ARTICLE

Selection between and within half-sibling progenies of *Ilex paraguariensis* for adventitious rooting of mini-cuttings

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Abstract: *This study aimed to evaluate half-sibling progenies for developing breeding strategies and to select mate clones with adventitious root competence, required for vegetative propagation by mini-cuttings. For rooting, single-bud mini-cuttings of 2 cm length were treated with indolebutyric acid at 2000 mg L^{-1}. At 60 days of cultivation, the mini-cuttings were evaluated for percentages of survival and rooting, number and mean length of the three largest roots, and number of rooted mini-cuttings per mini-stump. These data were analyzed considering the mixed model described for a completely randomized design, an environment, half-sibling progenies, and a single plant per plot, by using the SELEGEN-REML/BLUP software. The 50 genotypes with the highest number of rooted mini-cuttings were selected. The genetic gain was 48.41% for the number of rooted mini-cuttings per mini-stump. Selection for adventitious rooting based on the number of rooted mini-cuttings can be used in mate breeding programs for vegetative propagation by mini-cuttings.*

Keywords: *Breeding; vegetative propagation; adventitious rooting; cloning.*

INTRODUCTION

Mate (*Ilex paraguariensis* A. St.-Hil.), belonging to the Aquifoliaceae family and native to the mixed ombrophilous forest (Dartora et al. 2013, Bergottini et al. 2015, Barbosa et al. 2020), presents socioeconomic, environmental, and cultural prominence in the southern areas of Brazil, Paraguay, Argentina, and Uruguay (Schmitt et al. 2018). Its leaves and thin branches are the main products marketed, mainly as raw material for the manufacture of teas and beverages (Santin et al. 2019). Mate is consumed in the form of energy drinks in California, and sold in combination with other herbs, as an energetic or weight loss tea, in Europe (Bracesco et al. 2011). Cardozo Junior and Morand (2016) reported an increase in consumption of products derived from mate in several countries such as Italy, Spain, United States, France, Korea, Australia, Japan, Germany, Russia, and Syria. At present, this species is gaining importance in the pharmaceutical, therapeutic, and nutraceutical industries (Dallabrida et al. 2016), because of its antioxidant, anti-inflammatory, antimutagenic, and hypolipidemic activities (Bracesco et al. 2011).

The demand of the mate market can be met by guaranteeing the continuous production of raw material. One of the factors directly influencing yield and quality of the produced raw materials is the genetic origin and good development of seedlings used for the establishment of new plantations. Thus, the production of seedlings can

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be considered as one of the most important factors and has direct effects on the productivity and quality of the final product in the mate chain (Santin et al. 2015). However, the expansion of the commercial mate plantations has been limited by the difficulties related to its propagation, which can be seminal (seedlings) or clonal (plantlet production). Seedling production is limited by low germination rate, mostly caused by embryo immaturity and dormancy of the tegument (Wendling and Santin 2015), and by using non-improved seeds that result in low-quality seedings (Pimentel et al. 2017). Thus, an alternative to assist in the multiplication of the species is the direct selection for characteristics related to propagation of improved populations or elite individuals, in order to explore the potential for cloning these genotypes. In addition, the vegetative propagation of superior individuals allows the multiplication of the best genotypes and the production of plants genetically identical to the stock one, contributing to the uniformity of populations (Wendling and Santin 2015). That is, genetic improvement is indispensable to obtain gains in productivity, homogeneity, and quality of the produced plantlet, through cloning of genetically superior individuals (Brondani et al. 2018).

Single-bud mini-cutting is an alternative technique for the production of high-quality plantlets of mate (Pimentel et al. 2020), but cloning genetically superior genotypes is still a challenge, because of the low rooting rate of vegetative propagules (Wendling and Santin 2015). Adventitious rooting formation is crucial for mini-cutting survival and is a critical and essential process for vegetative propagation. Adventitious rooting is a complex, genetically controlled, and environmentally affected process, involving phytohormone reprograming and differentiation of specific cells of its base, with coordinated resource reallocation of the entire mini-cutting (Druege et al. 2019). The formation of adventitious roots is an especially complex process in woody species (Negishi et al. 2014), which is genotype-dependent and is regulated by multiple endogenous factors, such as genetic characteristics and propagule quality, including phytohormones, and environmental factors (Zhang et al. 2016). Thus, identifying the factors that alter the formation of roots is important for the success or failure of plantlet production via mini-cuttings, which depends on adventitious rooting (Cunha et al. 2009).

