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FORESTRY SCIENCE

Selection of *Acacia mearnsii* for mini-cutting propagation

Denise Gazzana[1] **Renata Smith Avinio**[1] **Thaíse da Silva Tonetto**[1] **Angélica Costa Malheiros**[2] **Kelen Haygert Lencina**[3] **Dilson Antônio Bisognin**[4*]

[1]Programa de Pós-graduação em Engenharia Florestal, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
[2]Curso de Engenharia Florestal, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
[3]Departamento de Agricultura, Biodiversidade e Florestas, Universidade Federal de Santa Catarina (UFSC), Curitibanos, SC, Brasil.
[4]Departamento de Fitotecnia, Universidade Federal de Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brasil. E-mail: dilson.bisognin@ufsm.br.
*Corresponding author.

ABSTRACT: *Acacia mearnsii* De Wild. (Black wattle) is a forest species with great social and economical importance for the southern region of Brazil. This species has multifunctional characteristics, being cultivated to produce tannin, cellulose and energy. This research identified black wattle genotypes with competence for adventitious rooting for mini-cutting propagation. Single-bud mini-cuttings were treated with IBA at a concentration of 2000 mg L^{-1}. After 30 days of cultivation in a humid chamber, the mini-cuttings were evaluated for percentages of survival, rooting, number of roots, average length of the three largest roots, and the number of mini-cuttings rooted per mini-stump. The identification of the best genotypes was performed with the Selegen REML/BLUP software. Selection between and within black wattle progenies can be performed based on the number of rooted mini-cuttings, making it possible to select genotypes with high competence for adventitious rooting while maintaining genetic variability for new selection cycles to develop clones for vegetative propagation by mini-cutting.
Key words: black wattle, adventitious rooting, superior genotypes, genetic improvement, number of rooted mini-cuttings.

Seleção de *Acacia mearnsii* para a propagação por miniestaquia

RESUMO: *Acacia mearnsii* De Wild. (Acácia-negra) é uma espécie florestal de grande importância social e econômica para a região sul do Brasil. Essa espécie apresenta características multifuncionais, sendo cultivada para produção de tanino, celulose e energia. Este trabalho teve por objetivo identificar genótipos de acácia-negra com competência ao enraizamento adventício para a propagação por miniestaquia. Miniestacas de gema única foram tratadas com AIB na concentração de 2000 mg L^{-1}. Aos 30 dias de cultivo as miniestacas foram avaliadas quanto às porcentagens de sobrevivência e enraizamento, número e comprimento médio das três maiores raízes e número de miniestacas enraizadas por minicepa. A identificação dos melhores genótipos foi realizada com o auxílio do software Selegen REML/BLUP. A seleção entre e dentro de progênies de acácia-negra pode ser realizada para o número de miniestacas enraizadas, sendo possível selecionar genótipos com alta competência ao enraizamento adventício, mantendo variabilidade genética para novos ciclos de seleção, visando desenvolver clones para a propagação vegetativa por miniestaquia.
Palavras-chave: acácia-negra, enraizamento adventício, genótipos superiores, melhoramento genético, número de miniestacas enraizadas.

INTRODUCTION

Acacia mearnsii De Wild., popularly known as black wattle, is a forest species belonging to the Fabaceae family with great social and economical importance for the southern region of Brazil. Part of the relevance of this species is due to its multifunctional characteristic, being cultivated for the production of tannin, cellulose, and energy (DUIN et al., 2017; IKEDA et al., 2019) with good results both in monoculture and agroforestry systems, in addition to being linked to environmental services (ENGEL et al., 2017).

Black wattle plantations are carried out, for the most part, from seminal seedlings, which results in forests with high genetic variability and, generally, low productive potential (DISARZ & CORDER, 2009). In addition, the species has a high degree of seed dormancy, making germination difficult, a factor caused by the impermeability of the seed coat (ROVERSI et al., 2002). To circumvent the problems inherent in the seminal multiplication of species, vegetative propagation appears as an alternative in genetic improvement programs, mainly due to the effectiveness in capturing additive and non-additive genetic gains (OLIVEIRA et al., 2015). The genetic gains obtained in forest improvement programs have provided great advances for the brazilian forestry sector, which can be confirmed mainly by the increase in productivity and quality of new plantations (OLIVEIRA et al., 2013).

