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Progeny selection of *Myrocarpus frondosus* for improved growth vigor of seedlings

Seleção de progênies de *Myrocarpus frondosus* para vigor de crescimento de mudas

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ABSTRACT

In this study, we aimed to evaluate half-sibling progenies of *Myrocarpus frondosus* based on morphological characteristics and to select seedlings with high growth vigor and quality. Seedlings were evaluated for shoot height and diameter, shoot height/diameter ratio, and number of leaves. The shoot height showed the highest estimations of linear correlation with the other characteristics. The linear correlation of shoot height between two consecutive evaluations was high from 60 days of cultivation, indicating that selection should be applied after this period. Selection of 101 seedlings within the best progenies resulted in a genetic gain of 15.13% for shoot height. The greatest indirect gain from selection was for the shoot height/diameter ratio, whereas the lowest indirect gain was for the stem diameter. *Myrocarpus frondosus* progenies can be selected for shoot height at 60 days of nursery cultivation, resulting in high direct gain for growth and indirect gain for stem height/diameter ratio.

Keywords: Early selection; Genetic gain; Seedling quality

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RESUMO

O objetivo deste trabalho foi avaliar progênies de meios-irmãos de cabreúva (*Myrocarpus frondosus Allemão*) com base em características morfológicas e selecionar mudas com maior vigor de crescimento em viveiro. As mudas foram avaliadas para a altura da parte aérea, diâmetro do coleto, relação da altura e diâmetro do coleto e número de folhas. A altura da parte aérea apresentou altas estimativas de correlação linear e altamente significativas com os demais caracteres avaliados. A correlação linear da altura do caule entre duas avaliações consecutivas foi alta a partir dos 60 dias de cultivo, indicando que a seleção deve ser aplicada após esse período. A seleção de 101 mudas dentro das melhores progênies resultou em um ganho genético de 15,13% para a altura do caule. O maior ganho indireto da seleção foi para a relação altura/diâmetro do caule, enquanto o menor ganho indireto foi para o diâmetro do caule. As progênies de *Myrocarpus frondous* podem ser selecionadas para a altura do caule aos 60 dias de cultivo no viveiro, resultando em alto ganho direto para o crescimento e ganho indireto para a razão altura/diâmetro do caule.

Palavras-chave: Seleção precoce; Qualidade de muda; Ganho genético

1 INTRODUCTION

Myrocarpus frondosus Allemão belongs to the Fabaceae family and is an arboreal species with hermaphrodite flowers, pollinated mainly by bees (Carvalho, 2003). Naturally occurring in Argentina, Paraguay, and Brazil, it is one of the best-known forest species in the south of Brazil for the different uses of wood, such as dormers, beams, and decorative covering and panels (Calegari et al., 2009). *Myrocarpus frondosus* is also used by the perfumery, pharmaceutical, and dye industries, in urban reforestation, in the reconstruction of altered ecosystems, and in the restoration of ciliary forests (Carvalho, 2003; Santi et al., 2017). The excellent quality of its wood has resulted in the disorderly exploitation of this species, making it scarce and therefore threatened with extinction, and it is currently listed under the vulnerable category, according to State Decree n° 42.099/2003 (Rio Grande do Sul, 2003).

Forest fragmentation, caused by indiscriminate exploitation, has highly impactful consequences, especially from the genetic point of view. Reducing effective population size results in the elimination of alleles and consequently genetic variability and the ability of the species to adapt to changes in the environment and to continue to generate viable offspring (Shimizu, 2007). Thus, knowledge about the genetic variability of natural populations makes defining strategies for species conservation and selection possible in forest improvement programs (Otsubo et al., 2015). Progeny tests make quantification of variability, estimation of genetic parameters, maintenance of the genetic representativeness of natural resources inside and outside their natural habitat, and the consequent increase of knowledge regarding the genetics of species possible by means of progeny tests (Kubota et al., 2015; Silva et al., 2013). In addition, progeny evaluation allows the detection of genetic variability associated with environmental factors and enables estimation of the expected genetic gains with the selection (Duarte et al., 2012).

