinction was made between sprouts and stems, the latter less than 50 cm in height.

Statistical analyses

Statistical analyses were performed using the mixed model procedure, Restricted Maximum Likelihood/Best Linear Unbiased Prediction – REML/BLUP, in the program Computerized Genetic Selection – Selegen and test for chi-square (Resende 2007a).

In the genetic evaluation, the procedure of mixed models was used by means of the following model (Resende 2007b): y = Xr + Zg + Wp + e, were y: data vector, r: vector of the fixed effects of repetition added to the overall mean, g: vector for genotypic random effects, p: vector for plot random effects and e: vector for residue random effects. Capital letters represent the incidence matrices for these effects.

From these analyses, the significance of random effects of the models was obtained by the test of the likelihood ratio (LRT) and the analysis of deviances (Resende 2007b). Accuracy estimates and variance components were obtained for each of the measured characteristics (Resende 2007b). Genotypic values (m+g) of all clones were predicted via BLUP (Resende 2007b).

Conclusion

The results show the presence of genetic variability between species and the possibility of future gains with selection. New evaluations will be carried out annually with a view to greater knowledge of the behavior of germplasms at different ages. This aspect is very important given the lack of research aimed at the classical genetic improvement of bamboo.

At the age of commercial cutting, i.e. at five years of age, selection of the best genotypes for experimental environmental conditions will be carried out. In this opportunity, the selected germplasm can be evaluated in different years of planting and in different types of soils, as a way to prove the superiority of the selected genotypes for the environmental conditions of Brasília and other sites with similar environmental conditions.

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