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Mini-cutting is a variation of cutting technique of vegetative propagation. Minicutting is considered the main technique for plantlet producing of forest species, as it enables the reduction of selection cycles, the establishment of more productive and uniform commercial plantations through the multiplication of superior genotypes (DIAS et al., 2012; XAVIER et al., 2013). However, the success in the production of plantlets by mini-cutting is intrinsically linked to the development of an adequate root system (SÁ et al., 2018), which in turn depends on several factors, ranging from propagation conditions to characteristics of the genotype and species.

With regard to black wattle, this technique still has limitations in the adventitious rooting rates of superior genotypes and; although, some studies on vegetative propagation have been developed, there is still a lack of efficient cloning protocols (ENGEL et al., 2017, 2019; IKEDA et al., 2019). Thus, further studies are needed with a focus on adventitious rooting of mini-cuttings (DIAS et al., 2012), a fact that has limited the success of genetic improvement of the species for vegetative propagation (ENGEL et al., 2019).

In this context, the early selection of genotypes with competence for adventitious rooting within forestry improvement programs can greatly contribute to further reducing the selection time, as it would circumvent the problem of maintaining the materials recalcitrant to vegetative propagation (OLIVEIRA et al., 2015). This can be done by selecting genotypes in improved populations or elite individuals due to their characteristics associated with vegetative propagation. However, the genetic gains arising from the use of this strategy depend on the heritability of such characters, such as the competence for rooting the evaluated genotypes (OLIVEIRA et al., 2015). In addition, carrying out progeny tests is an efficient strategy for genotype selection, which allows the determination of the genetic value of individuals and the estimation of genetic parameters and gain by selection (GAZZANA et al., 2020). Among the estimated genetic parameters, the most relevant and most used for studies in progeny tests are genetic variances, coefficients of variation, heritability in the broad and narrow sense, and genetic gain (BORÉM et al., 2017).

When working with selection between and within half-sib progenies, the number of rooted mini-cuttings has been considered adequate for the selection of genotypes with competence for adventitious rooting. The number of rooted mini-cuttings per mini-stump combines the productivity of mini-cuttings per mini-stump and the percentage of adventitious rooting; being therefore, indicative of the number of plantlets produced per mini-stump and necessarily considered for the selection of genotypes with satisfactory mass production of plantlets by mini-cutting (PIMENTEL et al., 2019). The number of rooted mini-cuttings per mini-stump was considered the best character for genotype selection for vegetative propagation by mini-cutting in species such as *Ilex paraguariensis* St. Hil. (PIMENTEL et al., 2019; GAZZANA et al., 2020) and *Cabralea canjerana* (Vell.) Mart. (BURIN et al., 2018), resulting in high genetic selection gain for adventitious rooting competence (BURIN et al., 2018; GAZZANA et al., 2020). Thus, early selection based on characters related to the competence for adventitious rooting is of paramount importance in forest improvement programs, as it allows greater selection gain for these characters (OLIVEIRA et al., 2015; GAZZANA et al., 2020).

The present study identified black wattle genotypes with competence for adventitious rooting for mini-cutting propagation.

MATERIALS AND METHODS

Thirteen black wattle half-sib progenies were evaluated. From each progeny, 200 seeds were sown in 50 cm³ polypropylene tubes, containing equal proportions of commercial substrate based on peat and medium-grain vermiculite in April 2019. The seedlings remained in the nursery of the Tanagro company, located in the city of Triunfo - RS until October, when they reached approximately 25 cm in height and the basal diameter grew close to 2.0 mm. The 51 most vigorous seedlings of each progeny, according to the highest values of height and diameter, were selected and used for the establishment of a mini-clonal garden in an acclimatized greenhouse at the Breeding and Vegetative Propagation Center (MPVP) of the Plant Science Department of Universidade Federal de Santa Maria (UFSM), Santa Maria (RS), Brazil. The clonal mini-garden was established in a closed soilless cultivation system, with coarse sand as substrate, as described in GAZZANA et al. (2020). The nutrient solution was supplied to the culture bed by flood fertigation twice a day, for 15 minutes at a flow of 8.67 L min^{-1}, with the following composition (in mg L^{-1}): 117.0 N in the form of nitrate; 15.75 N in the form of ammonium; 14.63 of P; 131.62 of K; 84.0 of Ca; 25.21 Mg; 73.28 of S; 0.01 of B; 0.02 Cu; 69.73 Fe; 0.03 Mn; 0.008 of Zn; and 0.0016 Mo. The pH of the solution was kept between 5.5 and 5.8, and the electrical conductivity at 1.5 dS m^{-1}, both adjusted weekly.