Breeding for vegetative propagation involves few possibilities for genetic recombination by sexual reproduction and many generations of selection for different traits and vegetative propagation (Bisognin 2011). Genetic tests assist in the selection of genotypes with greater competence for mini-cutting rooting, among which, progeny tests allow the estimation of genetic parameters between and within families and assessment of gains through selection. However, the genetic gains resulting from the use of this selection strategy depend on the heritability of the vegetative propagation-associated traits (Oliveira et al. 2015) used for the identification of superior genotypes for cloning. Although adventitious rooting competence is essential for vegetative propagation of mate, information on the use of any associated trait in the selection of superior genotypes for plantlet production by mini-cuttings is lacking. This information is still more relevant for mate, since it is considered a recalcitrant species for vegetative propagation, because its ability to form adventitious roots in vegetative propagules is very restricted, and the selection of superior genotypes may enable the vegetative propagation of this species.

Since mini-cuttings is a highly feasible technique for vegetative propagation of native tree species, especially when propagules are produced in mini-stumps of seedlings or young plantlets, because mini-cuttings are more responsive for adventitious rooting (Dias et al. 2012), as shown for mate (Pimentel et al. 2019, Pimentel et al. 2020), this study aimed to evaluate half-sibling progenies for developing breeding strategies and to select mate clones with adventitious root competence, required for vegetative propagation by mini-cuttings.

MATERIAL AND METHODS

The experiments were performed in a climatized greenhouse and in a humidity chamber at the Center of Plant Breeding and Vegetative Propagation (MPVP) of the Plant Science Department of the Federal University of Santa Maria (UFSM), Santa Maria (RS), Brazil. The mini-clonal garden was established in a closed soilless cultivation system by using seedlings of seminal origin of six mate half-sibling progenies, named 17SM1, 17SM3, 17SM4, 17SM7, 17SMLZ, and 17SMJS. The stock plants are centenary native individuals selected for seedling production and marketing, based upon progeny uniformity, seedling quality, leaf and branches yield, and commercial quality of established plantations. In the clonal mini-garden, 50 seedlings (genotypes) of each progeny were planted in a row, with 10 cm spacing between seedlings and rows.

The nutrient solution, consisting of macro- and micronutrients, was supplied twice a day for 15 min at a flow rate of 8.67 L min^{-1}; the solution contained the following macronutrients (mmol L^{-1}): 6.13 potassium nitrate, 10.0 calcium nitrate, 16.78 magnesium sulfate, 1.44 ammonium nitrate, 1.39 potassium monophosphate, and 1.68 iron. The micronutrients were added

to the nutrient solution (7.5 mL) as a previously prepared mixture containing (mmol L^{-1}) 0.15 sodium molybdate, 0.89 boric acid, 1.25 copper sulfate, 1.23 manganese sulfate, and 0.28 zinc sulfate. The nutrient solution was distributed in the cultivation bed by using a submerged motor pump, equipped with a timer, until the substrate formed by coarse sand was completely soaked. The pH of the nutrient solution was maintained between 5.5 and 5.8 and the electrical conductivity at 1.5 dS m^{-1}; both were weekly adjusted.

