

## Genetic divergence among provenances of *Mimosa scabrella* Benth. based on seed analysis

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### ABSTRACT

It was aimed through this work to evaluate the genetic divergence among four provenances of bracatinga (*Mimosa scabrella* Benth.) belonging to the state of Santa Catarina, namely: Abelardo Luz (AB), Chapadão do Lageado (CL), Lages (PB) and Três Barras (TB) by means of multivariate analyses, they are the principal components analysis and hierarchical clustering based on the Euclidian distance. Seeds of 40 mother trees, ten from each site, were evaluated as to the biometric aspects of seeds and variables coming from germination test. The traits which contributed for the most toward divergence among the provenances were the length and breadth of seeds, % of normal seedlings, % of germination and germination velocity index. Genetic divergence among the provenances was found and separated into two groups. The cross between the superior individuals of the provenances Abelardo Luz and with the superior individuals from the provenances of group I (CL and PB) are the most promising for future genetic breeding programs.

**Key words:** bracatinga; clustering analysis; multivariate analysis; principal components analysis

## *Divergência genética entre procedências de Mimosa scabrella Benth. com base em análise de sementes*

### RESUMO

Objetivou-se com este trabalho avaliar a divergência genética entre quatro procedências de bracatinga (*Mimosa scabrella* Benth.) pertencentes ao estado de Santa Catarina, sendo elas: Abelardo Luz (AB), Chapadão do Lageado (CL), Lages (PB) e Três Barras (TB), por meio de análises multivariadas, sendo elas a técnica de componentes principais e agrupamento hierárquico, baseado na distância euclidiana. Sementes de 40 matrizes, 10 de cada local, foram avaliadas quanto aos aspectos biométricos de sementes e variáveis oriundas do teste de germinação. Os caracteres que mais contribuíram para a divergência entre as procedências foram o comprimento e largura de sementes, % de plântulas normais, % de germinação e índice de velocidade de germinação. Constatou-se divergência genética entre as procedências, e foi possível separá-las em dois grupos. O cruzamento entre os indivíduos superiores das procedências Abelardo Luz e / ou com os indivíduos superiores das origens do grupo I (CL e PB) são os mais promissores para futuros programas de melhoramento genético.

**Palavras-chave:** bracatinga; análise de agrupamento; análise multivariada; análise dos componentes principais

## Introduction

The supply of seeds of native forest species with genetic quality to compose high forest plantings is one of the factors which at present has been limiting the utilization of these as an alternative to the use of exotic species (Silva & Higa, 2006). It is known that the great majority of the seedlings of forest species is coming from the propagation via seeds (Sarmiento & Villela, 2010), in this way, to know the sources of genetic variability of the propagation materials available to the breeder is the basis for the genetic progress of particular traits related to the seed supply and formation of high-quality seedlings.

The tests for evaluation of physical and physiological quality in seeds of forest species are simple and low-cost tools and enable to estimate the variability and compare lots of seeds among provenances, offering to the researcher information for the early phase of the genetic breeding program (Santos & Paula, 2009; Lazarotto et al., 2013).

To obtain genetically superior seeds which present satisfactory silvicultural performance in commercial plantings, it is necessary firstly to sample the sources of genetic variation of the species (Cruz et al., 2012), to select afterwards the individuals with superior traits and to conduct genetic recombinations which aim to obtain high quality seeds allied to the addition of yield.

One of the tools which aid the determination of the genetic divergence among populations of one species is the biometric analysis (Matheus & Lopes, 2007; Costa et al., 2016). This analysis allows knowing the genetic variability of the populations from the characterization of seeds which will be able to be utilized in the selection of superior genotypes and the prediction of crosses with greatest heterotic potential (Cruz et al., 2012).

Biometric analyses, in addition to serving as an important instrument to detect the genetic variability within and between the populations of the same species, which can result from environmental alterations or due to genetic factors, are widely utilized to evaluate the physical quality of seeds (Gusmão et al., 2006).