About 30 days after planting, seedlings were first pruned for apical dormancy breaking and inducing growth of lateral shoots (Figure 1a). The first formation pruning was carried out in the form of a bevel, about one centimeter above two buds that had well-developed leaves. Approximately 30 days after pruning to form the mini-stumps, the shoots formed above the first bud were collected (Figure 1b). After that, three consecutive collections of mini-cuttings were performed at approximately 30-day intervals, being in January, February, and March 2020. The collected shoots were sectioned in single-bud mini-cuttings and the leaf area was reduced to 50% of the original surface (Figure 1c). In each collection, the number of mini-cuttings produced per mini-stump was counted.

The mini-cuttings were treated with a hydroalcoholic solution (1:1 v/v) of indolebutyric acid (IBA) at the concentration of 2,000 mg L^{-1} for 10 s and cultivated in 100-well polyethylene trays containing equal proportions of pine bark substrate, medium vermiculite and coarse-grained sand (Figure 1d). The mini-cuttings were kept in a humidity chamber with a relative humidity above 95%, maintained by automated nebulization.

After 30 days of cultivation in the humidity chamber, the mini-cuttings were evaluated for survival and rooting percentages, the number of roots, the average length of the three largest roots (cm), and the number of mini-cuttings rooted per mini-stump. Mini-cuttings were considered rooted when they had at least one root with a length equal to or greater than 0.1 cm (Figure 1e).

The assumption of data normality was verified by the Shapiro-Wilk test ($P > 0.05$) and the homogeneity of variance by the Bartlett test ($P > 0.05$). Subsequently, Pearson's correlation analysis was performed between the characters evaluated, aiming to identify the characters with the greatest influence on mini-cutting collections. For these analyses, the Action Stat software was used (EQUIPE ESTATCAMP, 2014).

The variance components were estimated by the restricted maximum likelihood method (REML) and the prediction of phenotypic and genotypic values by the best linear unbiased prediction (BLUP) (RESENDE, 2002). Statistical model 82 was used, which corresponds to the model for evaluating individuals in half-sib progenies in one place, one plant per plot, in a completely randomized design, with the aid of the Selegen REML/BLUP software (RESENDE, 2016). The statistical model is expressed by: $y = Xu + Za + e$, where: y is the data vector; u is the vector of the effects of measurement-repetition combinations (assumed to be fixed) added to the overall mean, a is the vector of individual additive genetic effects (assumed to be random), and e is the vector of errors or residuals (random). Capital letters represent incidence matrices for these purposes.

