Early selection of *Cabralea canjerana* for propagation by mini-cutting

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Abstract – The objective of this work was to define an early selection strategy to identify *Cabralea canjerana* (Meliaceae) clones with high multiplication rate. A mini-garden of 109 clones of canjerana seedlings was established in a completely randomized design, in an acclimatized greenhouse. From seedlings, the mini-stumps and mini-cuttings were obtained. Mini-cuttings were collected at five different times, and the number of mini-cuttings per mini-stump, rooting percentage, and number of rooted mini-cuttings were quantified. The number of rooted mini-cuttings per mini-stump was the only trait that showed high correlation with the others. Five groups of clones based on the number of rooted mini-cuttings per mini-stump were separated using k-means clustering, and the genetic gain from selection and Pearson correlation were estimated. The selection of the two best groups in each evaluation period resulted in high genetic gains from selection for all evaluated traits. Early selection for the number of rooted mini-cuttings discarded 65% of the evaluated clones, which increases experimental precision in evaluations of traits associated with plantlet growth and quality. Early selection for the number of rooted mini-cuttings per mini-stump at different times allows the identification of *Cabralea canjerana* clones with high multiplication rate.

Index terms: breeding, canjerana, mini-stump, plantlet production, rooting.

Seleção precoce em *Cabralea canjerana* para a propagação por miniestaquia

Resumo – O objetivo deste trabalho foi definir uma estratégia de seleção precoce para identificar clones de *Cabralea canjerana* (Meliaceae) com alta taxa de multiplicação. Um minijardim com 109 clones de plântulas de canjerana foi estabelecido com um delineamento inteiramente casualizado em casa de vegetação climatizada. Das plântulas, foram obtidas as minicepas e as miniestacas. As miniestacas foram coletadas em cinco épocas, e foram quantificados o número total de miniestacas por minicepa, a percentagem de enraizamento e o número de miniestacas enraizadas. O número de miniestacas enraizadas por minicepa foi o único caráter que apresentou alta correlação com os demais. Foram estabelecidos cinco grupos de clones com base no número de miniestacas enraizadas por minicepas, pelo agrupamento das k-médias, e estimados o ganho genético de seleção e a correlação de Pearson. A seleção dos dois melhores grupos em cada época resultou em altos ganhos de seleção para os caracteres avaliados. A seleção precoce para o número de miniestacas enraizadas descartou 65% dos clones avaliados, o que aumenta a precisão experimental em avaliações de caracteres associados ao crescimento e à qualidade das mudas. A seleção precoce do número de miniestacas enraizadas por minicepas, em diferentes épocas, permite identificar clones de *Cabralea canjerana* com alta taxa de multiplicação.

Termos para indexação: melhoramento, canjerana, minicepa, produção de mudas, enraizamento.

Introduction

Cabralea canjerana (Vellozo) Martius, known as canjerana, is a Brazilian native tree species of the Meliaceae family with great economic importance. Canjerana is considered one of the most valuable species, because of its high quality and durability of wood, even when exposed to drastic weather conditions (Aimi et al., 2016) and because of its number of usages as timber products, landscaping, restoration of degraded areas and medicinal purposes (Gasparin et al., 2014; Rovedder et al., 2016). In this context, canjerana has the potential of being a native tree species of choice for using in clonal silviculture.

Production of canjerana plantlets can be hampered because the seed is recalcitrant (Medeiros & Abreu, 2007) and sensitive to dehydration, which leads to a rapid loss of viability. Seed storage in paper envelopes at 25ºC ensures a good state of conservation only for

ten days (Grunennvaldt et al., 2014). The species shows high variability for fruit maturation, which hinders the formation of seed lots. Seed germination is not uniform and ranges between 22 and 86%. In many cases, low percentage of seed germination can be attributed to differences in maturation of harvested fruits (Felippi et al., 2015). These factors limit seminal plantlet production of canjerana, demonstrating the need to carry out vegetative propagation studies. However, there is little information available in the literature.