The germination test, considered the main parameter for the evaluation of the physiological potential, is also utilized for the comparison of the quality of seeds among provenances, since, both the intensity and in the germination process velocity, differences depend upon genetic and/or environmental factors (Araújo Neto et al., 2013; Silva et al., 2013; Oliveira et al., 2016). The genetic divergence among provenances is of great importance for plant breeding, for the more divergent the parents are, the greater the possibility of obtaining favorable combinations (Rodrigues et al., 2010; Cruz et al., 2012; Souza et al., 2014).

Different techniques of multivariate analysis utilize physiological and morphological variations of plants, fruits, and seeds to estimate the genetic divergence in provenances, among them, the principal component analysis and the clustering methods (Bahia et al., 2008; Rodrigues et al., 2010; Lazarotto et al., 2013). The utilization of the multivariate techniques allows the evaluation of the genetic material from a set of measured characteristics to select promising materials

and evaluate the divergence among provenances, considering the importance of each variable in the existing total variation (Costa et al., 2016).

A study carried out by Lazarotto et al. (2013) utilizing multivariate analysis techniques to quantify the genetic divergence among provenances for several exotic forest species proved to be possible to quantify the genetic divergence among populations by traits coming from seed analyses.

The bracatinga is a native forest species belonging to the family Fabaceae which has become a forest extract prioritized in some regions of Southern Brazil, taking over economic importance due, mainly, to its multiple uses. In that way, studies addressing the genetic divergence between the species are highly important, since its diversity is still little explored.

The objective of this work was to estimate the genetic divergence among four provenances of bracatinga, coming from Santa Catarina state (Brazil) based on physical and physiological variables from seed analyses.

## Material and Methods

Ripened seeds of *M. scabrella* were collected in December of 2013, in four provenances belonging to the Santa Catarina state (Brazil), namely: Abelardo luz (AB), Chapadão do Lageado (CL), Lages (PB) and Três Barras (TB). In each site, seeds of 10 mother trees were collected, the choice of the mother trees was based upon recommendations prescribed by Sebbenn (2006) obeying the minimal distance of 100 m between the trees, aiming to decrease the possibility of sampling related individuals.

In the wake, the fruits were exposed to the sunshine (two days) to force the natural opening and aid in the later extraction of seeds, which consisted of putting the fruits into sackcloth bags and submitting them to beatings with the aid of a wooden instrument. Next, these seeds were taken to the Forest Ecology Laboratory of the Agroveterinary Science Center of the Santa Catarina State University (UDESC), localized at Lages, where they were processed in a hand manner with the utilization of sieves and immediately utilized in the conduction of the experiment.

Biometric analyses and germination test of seeds were carried out from the completely randomized experimental design adopting four replications of 25 seeds for each analysis. In the biometric evaluations, the seeds were taken randomly for the evaluation of the traits length, breadth, and thickness, utilizing a digital pachymeter (precision of 0.01 mm).

For the germination test, firstly break of dormancy of seeds by the method of immersion into the water at the initial temperature of 80 °C was performed, followed by rest in the same water out of the heating for 18 hours (Brasil, 2013). Soon afterward, the seeds were placed onto germitest paper rolls, wetted with water equivalent to 2.5 times the weight of the substrate with three sheets per roll. The paper rolls were identified and incubated in type B.O.D oven (Biological Oxygen Demand), containing three fluorescent lamps of 15 W and 12 h photoperiod at a constant temperature of 25 °C according to the Instructions for forest species analysis (Brasil, 2013).

The germinated seeds were evaluated daily for eight days. After obtaining the number of germinated seeds, the following characteristics were evaluated: germination velocity index – resulting from the summation of the ratio of the daily germination by time, in days elapsed from the start of the test; germination percentage – considering seeds germinated as those which presented the radical protrusion of at least 2 mm according to the methodology of (Brasil, 2009).