After a period of adaptation of the seedlings, the plants were drastically pruned to promote the overcoming of the apical dominance of the mini-stumps and induce the growth and development of lateral shoots. In each mini-stump, two pairs of leaves were retained to guarantee their photosynthetic activity. The produced shoots were collected and sectioned to single-bud mini-cuttings of 2 cm length, with leaf and area reduced to 50% of the original surface. The mini-cuttings were treated with hydroalcoholic solution (1:1 v/v) of indolebutyric acid at 2000 mg L^{-1}; they were grown in 100-well polyethylene trays containing an equal proportion of commercial substrate of pine bark-based medium, vermiculite, and coarse-grained sand (Pimentel et al. 2017). The trays were kept in a humidity chamber with a relative humidity of approximately 85%, provided by automated nebulization 8 times a day for 1 min. During each collection, the number of mini-cuttings produced per mini-stump was recorded to obtain the productivity of the mini-cuttings per mini-stump.

At 60 days of cultivation in the humidity chamber, mini-cuttings were evaluated for the percentages of survival and rooting (over total mini-cuttings), number of roots, mean 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 adventitious root of 0.1 cm or more in length. Four consecutive collections were performed in December of 2017 and February, May, and September of 2018, in periods ranging from 75 to 120 days, according to the availability of shoots in the mini-stumps.

The experiment had a completely random design, with 4 replications (collection times) and number of mini-cuttings ranging from 1 to 43, depending on the mini-cutting productivity per mini-stump. The assumption of data normality and homogeneity of variance were verified using the Shapiro–Wilk test ($p > 0.05$) and Bartlett test ($p > 0.05$), respectively, by using Action Stat software. The percentage, number, and length data that did not meet the assumptions of normal error and homogeneity of variances were transformed using Box–Cox and $\sqrt{(x + 0.5)}$, respectively. Subsequently, Action software was used to perform Pearson's correlation analysis among the evaluated traits, and the significance was verified using Student's t-test, at 5% probability of error.

The estimation of the variance components and the prediction of genetic values were performed using the procedure of maximum restricted likelihood/best impartial linear predictors (REML/BLUP), by using the SELEGEN–REML/BLUP (Statistical System and Computerized Genetic Selection) software (Resende 2016). For the evaluation of the individuals, the characters were analyzed using the mixed model, described for a completely randomized design, an environment, half-sibling progenies, and single plant per plot. The selection was performed between and within progenies for the 50 best evaluated genotypes for adventitious rooting competence, since this trait has greater importance for the mass production of plantlets by mini-cuttings, considering the number of rooted mini-cuttings per mini-stump. Resende and Barbosa (2005) stated that the best 50 genotypes are those having an adequate size to contain superior plants, when general selection is desired. According to the authors, when the number of genotypes is increased to above 50, many mean individuals are added, which contributes little to the selection. The selection gain in percentage with the best 50 genotypes was calculated using the formula: $G (\%) = \dfrac{MM - MO}{MO} * 100$, where $G (\%)$ is the selection gain in percentage; MM, improved mean; and MO, original mean observed.

RESULTS AND DISCUSSION

In this study, significant ($p < 0.05$) and positive correlations were noted between the mini-cutting survival from mate half-sibling progenies and the rooting percentage (0.61; Table 1). This positive relationship was also observed in the rooting of cuttings of *Averrhoa carambola* L. (Bastos et al. 2005) and in *Cabralea canjerana* clones (Burin et al. 2020). This result can be explained by the fact that rooted mini-cuttings have greater capacity for absorbing water and nutrients from the substrate, which reduces the mortality over the period of cultivation in a humidity chamber. In addition, mini-cuttings with a greater number of roots have higher survival rates, since a well-formed root system facilitates greater initial plantlet growth and plant development when transplanted in the field, providing better fixation and reducing mortality (Dias et al. 2012, Timm et al. 2015).