Vegetative propagation studies show that canjerana has rooting competence in vitro (Rocha et al., 2007), but the multiplication stage requires optimization. Because of the high cost, in vitro propagation is indicated mainly when other techniques of vegetative propagation do not succeed. Some canjerana clones show rooting competence of mini-cuttings, which could facilitate plantlet production (Gimenes et al., 2015). Plantlet production by mini-cuttings may recover the necessary juvenility for vegetative propagation, since maturation is a limiting factor for adventitious rooting (Wendling et al., 2014) and provide production of a large quantity of high quality plantlets in a small amount of time, depending on the rooting competence of each species (Stuepp et al., 2015). Adventitious rooting competence is also affected by recalcitrance to vegetative propagation, which demonstrates the need to adopt early selection strategies to circumvent this problem (Oliveira et al., 2015). The productivity of mini-cuttings per mini-stump together with rooting percentage defines the multiplication rate (Rocha et al., 2015), which corresponds to the number of rooted mini-cuttings and indicates potential quantity of plantlets. As canjerana clones vary in rooting competence (Gimenes et al., 2015), it is necessary to explore these differences to maximize plantlet production by mini-cuttings and to associate early selection strategies, which discard clones recalcitrance to vegetative propagation.

The advantages of genetic improvement strategies in the development of new cultivars and the maximization of plantlet production have been discussed for the main vegetable and tree species for which vegetative propagation is used (Assis & Resende, 2011; Bisognin, 2011). Forest improvement has been concentrated on exotic species, mainly on the genera *Eucalyptus*, *Pinus*, *Acacia* and *Tectona*. Among these, genetic improvement strategies for vegetative propagation are relatively well defined for *Eucalyptus* (Assis & Resende, 2011). In native species, studies have shown the viability of vegetative propagation by mini-cutting of *Anadenanthera macrocarpa* (Dias et al., 2012), *Araucaria angustifolia* (Pires et al., 2015) and *Cabralea canjerana* (Gimenes et al., 2015); however, strategies of early selection have not yet been investigated. Due to the economic importance of canjerana, the difficulties of seedling production and the need to select clones for vegetative propagation.

The objective of this work was to define an early selection strategy to identify *Cabralea canjerana* clones with high multiplication rate.

Materials and Methods

The experiments were conducted in an acclimatized greenhouse of polycarbonate 10 mm, panels with maximum temperature set to 32°C and minimum temperature of 17°C, at the Center for Plant Improvement and Vegetative Propagation, at the Departamento de Fitotecnia of the Universidade Federal de Santa Maria, in the state of Rio Grande do Sul, Brazil (29º42'S, 53º49'W and 95 m over the sea level).

For this study, one-year-old seedlings of canjerana were established in a clonal mini-hedge in a completely randomized design. One hundred and nine seedlings were arranged in polyethylene trays (55x34x15 cm) in a closed soilless system, with coarse sand as substrate, adapted from Bisognin et al. (2015). In each tray, a layer of approximately 4 cm of medium gravel was laid and covered by a polyethylene screen (1 mm), and on this layer approximately 11 cm of coarse sand was laid (growing bed). Twelve seedlings were planted in each tray, with a spacing of 10x10 cm. A transversal polyvinyl chloride tube (20 mm diameter) with two holes was placed over the growing bed to distribute nutritive solution, which was supplied once a day during 15 minutes with the aid of a timer and low flow pump. The nutrient solution completely soaked the substrate and formed a surface layer, which was drained through two holes, one at the base and the other at the top of the tray. The nutritive solution consisted of quantities of macro and micronutrients as described by Kielse et al. (2015). Solution pH was kept between 5.5 and 6.0, and electrical conductivity at 1 dS m^{-1}, both adjusted weekly. After acclimatization, seedlings were submitted to drastic pruning (coppice)

to form the mini-stumps that produced the shoots used for preparing the mini-cuttings.