The percentage of normal and abnormal seedlings were also evaluated according to the criterion by Brasil (2009); the length, green mass and dry matter mass of normal seedlings – evaluated on all normal seedlings of each replication/treatment, however the length was obtained with the use of a digital pachymeter (0.01mm) and for obtaining of the dry mass and green mass, the cotyledon was removed and next the seedling was weighted with the utilization of an analytical balance (precision of 0.0001g). For obtaining of the dry mass of seedlings, the seedlings were submitted to oven-drying regulated at 80 °C for 24 hours with the results expressed in dry mass (g) per seedling.

In the whole, 10 variables concerning the seed analysis, among them, the traits of the biometric evaluations: length (LS), breadth (BS) and thickness (TS) of seeds and the variables concerning the germination test: % of normal seedlings (NS), % of abnormal seedlings (AS) and % of germination (GER), in addition to germination velocity index (GVI), seedling length (SL), green mass of the seedlings (GMS) and dry mass of the seedlings (DMS).

Initially, the data collected were tested as to normality and soon afterward, submitted to the univariate analysis of variance of the within and between type, followed by the multivariate variance analysis to aid in the evaluation of the divergence among the provenances, with the help of the SISVAR software (Ferreira, 2011). All the traits were submitted to the principal component analysis, estimating the relative contribution of each variable for the discrimination of the provenances, eliminating the variables which presented the smallest explanation power from the same method employed by Lazarotto et al. (2013), in which the components which were capable of synthesizing a variance accumulated in around 70% were utilized.

With the variables of greatest importance elected, the genetic divergence dendrogram was generated, using hierarchical clustering method of the nearest neighbor by the Euclidian distance, with the aid of the Statistica software version 8.0 (Statsoft, 2007).

**Table 2.** Eigenvalues and eigenvectors of the multivariate principal by principal components obtained from ten variables coming from seed analysis in four origins of *Mimosa scabrella* Benth., belonging to the state Santa Catarina.

Factor	Eigenvalue	Total variance (%)	Accumulated eigenvalue	Accumulated total variance (%)
1	4.61	46.12	4.61	46.12
2	1.56	15.60	6.17	61.72
3	1.14	11.43	7.32	73.15
4	0.93	9.26	8.24	82.41
5	0.70	7.02	8.94	89.43
6	0.53	5.28	9.47	94.71
7	0.41	4.12	9.88	98.82
8	0.08	0.81	9.96	99.63
9	0.04	0.35	10.00	99.99
10	0.00	0.01	10.00	100.00

## Results and Discussion

The univariate analysis of variance showed that there were significant differences among provenances ( $p < 0.01$ ) for most traits evaluated, except for the variables seed thickness and percentage of abnormal seedlings (Table 1), pointing out, that, at first, the populations are divergent. Using the multivariate variance analysis, the values of the Wilks statistics  $\Lambda = 0.000013$ , corresponding to a  $F = 194.81m$  was obtained, which was significant ( $p < 0.01$ ), confirming the result of the univariate analysis and indicating that there is genetic divergence among the populations coming from seed analysis.

The variation coefficient differed among the variables surveyed; values considered satisfactory ( $CV \leq 22\%$ ), confirming the good experimental precision for all the characteristics, except for the variable dry mass of seedlings, which displayed variation coefficient regarded as high (64.1%).

The principal component analysis revealed that the first three components were sufficient to explain 73% of the observed variation (Table 2), the first one being the most important of all, which explained 46.12%, the second 15.60% and the third 11.43%. Thus, the first three components were utilized for the identification of the traits of greatest importance.

The importance of the different variables collected to group the provenances of bracinga together (Table 3), determined through their eigenvectors, establishes that (i) in main component 1, the % of normal seedlings, % germination, germination velocity index and green mass of seedlings were

**Table 1.** Summary of the variance analyses of the ten traits obtained from the analysis of the seeds in the evaluation of four provenances of *Mimosa scabrella* Benth., belonging to the state of Santa Catarina (Brazil).