Significant and positive correlation was also observed between the number of rooted mini-cuttings per mini-stump and the number of mini-cuttings produced, the percentages of survival and rooting, the number of roots, and the mean length of the

Table 1. Pearson's correlation matrix among number of produced mini-cuttings per mini-stump (CN), percentage of mini-cutting survival (S, %), percentage of rooting (R, %), number of roots per mini-cutting (RN), mean length of the three largest roots (RL, in cm), and number of rooted mini-cuttings (CNR) of four consecutive collections in mate half-sibling progenies

Variables	CN	S (%)	R (%)	RN	RL (cm)
S (%)	-0.08[*]				
R (%)	0.00[*]	0.61[**]			
RN	0.15[**]	0.24[**]	0.61[**]		
RL (cm)	0.02[*]	0.09[*]	0.50[**]	0.67[**]	
CNR	0.63[**]	0.39[**]	0.67[**]	0.53[**]	0.34[**]

* Not significant and ** significant at 5% probability of error, by Student's *t*-test.

three largest roots (Table 1), indicating a linear relationship between the productivity of mini-cuttings and adventitious rooting competence in mate half-sibling progenies. The mini-stumps capable of producing vigorous shoots could also contribute to the greater productivity of mini-cuttings per mini-stump, which in turn generates clonal seedlings with faster growth, both in a humidity chamber and greenhouse. This greater vigor of mini-stumps may be attributed to the higher amount of carbohydrate reserve and co-factors of adventitious rooting that maximize the rhizogenic process (Dias et al. 2011). Although the size of mini-cuttings was standardized during preparation, the greater interval between collections facilitated the greater accumulation of reserves in the collected shoots, thereby accelerating the development and rooting of the mini-cuttings. Paiva and Gomes (1995) indicated that reserves are indispensable for the survival of the propagule until it forms roots and develops. At appropriate levels, reserves are responsible for increasing the photosynthetic area and favoring root formation, since most of the reserves are transferred to the base of the mini-cuttings, contributing to the induction and formation of the root primordium (Paiva and Gomes 1995). These results are relevant, since the balance between productivity and rooting of mini-cuttings is extremely important for the production of vigorous and high-quality plantlets.

Since mini-cutting productivity is associated with adventitious rooting competence in mate half-sibling progenies, the greater the number of mini-cuttings rooted per mini-stump, the greater would be the percentage of rooting and the number and length of roots. Similar results have been reported in herbaceous mini-cuttings of *Prunus persica* (Timm et al. 2015) and mini-cuttings of *C. canjerana* (Burin et al. 2018), where high correlations were found between the number and length of roots. Considering the linear correlation among evaluated traits, the number of rooted mini-cuttings per mini-stump can be used to identify mate genotypes with high adventitious rooting competence within and between half-sibling progenies, which would facilitate and simplify the selection process of superior clones for vegetative propagation by mini-cuttings. These results are in agreement with those observed in *C. canjerana*, suggesting that the selection of clones should be performed based on the number of rooted mini-cuttings during the period of vegetative growth (Burin et al. 2018).

The REML/BLUP analysis procedure enabled the estimation of the genetic parameters by their genotypic values and predicted gains, being effective in identifying superior genotypes, as found by Bezerra et al. (2020). The estimates of individual heritability coefficients in the strict sense (h^2) were higher than those obtained in individuals within the progeny (h^2ad), for all evaluated traits (Table 2). In *Eucalyptus camaldulensis*, higher heritability estimates were observed for progeny mean than for individuals within progeny, suggesting the selection of the best progenies and genotypes within them (Azevedo et al. 2015). However, these results indicate that the selection must be performed based on the genotypes, regardless of their progeny, for the percentage of survival and rooting of mini-cuttings, number of roots, mean length of the three largest roots, and number of rooted mini-cuttings. This result is very important, since this study aimed to identify genotypes with high competence for adventitious rooting, to develop mate clones for vegetative propagation by mini-cuttings.