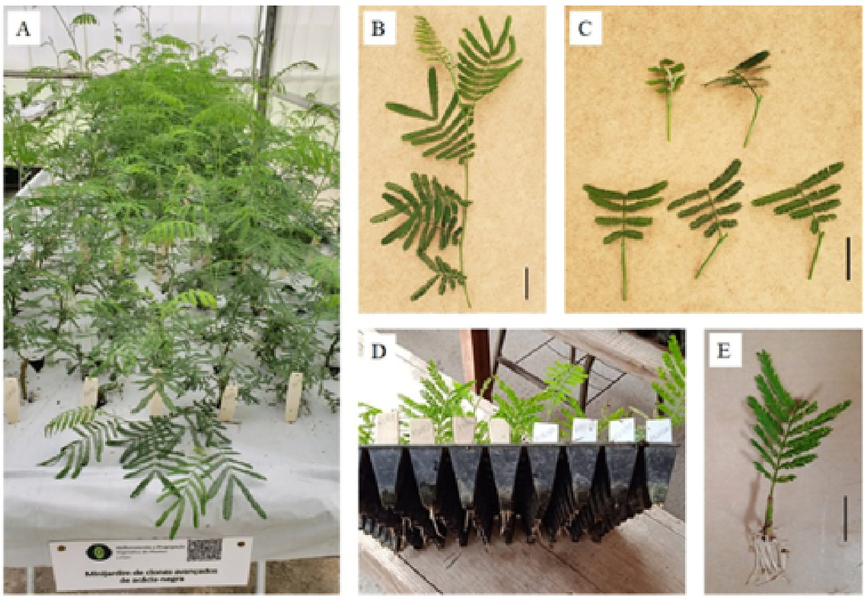

Figure 1 - Black wattle mini-stumps established in a mini-clonal garden (A), collected shoot (B), single-bud mini-cuttings (C), mini-cutting cultivated in 100-well polyethylene trays (D), and rooted mini-cutting (E). Bar = 3cm.

The average components (individual BLUPs), based on the permanent phenotypic effect, were obtained in order to classify and identify superior genotypes for vegetative propagation. The selection was carried out between and within progenies for the most representative trait, considering the correlation estimates and genetic parameters, being selected those genotypes that provided gain in relation to the general mean. In addition, the selection gain in percentage was calculated using the formula: G (%) = [(MM – MO) / MO] x 100, where G (%) is the selection gain in percentage, MM a improved mean, and MO the observed mean.

RESULTS AND DISCUSSIONS

Pearson's correlation analysis was significant (P < 0.05) and positive among all characters, except between the number of mini-cuttings produced and the percentages of survival and rooting and the number of roots (Table 1). Correlation estimates ranged between 0.133 and 0.852, being considered between low and high magnitude, respectively (RESENDE, 2002). The highest positive linear correlation estimates were observed between survival and rooting percentages and between rooting percentage and number of mini-cuttings rooted. The latter still showed a moderate correlation with survival. These results showed that the characters vary in the same direction, which denotes dependence between them (CRUZ et al., 2004), as well as showing the relevance of these three characters for the selection of black wattle for mini-cutting propagation. In addition, such responses may have occurred due to the rooted vegetative propagules having a greater capacity to absorb nutrients and water from the substrate, reducing mortality throughout the period of cultivation in the humidity chamber (GAZZANA et al., 2020). These results are in agreement with those obtained with mini-cuttings of *Eucalyptus benthamii* Maiden & Cambage x *E. dunnii* Maiden, in which the mini-cutting survival and rooting also presented a high and positive correlation estimate (BRONDANI et al., 2009).

It is still possible to consider two important aspects regarding the linear correlation. The first is the obtainment of high and positive estimates among the characters regarding adventitious rooting of mini-cuttings. These results are in agreement with those obtained with *Prunus persica* L. Batsch genotypes, in which high positive correlations were observed between rooting, mean number, and length of roots (TIMM et al., 2015; OLIVEIRA, 2018), and *Cabralea canjerana*, in which positive and high magnitude correlations were observed between the percentage of rooting and the number and average length of roots (BURIN et al., 2018). The second aspect to consider is that the number of mini-cuttings rooted is positively correlated with the number of mini-cuttings produced and with all other characters associated with rooting (Table 1). These results indicated that the number of rooted mini-cuttings is an extremely important character, as it defines the potential multiplication rate of a clone and; consequently, the potential number of plantlets produced (BURIN et al., 2018).

The number of rooted mini-cuttings is very important when the objective is to maximize the multiplication rate and; consequently, increase plantlet productivity. In addition, knowing the number of rooted mini-cuttings allows for the operationalization of expedition activities and planting of plantlets in the field. Thus, these results allowed us to infer that the number of rooted mini-cuttings is the most suitable character to carry out the selection between and within black wattle progenies. These results are in agreement with those observed in *Cabralea canjerana* (BURIN et al., 2018) and progenies of *Ilex paraguariensis* half-sib progenies (GAZZANA et al., 2020), in which the selection should be performed based on the number of mini-cuttings rooted throughout the vegetative growth period.