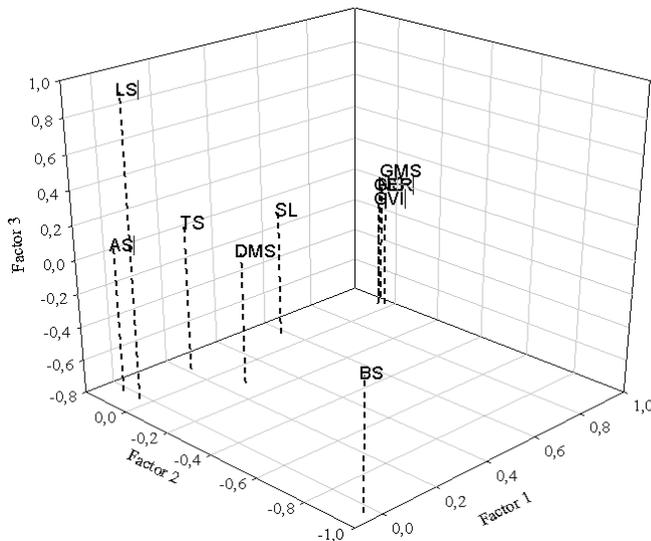
Variable	Mean squares		Mean	CV%
	Between	Within		
LS (mm)	0.801182**	0.268734**	4.982	2.96
BS (mm)	1.290894**	0.964056**	3.494	1.31
TS (mm)	0.459514 <sup>ns</sup>	0.27752 <sup>ns</sup>	1.363	5.25
NS (%)	262.966667**	171.580556**	54.730	16.64
AS (%)	3.541667 <sup>ns</sup>	8.625000 <sup>ns</sup>	7.135	17.52
GER (%)	4104.666667**	2483.155556**	66.375	14.30
GVI	436.435252**	132.411712**	11.107	21.86
SL (cm)	31.912537**	6.809728**	11.092	7.10
GMS (g)	1.014638**	1.074855**	1.078	12.77
DMS (g)	0.000003**	0.000002**	0.063	64.08

\*\* Significant by the F test at 1% of probability; ns: Non-significant; CV% = Coefficient of variation, namely: length (LS), breadth (BS) and thickness (TS) of seeds, % of normal seedlings (NS), % of abnormal seedlings (AS) and % of germination (GER), in addition to the germination velocity index (GVI), seedling length (SL), green mass of the seedlings (GMS) and dry mass of the seedlings (DMS).

**Table 3.** Contribution of the ten variables surveyed in seeds of *Mimosa scabrella* Benth., from different provenances of the state of Santa Catarina (Brazil), in each of the principal components.

Variable	Weight factor 1	Contribution factor 1 (%)	Weight factor 2	Contribution factor 2 (%)	Weight factor 3	Contribution factor 3 (%)
LS (mm)	-0.04	0.01	0.01	13.08	0.99*	49.43*
BS (mm)	-0.05	0.12	-0.98*	23.35*	-0.01	21.57
TS (mm)	0.19	2.32	0.06	21.42	0.12	0.86
NS (%)	0.97*	20.51*	0.04	0.07	-0.03	0.81
AS (%)	-0.04	0.04	0.09	22.45	0.10	4.89
GER (%)	0.97*	20.46*	0.05	0.10	-0.04	0.50
GVI	0.93*	19.40*	0.01	0.30	-0.09	1.55
SL (cm)	0.55	13.10	0.06	1.00	0.01	0.16
GMS (g)	0.94*	20.43*	-0.01	0.02	0.09	1.01
DMS (g)	0.24	3.60	-0.14	18.20	0.03	19.22

\* Variables with a significant contribution of the factors. They are: length seeds (LS), breadth seeds (BS) and thickness seeds (TS), % of normal seedlings (NS), % of abnormal seedlings (AS) and % of germination (GER), in addition to the germination velocity index (GVI), seedling length (SL), green mass of the seedlings (GMS) and dry mass of the seedlings (DMS).