Among the evaluated traits, the number of produced mini-cuttings, percentages of survival and rooting, and mean length of the three largest roots showed heritability estimations of low magnitude (Resende 2016), which resulted in low gain from selection (Table 2). However, heritability estimations considered by Resende and Barbosa (2005) of moderate to high magnitude were found for the number of roots and number of rooted mini-cuttings. These results indicate that the selection should be made for the number of rooted mini-cuttings, an easily measured trait, with the possibility of satisfactory genotype selection gains, both between and within mate half-sibling progenies propagated by mini-cuttings. This is because the expected progress of selection depends directly on heritability (Resende 2016, Matos Filho et al. 2019), and moderate to high estimates of heritability favor the initial selection among and within the best progenies (Carias et al. 2016). Individual heritability estimates in the narrow sense of

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 mate half-sibling progenies

Parameters[1]	CN	S (%)	R (%)	RN	RL (cm)	CNR
h^2a	0.27	0.01	0.28	0.42	0.23	0.51
h^2ad	0.22	0.01	0.22	0.35	0.18	0.43
$CV\,gi$ (%)	17.31	3.26	24.41	37.18	71.78	45.06
$Acgen$	0.41	0.08	0.42	0.49	0.38	0.53
Mean[2]	13.11	72.29	48.97	4.36	0.96	6.44

[1] h^2a: individual heritability in the strict sense, that is, of the additive effects; h^2ad: heritability within progenies; $CV\,gi$ (%): individual additive genetic variation coefficient; $Acgen$: accuracy in the selection of progenies; and overall mean.

[2] Overall mean observed for each trait without transformation.

moderate ($0.15 < h^2 < 0.50$) to high ($h^2 > 0.50$) magnitudes have already been observed for the production of leaf mass (Simeão et al. 2002), and those of high magnitude were noted for seedling height and diameter of mate progenies (Costa et al. 2005).

The individual additive genetic variation coefficient ($CV\,gi$) quantifies the magnitude of genetic variation available for selection (Oliveira at al. 2018); therefore, the higher its value, the more appropriate is the trait for selection. In this study, the highest $CV\,gi$ values were obtained for the mean length of the three largest roots and number of rooted mini-cuttings (Table 2). Considering that $CV\,gi$ values above 10% are sufficient for effective selection (Resende 2016), only the percentage of survival could not be considered in the selection of mate genotypes and progenies. These results indicated a high magnitude of genetic variation available for selection (Pimentel et al. 2014), enabling significant genetic gains from selection between and within mate progenies based on adventitious rooting competence. According to Bezerra et al. (2020) this shows the existence of variability that can be exploited by selection and, consequently, the possibility of identifying superior genotypes for these traits. The $CV\,gi$ values between 25.9% and 41.6% were observed for rooting traits of *P. persica* cuttings (Oliveira et al. 2018). $CV\,gi$ values between 10.4% and 17.6% resulted in genetic gains from selection of 4.2% and 7.2%, respectively, for height and diameter of mate seedlings (Costa et al. 2009).

The selection accuracy ($Acgen$) is directly proportional to the heritability of the respective trait, which refers to the correlation between the predicted and true genetic values of the individuals (Resende and Duarte 2007, Lopes et al. 2018). As expected, one of the highest values of $Acgen$ was obtained for the number of rooted mini-cuttings (Table 2), also indicating that the selection for rooting competence of mini-cuttings should be performed based on this trait. These results are in agreement with those of Burin et al. (2018), who performed the selection of *C. canjerana* clones based on the number of rooted mini-cuttings over three successive collections, highlighting the feasibility of using this trait to identify genotypes with high competence for adventitious rooting of mini-cuttings.

The REML/BLUP procedure allows the prediction of genetic values (g) and, consequently, ranking of the best genotypes for a given trait (Resende 2016), in this case, for the number of rooted mini-cuttings per mini-stump. Following this order, the 50 genotypes with the highest genotypic values were selected, revealing that progenies 17SM1, 17SM3, 17SM4, 17SM7, 17SMLZ, and 17SMJS had at least five selected genotypes each, and progenies 17SM3 and 17SMLZ contributed to the largest number (11) of selected genotypes (Table 3). Selection of the number of rooted mini-cuttings within the progenies resulted in selection intensities ranging from 9.80% to 22.90%, corresponding to an intensity of selection of 17.86%, considering all evaluated individuals. This selection intensity can be considered to be adequate for maintaining sufficient genetic variability for the selection of other traits. Higher selection intensities (1.26% and 2.14%) were used for *Pinus caribaea* var. *hondurensis* in different sources of clonal seed orchard (Sampaio et al. 2000), and, for *E. camaldulensis*, selection intensity was 15.15% among progenies and 6.67% within progenies (Azevedo et al. 2015).