In addition to the need to identify the most suitable character to carry out the selection, the interpretation of genetic progress and estimates of genetic parameters is also essential to identify the nature of genes involved in controlling quantitative traits. Furthermore, it makes possible to evaluate the selection efficiency of different breeding strategies, seeking to maximize genetic gain and maintain the variability necessary for new selective cycles (PIRES et al., 2014; LI et al., 2017). This is because through the estimation of genetic parameters in the forest improvement process to predict the selection gain and the genetic value of individuals, in addition to the heritability, which quantifies the fraction of phenotypic variance of heritable nature and capable of being exploited in the selection (RESENDE, 2002).

Individual heritability in the narrow sense (h^2a) quantifies the relative importance of the additive proportion of genetic variance that can be transmitted to the next generation (RESENDE, 2002). Values below 0.15 are considered of low magnitude, according to Resende's classification (2016). These values were obtained from the number of mini-cuttings produced, the percentage of survival, and the average length of the three largest roots (Table 2). This indicates that little genetic gain is expected by selection between and within progenies for these traits in terms of additive

Table 1 - Pearson correlation matrix between characters, number of mini-cuttings produced (PROD), percentage of survival (SOBR), percentage of rooting (ENR), number of roots (NR), average length of the three largest roots (MC3MR), and number of rooted mini-cuttings (NME) in black wattle mini-cuttings.

Characters	--------PROD--------	---------SOBR--------	---------ENR---------	----------NR----------	-------MC3MR-------
SOBR	-0.032^{ns}				
ENR	-0.011^{ns}	0.852*			
NR	0.034^{ns}	0.368*	0.455*		
MC3MR	0.133*	0.438*	0.465*	0.347*	
NME	0.463*	0.658*	0.775*	0.394*	0.422*

^{ns}not significant and *significant at 5% probability of error, by Student's t test.

variance, since low values indicate that genetic factors contributed little to the expression of the phenotype and; therefore, the greater the difficulty for obtaining genetic gains with selection (HUNG et al., 2015).

Conversely, when moderate to high heritability values are observed, greater genetic gain is expected by selection for a particular character (ZARUMA et al., 2015; CANUTO et al., 2016). This is expected as a result of the selection for the number of mini-cuttings rooted per mini-stump, a character that presented the highest heritability estimates (Table 2), corroborating the considerations regarding the correlation between the characters. According to Henriques et al. (2017), the magnitude of its value indicates good genetic control in its expression and shows high potential for selection, with prospects of obtaining satisfactory genetic gains. Similar results were observed among *Prunus persica* genotypes, with low to moderate estimates of heritability for the percentage of rooting, the number of roots, and the average length of roots (OLIVEIRA, 2018).

As for the individual additive genetic variation coefficient (CVgi), which expresses the percentage of additive genetic variation existing within the progeny, it was reported that all characters showed variability among the evaluated plants, which enables selection. This is due to the fact that values above 10% are sufficient to practice effective selection of genotypes (RESENDE, 2002), and the higher this value, the greater the ease of finding superior individuals that can provide gains from selection (AGUIAR et al., 2010). It is also noteworthy that the main advantage of the coefficient of variation is to enable the quantification and weighting of the proportion of variation that exists, due to environmental and genetic factors, as well as the proportion that will be inherited in the next generation (KAMPA et al., 2020). In this sense, the number of mini-cuttings rooted per mini-stump (CVgi = 20.76%) stands out as the highest value observed when compared to other characters related to rooting (Table 2).

Regarding selective accuracy (Acgen), which is associated with selection accuracy and refers to the correlation between predicted genetic values and true genetic values of individuals, low to moderate values were found for all characters, according to the classification of RESENDE (2002), being the highest

Table 2 - Estimates of genetic parameters for the number of produced mini-cuttings per mini-stump (CN), percentage of mini-cutting survival (S%), percentage of rooting (R%), number of roots (RN), mean length of the three largest roots (RL cm), and number of rooted mini-cuttings (CNR) in black whattle half-sibling progenies.