Five evaluations were done according to the availability of shoots on the mini-stumps. The first evaluation was performed in September 2013, and the last (fifth) one in May 2015. In each collection, just the clones with shoots availability were evaluated. At each collection, mini-cuttings 1.5 to 2.0 cm long, with one pair of leaflets, had their leaflets cut to be reduced to half of their original size. The number of mini-cuttings produced per mini-stump depended upon the availability of shoots in each collection. Based on preliminary tests, the basal part of the mini-cuttings was immersed in a solution of 3,000 mg L^{-1} of indolebutyric acid (AIB), for 10 seconds.

The mini-cuttings were grown in a vertical position in polyethylene trays, containing a mixture of commercial substrate, coarse sand and carbonized rice bark (1:1:1 v/v) and kept in the nebulization chamber, with an average temperature of 25°C and relative humidity of about 80%. In each of the evaluations, the total number of mini-cuttings produced per mini-stump was recorded (mini-cutting per mini-stump productivity). At 60 days of cultivation in the nebulization chamber, the percentage of rooted mini-cuttings and the number of rooted mini-cuttings per mini-stump were assessed. The mini-cuttings were considered rooted when they showed at least one adventitious root with a length equal to or greater than 0.1 cm.

To identify the best clones of canjerana for vegetative propagation by mini-cutting, in each of the five successive collections, five groups were previously established according to the number of rooted mini-cuttings per mini-stump by the method of non-hierarchical k-means clustering (Mingoti, 2005). Group means of each collection were compared by Student's t-test for independent samples, at 5% probability. To validate the clustering, univariate analysis of variance was employed with a variable number of replicates (clones) ranging from three to 54 clones. Pearson linear correlation was performed between the traits of number of mini-cuttings produced per mini-stump, rooting percentage and number of rooted mini-cuttings.

The indirect genetic gain of selection (GS) for the three traits evaluated was calculated by the difference between the mean of the selected clones (MSC) and the mean of the original clones (MOC), that is, GS = MSC - MOC. GS percentage was also calculated, using the equation GS% = (GS/MOC) × 100. All analyses were carried out with the aid of Genes software (Cruz, 2013) and Office Excel.

Results and Discussion

For all traits and evaluations, differences were observed between the five groups and clones within the groups, except for number of mini-cuttings produced per mini-stump in the second and fifth evaluations, in which differences were not detected by the F-test (Table 1). The percentage of rooting and the number of rooted mini-cuttings per mini-stump showed differences between groups of clones in all evaluations (Table 1). Therefore, the non-hierarchical k-means clustering method was efficient in separating canjerana clones into five groups for all mini-cutting rooting traits. Besides the fact that canjerana clones differed in their competence for adventitious rooting of mini-cuttings, the non-hierarchical k-means clustering was effective in separating them into groups based on the number of rooted mini-cuttings per mini-stump, which best defines the multiplication rate of canjerana clones. The efficiency of non-hierarchical k-means clustering method in separating groups of clones was also observed in hybrids of *Eucalyptus* (Beltrame et al., 2012).

The rooting percentage and the number of mini-cuttings produced per mini-stump are independent traits, since the estimation of Pearson linear correlation was low ($r = 0.11$, $p<0.05$). This is an indication that early selection based on only one of these traits may result in the identification of clones with a low multiplication rate. This is because the multiplication rate of a clone depends on the number of mini-cuttings produced and the rooting percentage, i.e., the production of mini-cuttings and the rooting competence. The mini-cuttings produced in a clonal mini-hedge is only considered adequate when there is a considerable high production of mini-cuttings associated with high adventitious rooting competence, resulting in high multiplication rate (Freitas et al., 2017). Among the strategies of selection based on one of these traits, the selection of clones with the highest number of mini-cuttings associated with the lowest percentage of rooting is the least advantageous combination for commercial production of plantlets,

due to the greater demand for materials, physical space and labor needed for rooting a small number of mini-cuttings.