Selection among and within progenies has been increasingly used in *Eucalyptus* species to select the best progenies based on the mean and the best genotypes of these progenies (Costa et al., 2015b; Rosado et al., 2009). In addition, the early selection, the result of the study of plants in the juvenile phase, comprises a strategy to reduce each breeding cycle and to maximize the genetic gain for perennial woody species. For this, the seedlings of these species are evaluated for morphological parameters and growth rates, and this information allows identification of adequate development of the plants and the potential of survival after field planting (Silva et al., 2012). Among the morphological variables used to verify the quality of seedlings are the height of the shoot and the diameter of the collection, which are important indicators of seedling survival because they reflect root development and the ability to adapt to adverse conditions in the field (Prates et al., 2012). In addition, they may be associated with the number of leaves, which provides an estimate of the photosynthetic capacity and the transpiration area of the plant (Ritchie et al., 2010).

The objective of this research was to evaluate progenies of half-siblings of *M. frondosus* and to select genotypes with the most vigorous growth in the nursery and thereby increase the quality of the seedlings.

2 MATERIAL AND METHODS

The present study was conducted between February 2015 and March 2016 in the Forest Nursery and in the Plant Breeding and Breeding Nucleus of the Federal University of Santa Maria, Santa Maria, RS (29°72’47” S e 53°72’14” W).

The *M. frondosus* diaspores were collected in December 2014 from seven parent plants in the municipality of Nova Palma (RS). Due to the difficulty of removing seeds without compromising and/or damaging the structure of the embryo, the fruit (diaspore) was used in the production of seedlings. The diaspores of each mother plant were kept in a covered and airy environment for two days, with daily stirring. Subsequently, they were placed in a brown paper bag inside kraft paper drums and packed in a cold and dry chamber (18 °C and 49% relative humidity) until sowing (April).

The seedlings were produced in commercial substrate Carolina Soil® Sphagnum peat base, expanded vermiculite, agricultural gypsum, nitrogen, phosphorus and potassium (NPK), and dolomitic limestone. As base fertilizer, 6 g L^{-1} of NPK controlled release fertilizer (18-05-09) were added. According to the manufacturer's technical specifications, this fertilizer releases nutrients gradually between five and six months when added to moist substrate and maintained at an average temperature of 21 °C. After moistening and mixing in a concrete mixer, the substrate was accommodated in the polypropylene tubes on a compacting table. Polypropylene conical tubes with a volume of 280 cm^3 were used and were packed in trays that were suspended 16 cm from the soil surface. Forty-two progeny seedlings from each parent plant were evaluated, disregarding the border rows.

In each tube two diaspores were placed, covered with a thin layer of the substrate, and after sowing, the trays were taken to the greenhouse. Irrigation was performed by a movable bar containing microsprinklers (flow rate of 8 mm dia^{-1}). At 40 days after sowing, thinning was performed and surplus seedlings were eliminated, leaving only one per tube. At 180 days after sowing, trays were removed from the greenhouse and taken to an area with shade (50%), where they remained until the end of the experiment (270 days). At the moment the seedlings were removed from the greenhouse, cover fertilization with solution prepared from the dissolution of 200 g of ammonium sulfate and 150 g of potassium chloride in 100 L of water (Gonçalves et al., 2005) was applied. This fertilization was carried out biweekly, with the presence and absence of potassium chloride in the solution being intercalated in each application.

Evaluations began 30 days after emergence when approximately 50% of the seedlings of all progenies emerged up to 270 days (a total of nine monthly assessments). The height of the aerial part (H), the collar diameter (CD), plant height/collar diameter ratio (H/CD), and number of leaves was evaluated. The height of the shoot was measured in cm from the substrate level to the apical bud. The sample diameter was measured in mm with the aid of a digital caliper. The number of leaves was counted visually. The experiment comprised a factorial scheme of 7 × 9 (progenies and monthly evaluations) in the completely randomized design, with 42 replications of one plant per progeny.

Data were submitted for analysis of variance, following the procedure of simple factorial analysis of the Genes program (Cruz, 2013). Pearson correlation was also performed between the characteristics evaluated and within each characteristic for the monthly evaluations. The correlation data between the evaluations and the mean square were used to define the number of evaluations that can be used for the analysis of *M. frondosus* seedlings in the nursery. The means of progenies and the seedlings of the best progenies were compared by the Scott-Knott test at 5% probability of error. These comparisons were used to select between and within progenies. Selection gains between progenies and seedlings within progenies were calculated, respectively, by the difference between the mean of the evaluated morphological attribute of the progenies or the selected seedlings (Mean of the selected ones: DM) and the average of the progenies or evaluated seedlings (Mean of all evaluated seedlings: MO) for height, collar diameter, height/collar diameter ratio, and number of leaves. The percentage selection gain (GS%) was also calculated. These analyses were performed using the Office Excel® application.