**Figure 1.** The plot of the three-dimensional plan, of the Principal Component Analysis, demonstrating the importance of the variables coming from the seed analysis for discrimination of the provenances of *Mimosa scabrella* Benth., in each factorial plan. They are: length (LS), breadth (BS) and thickness (TS) of seeds, % of normal seedlings (PN), % of abnormal seedlings (AS) and % of germination (GER), in addition to the (GVI), seedling length (SL), green mass of the seedlings (GMS) and dry mass of the seedlings (DMS).

statistically significant and account for 80.8% of the total variance of component 1; (ii) in component 2, only the breadth of seeds was significant and accounts for 23.3% of the total variation of that principal component; (iii) while the variable length of seeds explained 49.4% of the variation of component 3 (Figure 1).

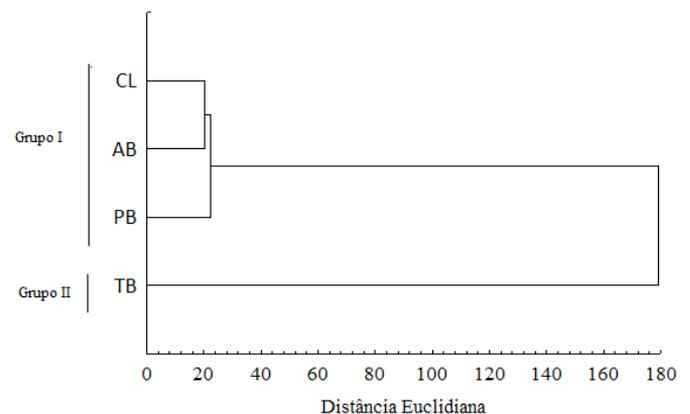
From the principal component analysis, it was possible to find that some of the parameters related to the germination test (for instance: germination velocity index, % of germination, green mass of the seedlings and % of normal seedlings) are of great importance to explain the variations among the different provenances of naturally occurring *M. scabrella*. These results indicate the existence of the variability of these parameters in the studied provenances.

On the other hand, vectors concerning the parameters coming from the germination test, such as the % of abnormal seedlings and the length and dry mass of the seedlings, in addition to the vector concerning the biometric characteristic seed thickness, indicate variables characterized by the little differentiation.

The principal component analysis allowed, then, eliminating the variables that little explain the variation existing among the provenances, including thick seeds, % of abnormal seedlings, seedling length and dry mass of seedlings. A similar result was found by Lazarotto et al. (2013) in which the variable percentage of abnormal seedlings was eliminated for discrimination of eight out of the nine exotic forest species studied and the variable percentage of normal seedlings was maintained for the distinction of all the species. Another similar point found in work cited above is that, as in the present work, the percentage of normal seedlings was also the variable that explained the greatest variability, with the highest value in component 1.

Santos et al. (2005) using variables coming from seed analysis for discrimination of the physiological quality among different mother trees of *Tabebuia chrysostricha* (Mart. ex A. DC) Stand, also considered the variables % GER, GVI, and % NS suitable for donor discrimination.