The number of rooted mini-cuttings per mini-stump showed high estimation of correlation with the percentage of rooting ($r = 0.79$, $p<0.05$) and with the number of mini-cuttings produced per mini-stump ($r = 0.52$, $p<0.05$), regardless of the genetic differences between clones. These results are very important for vegetative propagation by mini-cutting, because the higher the percentage of rooting and rooted mini-cuttings, the greater the potential for plantlet production, due to the high multiplication rate. These results support the idea that canjerana clones can be selected considering only the number of rooted mini-cuttings per mini-stump, which facilitates the assessment and the identification of superior clones for propagation by mini-cutting. Early selection based upon the number of rooted mini-cuttings should improve genetic gain, since it facilitates the discard of clones that are recalcitrant to vegetative propagation (Oliveira et al., 2015).

There were significant differences between groups of canjerana clones, except for the number of mini-cuttings per mini-stump in the fifth evaluation (Table 2). The significant differences between groups are fundamental for the identification of superior clones for multiplication rate. Based on Pearson correlation analysis, the number of rooted mini-cuttings is the most important trait. As already discussed, it is possible that some clones stand out for their number of mini-cuttings, and others for their rooting percentage, but what is important in vegetative propagation is the multiplication rate, which is a combination of both traits and is quantified by the number of rooted mini-cuttings. This is because of the fact that adventitious

Table 1. Analysis of variance of clusters for the traits number of mini-cuttings produced per mini-stump, rooting percentage, and number of rooted mini-cuttings per mini-stump of canjerana clones.

Sources of variation	Degrees of freedom	Mean squares		
		Mini-cutting/ mini-stump	Rooting (%)	Rooted mini-cuttings
		Evaluation 1		
Cluster	4	88.32*	10,831.86*	68.18*
Residue	74	8.50	170.91	0.10
		Evaluation 2		
Cluster	4	16.42^{ns}	6,326.90*	49.19*
Residue	32	8.00	215.62	1.92
		Evaluation 3		
Cluster	4	41.54*	8,883.53*	49.46**
Residue	58	6.53	163.82	0.09
		Evaluation 4		
Cluster	4	248.22*	8,533.80*	122.53*
Residue	79	9.25	104.78	0.10
		Evaluation 5		
Cluster	4	20.19^{ns}	4,384.65*	28.37*
Residue	62	18.86	70.45	0.02

*Significant by the F test, at 5% probability. ^{ns}Nonsignificant.

Table 2. Mean number of mini-cuttings produced per mini-stump, mean rooting percentage, mean number of rooted mini-cuttings, and number of clones per cluster in each of the five groups formed by k-means clustering of canjerana clones evaluated in different periods.

Cluster[(1)]	Mini-cutting/ mini-stump[(2)]	Rooting (%)	Rooted mini-cuttings	Clones/ cluster
		Evaluation 1		
1	10.45a	58.29a	5.55a	11
2	6.50b	61.79a	3.42b	12
3	7.71ab	30.99b	2.00c	17
4	5.27b	21.84c	1.00d	15
5	4.13bc	0.00d	0.00e	24
		Evaluation 2		
1	8.83a	84.21a	7.17a	6
2	7.00ab	60.67b	4.00a	5
3	6.20ab	46.99b	2.50b	10
4	4.82b	23.37c	1.00c	11
5	6.00ab	0.00d	0.00d	5
		Evaluation 3		
1	9.50a	71.82a	6.50a	4
2	7.80a	59.14ab	4.00b	5
3	6.00ab	42.95b	2.00c	9
4	3.88b	31.10b	1.00d	17
5	4.25b	0.00c	0.00e	28
		Evaluation 4		
1	16.50a	54.63a	8.50a	6
2	11.14b	46.95ab	4.57b	7
3	7.71b	38.57ab	2.57c	7
4	5.20b	27.51b	1.00d	10
5	4.48bc	0.00c	0.00e	54
		Evaluation 5		
1	10.60a	48.36a	4.40a	5
2	8.00a	46.98a	3.00b	3
3	10.50a	28.85a	2.00c	4
4	7.73a	14.87b	1.00d	11
5	7.27a	0.00c	0.00e	44

[(1)]k-means clustering. [(2)]Clusters followed by equal letters in the column do not differ at 5% probability, by Student's t-test, for independent samples.

rooting percentage becomes an efficient selection criterion, since, together with the production of mini-cuttings per mini-stump, it allows estimating the multiplication rate or the productivity index of the clones (Rocha et al., 2015). With these results, the study demonstrated the suitability of the selection of canjerana clones for vegetative propagation only based upon the number of rooted mini-cuttings per mini-stump.