3 RESULTS

Analysis of the variance showed that the evaluations at 210 and 240 days after emergence had very high average squares of the residue, and consequently, the variances were not uniform for the analysis of the data as factorial design. Thus, data from these two evaluations were discarded for all characteristics studied and analysis of variance was performed only with monthly assessments up to 180 days after emergence. The analysis of variance showed significant interaction between the progenies of *M. frondosus* and the different evaluations for all characteristics (Table 1). The values of the genotypic variation coefficient, which is expressed as a percentage of the general average of the amount of genotypic variation, were relatively low for the variables studied in the *M. frondosus* seedlings. The coefficients of experimental variation was low for all characteristics studied, meaning good accuracy in the data collection.

There was a significant and positive correlation between all characteristics evaluated, except between the number of leaves and the shoot height:collar diameter ratio (data not shown). Among the characteristics studied, the shoot height showed high linear and significant correlation estimates at the 1% error probability level with the collar diameter (0.98), with the shoot height:collar diameter ratio (0.83), and with the number of leaves (0.91). A linear correlation analysis was also performed between the monthly evaluations of shoot height of *M. frondosus* progenies to study this characteristic throughout the experimental period (Figure 1). Correlation estimations between two evaluations considering evaluation at 30 days decreased until the fourth evaluation (third pair of correlation between first and fourth evaluations), when correlation estimations start being very similar between evaluations. The estimations of linear correlation decreased from r^2 = 0.94 (between first and second evaluations) to r^2 = 0.72 (between first and sixth evaluations). Less reduction among correlation estimations were found when data pairs were compared between the second (r^2 = 0.87), third (r^2 = 0.92), and fourth months after emergence (r^2 = 0.96) with the sixth month (180 days after emergence).

Table 1 - Summary of variance analysis for shoot height (cm), collar diameter (mm), shoot height/collar diameter ratio (H/CD), and number of leaves (Nº leaves) of progenies of *Myrocarpus frondosus* evaluated for 180 days after emergence in the nursery

Sources of variation	**Degrees of freedom**	**Medium squares**			
		Height	**Diameter**	**H/CD**	**Nº leaves**
Progenies (p)	6	214.8168*	5.0977*	19.4784*	23.9159*
Evaluation (a)	5	1031.8825*	53.5539*	6.6329*	408.3003*
Prog × Eval	30	8.1624*	0.5308*	1.9617*	23.3427*
Residue	1722	3.8513	0.1388	0.4366	4.9009
Mean		10.08	2.67	3.79	9.15
CV_g (%)[1]		9.07	5.24	7.25	3.00
CV_e (%)		19.46	13.92	17.42	24.19
CV_{rel}		0.4662	0.3760	0.4160	0.1241
h^2		0.982	0.973	0.977	0.795

* Significant by test F (p<0,05). [1] CV_g = genotypic coefficient of variation (%); CV_e = experimental coefficient of variation (%); CV_{rel} = relative coefficient of variation (CV_g/ CV_e); e h^2 = heritability.

The Scott-Knott test showed significant differences among the means of the *M. frondosus* progenies for all the characteristics evaluated (Table 2), and the averages could be classified into four groups for the height of the shoot, in three groups for the collar diameter and for the shoot height:collar diameter ratio and in two groups for the number of leaves. Despite the differences between the means of the progenies for shoot height (P5 with 11.66 and P1 with 8.68 cm), there was grouping of four progenies together and one progeny in each of the other groups. Based on these results and considering that the selection within progenies is more important, only the progeny with the lowest mean shoot height (P1) was discarded in the selection.