Parameters[1]	-------CN-------	------S(%)------	-----R(%)-----	-------RN-------	----RL(cm)----	-----CNR-----
h^2a	0.076	0.110	0.377	0.259	0.078	0.404
CVgi%	11.745	13.815	18.224	18.414	17.592	20.759
Acgen	0.243	0.114	0.216	0.110	0.088	0.420
Average[2]	6.017	44.975	34.601	3.385	1.231	2.080

[1]h^2a: individual heritability in the narrow sense, that is, of additive effects; CVgi%: individual additive genetic variation coefficient; Acgen: accuracy in progeny selection; and overall average.
[2]Overall average of each of the characters observed, without transformation.

value observed for the number of rooted mini-cuttings. The greater the selective accuracy in the assessment of an individual, the greater the confidence in the assessment, and in the predicted genetic value for the individual. Thus, enabling greater gains with selection (RESENDE, 2002; BORÉM et al., 2017). According to accuracy, the number of rooted mini-cuttings is the most suitable character to practice selection, corroborating the results obtained with linear correlation.

Based on the information obtained in this study, the selection of the best genotypes within the progenies was performed based on the number of rooted mini-cuttings (Table 3). The ordering of individuals by their genetic values related to rooting is important for the selection of genotypes intended for propagation by mini-cuttings (PIRES et al., 2017). Thus, the estimated genetic gains with the selection of the best individuals can be obtained for the number of mini-cuttings rooted through the cloning of genotypes with high competence for adventitious rooting (BURIN et al., 2018; GAZZANA et al., 2020).

The selection of genotypes in the different progenies evaluated, carried out with the aid of mixed models, presents an optimal property and maximizes genetic gain. However, special attention must be paid to the number of selected progenies and individuals, as the selection of few individuals can lead to the early elimination of important alleles, although it increases genetic gain (PAGLIARINI et al., 2016). In this sense, it is necessary to reconcile genetic gains with the maintenance of variability through the construction of scenarios that will optimize the selection process, such as those carried out in this research, where 72 genotypes from 12 progenies were selected (Table 3). For this, the genotypes were classified according to the predicted genotypic value (g) and revealed that only the 19SM102B progeny did not contribute with any selected genotype. The number of genotypes selected in each progeny ranged from 2 to 16, which corresponds to a selection intensity within progenies between 3.9% and 31.4% and an average selection intensity of 10.9%.

The number of selected genotypes can be considered adequate to maintain sufficient genetic variability for selection of other characters in the subsequent generations of clonal selection. These values are similar to those obtained with *Bactris gasipaes* Kunth with an average intensity of 10% (BORGES et al., 2017), and for *Eucalyptus camaldulensis*, with selection intensities of 6.67% within progenies (AZEVEDO et al., 2015). The decision regarding which selection intensity should be applied to each genetic material is essential for maximizing genetic gains, since at higher intensities, the genetic gain will also be greater. However, the use of high selection intensity can negatively affect genetic gain in new selection cycles by drastically reducing genetic variability (STURION et al., 2017).

The selection based on the number of rooted mini-cuttings was essential to identify the genotypes with greater competence for adventitious rooting during the vegetative growth period of the mini-stumps (Table 3), since this parameter varies within the progenies of half-sib black wattle (Figure 2).

Table 3 - Number of selected genotypes and new mean of black wattle half-sib progenies, with the respective improved mean of all selected genotypes, original mean and genetic gain in percentage for mini-cutting productivity per mini-stump (CN), percentage of mini-cutting survival (S), rooting percentage (R), number of roots (RN), average length of the three largest roots (RL), and number of rooted mini-cuttings (CNR) in three samples collected at 30 days of cultivation in wet chamber.