From the two best groups of clones for number of rooted mini-cuttings selected in each evaluation, there were 23, 11, 9, 13, and 8 clones respectively selected in the five consecutive evaluations (Table 2). These numbers were equivalent to 29.1, 29.7, 14.3, 15.5, and 11.9% of selected clones respectively in each evaluation. In consecutive evaluations, some clones were consistently selected, while others were selected only in a few cases, which resulted in the selection of 38 canjerana clones, equivalent to a selection index of 35.0%. These differences can be explained by the variation between clones associated with seasonality, which interferes with the number of mini-cuttings per mini-stump and the rooting percentage, as observed in other native tree species, such as *Araucaria angustifolia* (Pires et al., 2015) and *Piptocarpha angustifolia* (Ferriani et al., 2011). This indicates that the evaluation of clones for mini-cuttings rooting should be performed at different collections, to enhance the selection process by reducing the effects of seasonality in mini-cuttings rooting.

In the case of selecting only the clones belonging to the best group for each evaluation, this would result in 24 clones, equivalent to a selection index of 22.0%. Thus, there would be a greater reduction in the genetic variability only for selecting clones for multiplication rate, which could restrict the genetic gain from selection for other traits, such as those associated with plantlets quality (shoot height, stem diameter, root, and shoot fresh weight, among others). Alternatively, when considering the top two groups, selected clones showed superior performance for the three evaluated traits and kept a large variation between the best and the worst clone (Table 3). The total number of mini-cuttings per mini-stump ranged from 3 to 19, the rooting percentage ranged from 23.1 to 100.0%, and the number of rooted mini-cuttings per mini-stump ranged from 3 to 10. Of the 38 selected clones, 11 clones showed less than 50% of mini-cutting rooting and 27 clones showed percentage of rooting ranging from 50 to 100%. These rooting percentages indicated high feasibility for the vegetative propagation of canjerana by mini-cutting. The identification of clones with higher adventitious rooting competence should increase the production of rooted mini-cuttings, which would result in high multiplication rate. The mean number of mini-cuttings

Table 3. Number of mini-cuttings produced per mini-stump, rooting percentage, and number of rooted mini-cuttings per mini-stump of 38 selected clones of *Cabralea canjerana*.

Clone	Mini-cutting/ mini-stump	Rooting (%)	Rooted mini-cuttings/ mini-stump
12SMI15[(1)]	15.0	64.1	10.0
12SMI28[(1)]	15.0	60.0	9.0
12SMI14[(1)]	10.5	61.4	6.5
12SMI36[(1)]	10.5	62.0	6.5
12SMI27[(1)]	14.3	52.6	6.3
10SM03[(1)]	19.0	31.6	6.0
12SMI17[(1)]	10.0	60.0	6.0
12SMI25[(1)]	6.5	91.7	6.0
12SMI42[(1)]	11.0	54.5	6.0
12SMI43[(1)]	9.0	68.1	6.0
12SMI44[(1)]	7.5	72.2	5.5
12SMI41[(1)]	13.7	38.9	5.3
10SM01[(1)]	12.7	39.4	5.0
10SM05[(1)]	10.8	48.7	5.0
12SMI09[(1)]	9.0	55.6	5.0
12SMI10[(1)]	8.0	62.5	5.0
12SMI12[(1)]	15.0	33.3	5.0
12SMI29[(1)]	6.0	83.3	5.0
12SMI57[(1)]	5.0	100.0	5.0
12SMI20[(1)]	9.0	54.7	4.3
12SMI51[(1)]	5.0	80.0	4.0
12SMI58[(1)]	6.0	66.7	4.0
12SMS22[(1)]	8.0	50.0	4.0
12SMI30[(1)]	9.0	44.4	4.0
10SM04	13.0	30.8	4.0
10SM240	4.0	100.0	4.0
12SMI16	7.0	58.3	4.0
12SMI19	14.0	28.6	4.0
12SMI22	13.0	30.8	4.0
12SMI24	8.0	50.0	4.0
12SMI39	6.7	66.8	4.0
12SMS44	7.0	57.1	4.0
12SMS16	3.0	100.0	3.0
12SMI01	4.0	75.0	3.0
12SMI05	5.0	60.0	3.0
12SMI46	5.0	60.0	3.0
10SM42	7.0	42.9	3.0
10SM30	13.0	23.1	3.0