Figure 1 - Linear correlation between the monthly evaluations of shoot height of *Myrocarpus frondosus* progenies. For each case, the correlations were estimated between the respective evaluation with the subsequent ones up to 180 days (six months) after emergence

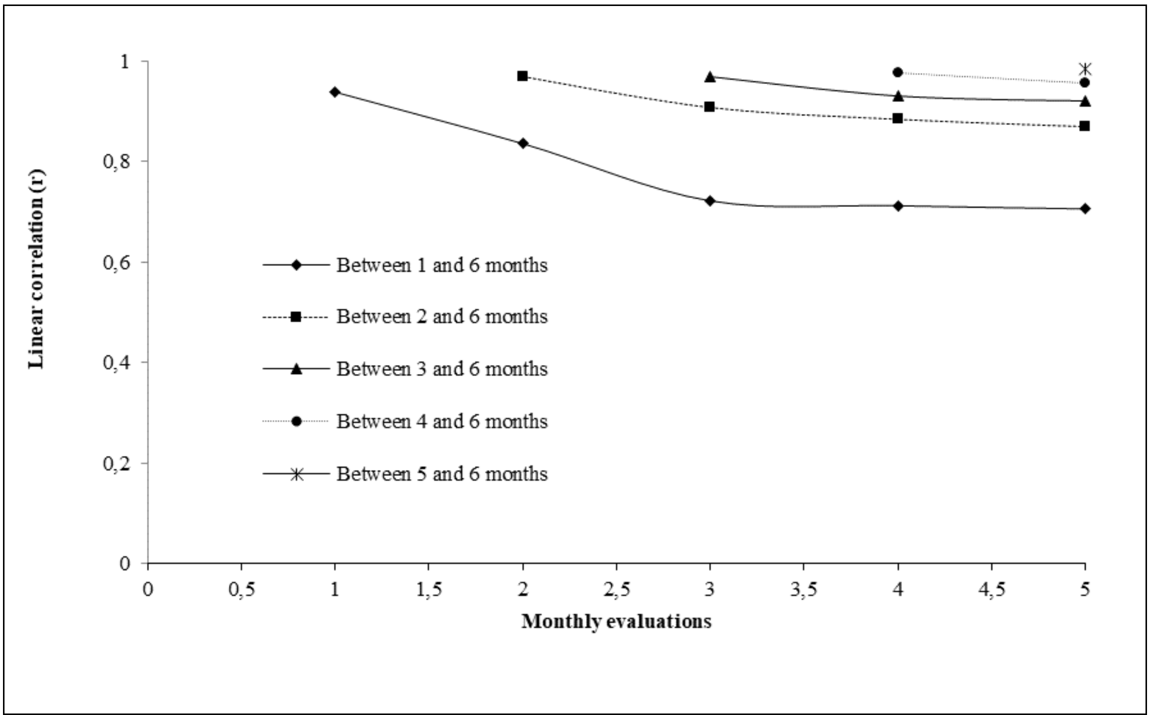

Table 2 – Means of *Myrocarpus frondosus* progenies for shoot height (cm), collar diameter (mm), height/collar diameter ratio (H/CD), and number of leaves (Nº leaves) evaluated for 180 days in the nursery

Progenies	Height	Diameter	H/CD	Nº leaves
P5	11.66 a*	2.79 a	4.21 a	9.44 a
P2	10.75 b	2.88 a	3.75 b	9.35 a
P7	10.13 c	2.74 a	3.70 b	9.17 a
P4	9.92 c	2.45 c	4.03 a	8.75 b
P6	9.78 c	2.61 b	3.76 b	9.45 a
P3	9.62 c	2.59 b	3.76 b	9.19 a
P1	8.68 d	2.65 b	3.33 c	8.71 b
Overall mean	10.08	2.67	3.79	9.15

* Progeny means followed by the same letter do not differ by the Scott-Knott test, at 5% probability of error

For the selection of the best seedlings within the selected progenies, the Scott-Knott test was initially performed to separate them into groups based on shoot height. The seedlings of the P4, P5, and P6 *M. frondosus* progenies were separated into two groups, and the plants were selected only from the highest height group, resulting in the selection of 18, 18, and 25 seedlings, respectively (Table 3). The seedlings of the P2 and P3 progenies were separated into four groups, and the P7 progeny into three groups, from which the plants belonging to the two groups of higher shoot height were selected.