The dendrogram for the hierarchical clustering by the nearest neighbor method based on the Euclidean distance is shown in Figure 2. It was found that the provenances of CL, AB and PB were formed in the same group (I), while TB was formed in a single group (II). The genetic breeding of forest species, especially those with lack of studies like the bracinga. They depend on the separation of the provenances into different



**Figure 2.** Dendrogram generated by the hierarchical method of the nearest neighbor from the Euclidean distance obtained on the basis of the five variables of analysis of analysis of seeds selected for the grouping of four provenances of *Mimosa scabrella* Benth., belonging to the state of Santa Catarina (AB: Abelardo Luz; CL: Chapadão do Lageado; PB: Lages; TB: Três Barras).

groups so interest can be carried out (Abreu et al., 2009). So, it happens mainly with the species that have greater diversity among populations. Thus, the provenances of bracinga that present seeds with best physical and physiological qualities and exhibit the most significant genotypic divergence will work as a basis for future breeding programs. The provenances AB and CL are part of group I and they deserve to be highlighted because they showed the highest values for the variables related to the germination test, with 74 and 67% germination, 68 and 61% of PN, demonstrating physiological quality of the seeds coming from those provenances which make them attractive to the composition of selection programs. Both Maguire (1962) and Brasil (2009) rely on the principle that the lots which present the greatest velocity of seed germination are the most vigorous, reinforcing the idea that the AB and CL populations are recommended for the formation of segregating populations because of their improved performances as to the germination capacity and vigor.

The PB provenance stood out with the greatest value concerning the length and breadth of seeds (Table 4), but with decreased values for the variables % of germination, germination velocity index, % of normal seedlings and green mass of the seedlings, showing that the size of bracinga seeds had no direct relation to the germination capacity and vigor.

Alves et al. (2005) found that the germination of seeds of *Mimosa caesalpinifolia* coming from different provenances was not affected by seed size either, showing that there can be no correlation between the biometric traits of the seeds and the variables of the germination process, justifying the non-adoption of size classes as an indicator of the success in seedling establishment. Similar results were obtained by Torres (1994) for *Acacia senegal* (L.) de Willd and by Castro & Dutra (1997) for *Leucaena leucocephala* (Lam.) de Wit, both belonging to the family Fabaceae.

Sturion (1990), in a study concerning the influence of the origin and the size of the seeds of *Mimosa Scabrella* Benth, concluded that for two provenances tested, the germination percentage was not affected by seed size but the germination and vigor were important to the distinction among the most promising provenances as to the seedling quality, survival and early development in nursery.

According to the results found, individuals of AB provenances can be considered the most promising as they present higher values for the physiological characteristics. Therefore, the crossing of this provenance with the best individuals from the same origin (AB) and with the superior individuals from the provenances of group I (CL and PB). For genetic breeding programs which aim to obtain new gene,

**Table 4.** Average values of the traits selected through the analysis of the principal components, namely, seed length (SD), seed breadth (SB), % of normal seedlings (NS), % of germination (GER), germination velocity index (GVI), green mass of the seedlings (GMS) for the four provenances of *Mimosa scabrella* Benth., belonging to the state of Santa Catarina.

Provenances	SD (mm)	SB (mm)	NS (%)	GER (%)	GVI	GMS (g)
Abelardo Luz (AB)	4.81	3.36	68	74	15.5	1.28
Chapadão do Lageado (CL)	4.99	3.44	61	67	11.1	1.14
Lages (PB)	5.17	3.45	45	53	9.4	0.94
Três Barras (TB)	4.93	3.44	49	55	8.1	0.95

combinations is recommended to cross between AB x AB and AB x CL. They allow the combination of the higher values of the germination test, and thereby increasing the probability of joining alleles responsible for characteristics of interest in the new combinations (Rodrigues et al., 2010).

## Conclusions

The biometric characteristics and the evaluated variables in the germination test of *M. scabrella* of the state of Santa Catarina highlighted the divergence among the surveyed provenances.

The provenance of Três Barras (TB) is the most genetically distant; about the other provenances (CL, AB, and PB).

Based on the evaluated traits, the cross between the superior individuals of the provenance Abelardo Luz and with the superior individuals from the provenance of group I (CL and PB) are the most promising for future genetic breeding programs. Of the ten traits evaluated five have a greater contribution to the total variation, highlighting length and width of seeds, % of normal seedlings, % of germination and germination velocity index.

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