[(1)]Clones selected based on the best cluster for each evaluation.

per mini-stump of selected clones was 9.4 every 60 days, considered a high production for a native species that exhibits slow growth. Collecting shoots of *Anadenanthera macrocarpa* every 26 days resulted in an average of 2.2 mini-cuttings per mini-stump (Dias et al., 2012) and every 35 days produced from 1.1 to 2.5 mini-cuttings per mini-stump of *Piptocarpha angustifolia* (Ferriani et al., 2011).

The selection of 24 or 38 clones with the highest number of rooted mini-cuttings resulted in high values of indirect genetic gain from selection. The gain from selection of 24 clones varied from 62.7 to 287.5%, and from selection of 38 clones varied from 46.9 to 225.7%. As expected, the number of rooted mini-cuttings per mini-stump showed the greatest genetic gain from selection, for both groups of 24 clones (287.5%) and 38 clones (225.7%). Even the smallest indirect genetic gain from selection can be considered high, which was estimated for the number of mini-cuttings per mini-stump (46.9%) when selecting 38 clones (Table 4). Therefore, the selection strategy was adequate for both numbers of selected clones, with an advantage of keeping higher genetic variability when selecting 38 instead of 24 clones. Thus, early selection for the number of rooted mini-cuttings per mini-stump would eliminate 65% of the clones, which would allow an association of strategies that promote increased experimental precision in the evaluation of other important traits for mass production of plantlets by mini-cutting, as is the case of those associated with growth vigor and the quality of plantlets. Appropriate number of mini-stumps increases experimental precision, which allows accurate inferences about evaluated traits (Cargnelutti Filho et al., 2016). Therefore, the early selection strategy to identify *Cabralea canjerana* clones with high multiplication rate cut down the number of clones for other traits evaluation, which allows evaluating more mini-stumps per clone, which results in higher experimental precision.

Table 4. Means of original clones (MOC), means of selected clones (MSC), indirect genetic gain (GS), and percentage (GS%) from selection of *Cabralea canjerana* clones for the number of mini-cuttings produced per mini-stump, rooting percentage and number of rooted mini-cuttings per mini-stump.

Traits	Mini-cuttings/ mini-stump	Rooting (%)	Rooted mini-cuttings/ mini-stump
	Selection of 24 clones		
MOC	6.4	21.6	1.5
MSC	10.4	60.9	5.8
GS	4.0	39.3	4.3
GS (%)	62.7	181.6	287.5
	Selection of 38 clones		
MOC	6.4	21.6	1.5
MSC	9.4	58.4	4.9
GS	3.0	36.8	3.4
GS (%)	46.9	170.1	225.7

Conclusions

1. Early selection for the number of mini-cuttings per mini-stump in different times allows the identification of *Cabralea canjerana* clones with high multiplication rate.

2. The early selection evaluated to identify *C. canjerana* clones with high multiplication rate cut down the number of clones for other traits, which allows evaluating more mini-stumps per clone, which results in higher experimental precision.

Acknowledgments

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), for financial support.

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Received on May 30, 2017 and accepted on November 23, 2017