The results of this study showed that the selection of genotypes based on the number of rooted mini-cuttings was important to identify those with high adventitious rooting competence during vegetative growth, since this trait varied between and within half-sibling progenies of mate. As expected, the highest genetic gain was observed for the number of rooted mini-cuttings (48.41%), because it was the directly selected trait (Table 3). The selection of the 50 genotypes with high adventitious rooting competence increased the original observed mean from 6.44 (Table 2) to 9.97 rooted mini-cuttings in four consecutive collections, equivalent to an increase of 54.81%. The highest values of indirect genetic gain of selection were observed for the

Table 3. Number of selected genotypes and mean of mate half-sibling progenies, with the respective mean of all selected genotypes, original mean, and percentage genetic gain for the number of mini-cuttings produced by mini-stump (CN), percentage of survival of mini-cuttings (S%), rooting percentage (R%), number of roots (RN), mean length of the three largest roots (RL cm), and number of rooted mini-cuttings (CNR) collected at different times throughout the year and evaluated at 60 days of cultivation in a humidity chamber

Progenies*	Nº of selected genotypes	CN	S (%)	R (%)	RN	RL (cm)	CNR
17SM1	6	2.19	61.51	49.50	1.57	0.22	1.90
17SM3	11	2.13	61.60	49.70	1.49	0.22	1.88
17SM4	8	2.11	61.56	49.03	1.44	0.22	1.88
17SM7	5	2.12	61.65	51.02	1.44	0.22	1.88
17SMLZ	11	2.08	61.72	53.18	1.51	0.23	1.83
17SMJS	9	2.,18	61.67	49.10	1.40	0.22	1.85
Improved mean		2.13	61.62	50.25	1.47	0.22	1.87
Original mean		1.90	61.43	43.99	1.17	0.17	1.26
Genetic gain (%)		12.11	0.31	14.23	25.64	29.41	48.41

* Original data were transformed. The number of mini-cuttings per mini-stump, number of roots, mean length of the three largest roots, and number of rooted mini-cuttings were transformed to $\sqrt{(x + 0.5)}$, and the percentage of survival and rooting of the mini-cuttings was transformed using Box–Cox.

number of roots and the mean length of the three largest roots (Table 3). These results were also expected, as the rooting traits showed the highest values of linear correlation with the number of rooted mini-cuttings.

The results of this study showed that Pearson correlation estimations and genetic parameters associated with the prediction of genetic values allowed the identification of the best trait to be used in the selection and order the genotypes based on their genetic value. The number of rooted mini-cuttings was the most suitable trait for the selection of genotypes with high competence for adventitious rooting between and within mate half-sibling progenies. The genetic gains provided with the selection between and within the progenies can be considered satisfactory as well as more efficient for choosing the best genotypes. Both the direct genetic gain for the number of rooted mini-cuttings and the indirect gains, mainly for the number of roots and the mean length of the three largest roots, can be considered to be of high magnitude. This is an important result, since a new plantlet must have a well-formed root system to increase its capacity to absorb water and nutrients, enabling good establishment and development in the field. The best clones from selected progenies should result in uniform growth and development, and high yield and quality of leaves and branches, positively impacting farmer profits and quality of industrial products. The breeding strategy developed in this study enabled the selection of mate genotypes with high competence of adventitious rooting within all evaluated half-sibling progenies and maintained variability for selecting other traits to develop new clones for vegetative propagation. The mass production of plantlets by using mini-cuttings requires, in addition to adventitious rooting, a well-formed and developed root system associated with high morphological and physiological quality of the produced plantlets. Therefore, selection for adventitious rooting competence based on the number of rooted mini-cuttings can be used in the breeding programs of mate for vegetative propagation by mini-cuttings.

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