Table 3 – Number and mean of selected seedlings of *Myrocarpus frondosus* progenies for shoot height (cm), collar diameter (mm), height/collar diameter ratio (H/CD) and number of leaves (Nº leaves) evaluated for 180 days in nursery

Progenies	Nº of plants selected	Height	Diameter	H/CD	Nº leaves
P2	13	13.07	3.04	4.31	10,18
P5	18	13.02	2.79	4.68	10,10
P7	12	12.47	2.89	4.29	9,94
P4	18	11.26	2.59	4.35	9,07
P3	19	11.16	2.72	4.16	9,79
P6	21	10.95	2.72	4.04	9,85
Overall mean of plants		11.87	2.77	4.30	9.80

Table 4 – Original mean (OM), mean of progenies or selected seedlings (MS), genetic gain (GG) and percentage (GS%) between progenies and within the best progenies of *Myrocarpus frondosus* for shoot height (cm), collar diameter (mm), height/collar diameter ratio (H/CD) and number of leaves (Nº leaves)

Characteristics	**OM**	**MS**	**GG**	**GG%**	**OM**	**MS**	**GG**	**GG%**
	Selection between progenies				**Selection within progenies**			
Height	10.08	10.30	0.22	2.18	10.31	11.87	1.56	15.13
Diameter	2.67	2.68	0.01	0.37	2.67	2.77	0.10	3.74
H/CD	3.79	3.87	0.08	2.11	3.86	4.30	0.44	11.40
Nº leaves	9.15	9.22	0.07	0.76	9.22	9.80	0.58	6.29

The selection of the best seedlings within the progenies resulted in a selection gain of 1.56 cm in height, which corresponds to a genetic gain of 15.13% (Table 4). For selection among progenies, the highest gain was also with the direct selection for shoot height (2.18%). The highest indirect selection gain was for shoot height:collar diameter ratio, both for progeny selection (2.11%) and seedling selection in the best progenies (11.40%). The collar diameter had the lowest indirect genetic gain of selection for progenies (0.37%) and for seedlings within progenies (3.74%).

4 DISCUSSION

The significant interaction between the progenies of *M. frondosus* and the different evaluations for all characteristics (Table 1) indicates that the progenies presented different behavior during the evaluation period, because of the interaction of the genotype with the nursery environment. In addition, both independent factors were also significant, indicating that the progenies of *M. frondosus* differed from each other, and also in relation to the behavior of the seedlings in nursery conditions. This result is relevant because the existence of genetic variability among seedlings is fundamental for genetic improvement, making it possible to obtain genetic gains with selection (Gouvêa et al., 2013), and in this case, also among the progenies studied (Costa et al., 2015b).

Even with relatively low coefficients of genotypic variation (Table 1), shoot height had the highest values of genotype variation coefficient, which indicates that it is possibly the most appropriate characteristic for the selection of progenies and seedlings within the progenies studied. On the contrary, the collar diameter had the lowest coefficient of genotype variation, which indicates that the selection is not very efficient for this characteristic in progenies of *M. frondosus*. The same trend was observed in seedlings of *Eremanthus erythropappus* (DC) MacLeish, in which the highest values of the genotype coefficient of variation were observed for shoot height (17.9%) and the lowest for the collar diameter (5.4%) (Silva et al., 2007). For seedlings of *Tectona grandis* L. f. at three months of age, the collar diameter had the highest coefficient of genotype variation (11.2%), followed by height (6.0%) (Costa et al., 2015a). For seedlings of *Ilex paraguariensis* St. Hil., the highest genotype coefficient of variation was for shoot height (27.2%), followed by number of leaves (23.7%) and collection time (15.1%), which were considered significant and suitable for plant selection (Costa et al., 2011).

All coefficients of experimental variation were higher than the coefficients of genotype variation (Table 1), resulting in coefficients of relative variation lower than unity, which was also observed in progenies of *Dipteryx alata* Vog. (Baru) with seven-month-old seedlings (Canuto et al., 2015). The heritability estimates were high and above 0.95 for all the evaluated characteristics, except for the number of leaves (0.79), which corroborates the possibility of obtaining genetic gain with the selection. The high magnitude of heritability also indicates that selection can be performed based on data from individual seedlings, which is important for the selection of the best genotypes in the progenies of *M. frondosus*.

The high estimates of positive correlation between all characteristics indicates that as the shoot height increases, a proportional increase of the other associated characteristics also occurs. Therefore, indirect genetic gains are expected for the collar diameter, the shoot height:collar diameter ratio, and the number of leaves with selection for shoot height of *M. frondosus* seedlings. This strategy maximizes the efficiency of the process since the selection will be performed only for one of the correlated characteristics (Cruz et al., 2004), besides being a characteristic of easy measurement in the nursery. These results confirm that linear correlation estimations can be used to define the characteristic to be used for plant selection (Olawuyi et al., 2014) and justifies its relatively recent application in breeding programs (Silva et al. 2016).