Progenies	Nº of selected genotypes	-----CN-----	----S (%)----	----R (%)----	-----RN-----	--RL (cm)--	----CNR----
19SMA18	16	6.330	48.006	38.451	3.710	1.305	2.423
19SMA5	14	6.350	48.096	38.169	3.642	1.320	2.414
19SM231A	9	6.374	48.059	38.338	3.736	1.320	2.456
19SMA14	7	6.380	47.187	37.317	3.646	1.320	2.345
19SM852A	6	6.442	48.034	38.820	3.590	1.288	2.456
19SM405C	5	6.158	49.020	38.918	3.774	1.302	2.334
19SM782E	4	6.282	48.270	37.871	3.732	1.395	2.350
19SMA10	3	6.347	47.890	37.835	3.519	1.288	2.388
19SMJCCS	2	6.263	48.002	38.052	3.660	1.286	2.385
19SMA17	2	6.240	47.187	37.380	3.564	1.316	2.327
19SM852I	2	6.280	47.755	37.524	3.637	1.304	2.308
19SM852G	2	6.285	47.860	37.650	3.526	1.327	2.301
Improved mean		6.311	47.947	38.027	3.645	1.314	2.374
Original mean		6.017	44.975	34.601	3.385	1.231	2.080
Genetic gain (%)		4.89	6.61	9.90	7.68	6.76	14.13

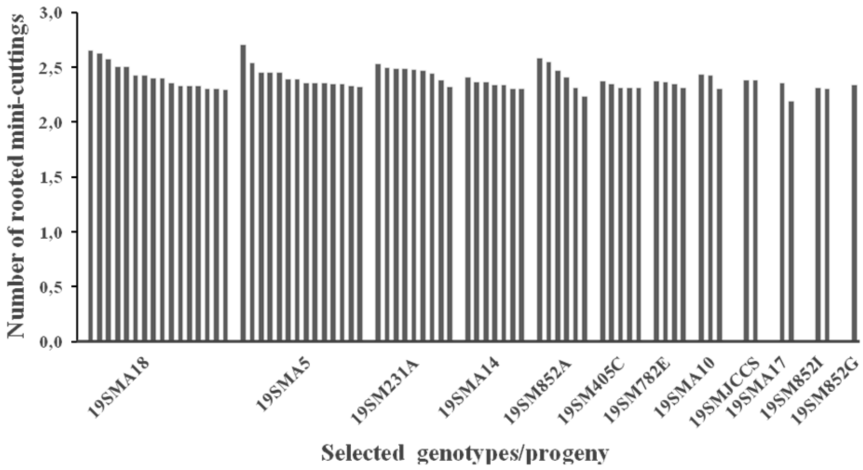

Figure 2 - Number of rooted mini-cuttings per mini-stump of 72 selected genotypes from twelve half-sib progenies of black wattle.

Considering all 72 selected genotypes, the number of rooted mini-cuttings per mini-stump varied between 2.71 to 2.19 (Figure 2). As expected, the greatest genetic gain was observed in the number of mini-cuttings rooted per mini-stump (14.13%) (Table 3), due to the fact that the selection was carried out for this character and presented a higher heritability value. For other characters related to rooting, the indirect gains with selection were of lesser magnitude, which also confirms the values reported for correlation and estimates of genetic parameters. This genetic improvement strategy has been tested in other perennial species and resulted in genetic gain values similar to those observed in this study, such as for *Prunus persica* genotypes in which the selection gains were 9.1, 17.5, and 24.0% for rooting, number of roots, and average length of the three largest roots, respectively (OLIVEIRA, 2018), and for *Myracrodruon urundeuva* Allemão in which the selection of individuals provided a gain of 7.5% (PUPIN et al., 2017). Furthermore, studies with *Ilex paraguariensis* and *Cabralea canjerana* observed gain values reaching 48.3 and 79.1%, respectively, when the selection for the number of mini-cuttings rooted per mini-stump was performed (BURIN et al., 2018; GAZZANA et al., 2020), which illustrates the potential of the improvement strategy for vegetative propagation that was applied in the present study and provided promising results. This places selection for adventitious rooting as the first step when one seeks clones with good development both in the nursery and in the field. Therefore, early selection for adventitious rooting competence for mini-cuttings propagation of black wattle can be performed based on the number of rooted mini-cuttings, with adequate genetic gains and desirable genetic variability for new cycles of selection aiming the development of clones for vegetative propagation.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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