To infer about the best time for shoot height selection, a linear correlation analysis was performed between pairs of monthly evaluations (Figure 1). Correlations with the evaluation at 30 days (first month) were the ones that presented the lowest estimates and the largest reduction among all evaluations. This reduction may be related to differences in seedling emergence, which is expected between and within progenies of unimproved native species. This is also evident from the fact that estimates of linear correlation with shoot height observations at 60 days after emergence showed a lower variation with all other evaluations. In addition, all correlation estimates with data from second month of evaluation were higher than those obtained when shoot height data at 30 days after emergence were considered. Regardless of the time of evaluation considered to define the pair of linear correlations, all the estimates were affected, considering the evaluation data at 180 days after emergence (sixth month). Thus, in this experiment, the selection of progenies and seedlings within progenies can be performed based on the monthly evaluations of the shoot height of the *M. frondosus* seedlings obtained up to 180 days of nursery cultivation. However, these results indicated that in future experiments, the shoot height at 60 days of nursery cultivation (second month) could be used, considering the high correlation ($r^2 = 0.87$) estimated between the observations made at 60 and at 180 days of cultivation.

The Scott-Knott test separated the progeny means of all seedlings and evaluations for shoot height (Table 2), being selected six progenies of the tree best groups (Table 3). This strategy would allow maintenance of greater genetic variability among the selected seedlings within the progenies and, consequently, greater possibility of selection for other characteristics. The selection of the best seedlings within the selected progenies resulted in 101 plants (Table 3), corresponding to the selection of 34.35% seedlings, which may be considered adequate to maintain sufficient genetic variability for selection of other characteristics. The selection applied to shoot height within the progenies resulted in intensities ranging from 28.6% (P7) to 50% (P6). In spite of the smaller number of seedlings selected, P2 contributes to seedlings of mean shoot height similar to P5, which presented the highest progeny mean, demonstrating the superiority of some seedlings of the P2 progeny. Higher selection intensities were used with species of the genus *Eucalyptus*. For the estimation of gains from selection in *E. camaldulensis* Dehnh, a selection intensity of 15.15% among progenies and of 6.67% within the best progenies was used (Azevedo et al., 2015). Similarly, for progenies of *E. urophylla* S. T. Blake, 6.25% of the genotypes of the population were selected (Rosado et al., 2009).

The genetic gains estimated with direct selection for shoot height of 101 seedlings of *M. frondosus* were higher within the progenies (15.13%) than among the progenies (2.18%), raising the average from 10.31 cm to 11.87 cm in one cycle of selection (Table 4). It should be noted that the *M. frondosus* seedlings were evaluated in the nursery, being necessary evaluations to the field and also to other characteristics, which should result in high genetic gain considering the number of progenies and selected seedlings for growth and vigor.

The results of this research show that progenies of half-siblings of M. frondosus vary for shoot height, collar diameter, shoot height/collar diameter ratio, and leaf number. The shoot height is a characteristic that is easy to measure in the nursery, is non-destructive, and presents a high coefficient of genotype variation and positive correlation with the other characteristics evaluated, which allowed high gains from selection. In the genetic improvement of forest species, the selection of genotypes that combine the highest number of desirable characteristics is often hampered by the long cycles of growth characteristics of these native tree species, and in this case, genetic gains in height were accompanied by indirect gains for the collar diameter, shoot height/collar diameter ratio, and for the number of leaves. The use of only one characteristic for progeny evaluation maximizes the efficiency of the evaluation and selection processes. Selection for shoot height can be applied at 60 days of nursery cultivation, which also facilitates the selection process. Therefore, selection among and within half-sibling of M. frondosus progenies provides high genetic gains for the morphological characteristics of seedlings. Assessments at more advanced ages and in field conditions are necessary, allowing the definition of more appropriate strategies to be adopted in breeding programs. The selected seedlings can also be evaluated for competence to adventitious rooting, which is necessary for the development of M. frondosus clones.

5 CONCLUSIONS

Progenies of *Myrocarpus frondosus* can be selected for shoot height at 60 days of nursery cultivation, resulting in high direct genetic gain for growth and indirect gain for height/collar diameter ratio.

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