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INFLUENCE OF STEM CUTTING POSITION, LEAVES CONDITION AND SIZE OF MINICUTTINGS IN ROOTING OF BLACK WATTLE

SUMMARY

Because of the commercial interest in forest resources provided by black wattle (*Acacia mearnsii* De Wildman), mainly for tannin extraction, the researches about clonal propagation techniques have been developed for this species. The objective of our study is to determine the type and size of minicuttings for the best adventitious rooting in three commercial clones of black wattle. The minicuttings were collected from a mini-clonal hedge located in the nursery at TANAGRO S.A. Company, located in Montenegro, Brazil. There were tested four sizes of minicuttings (5, 8, 11, 14 and 17 cm); two types depending on the stem cutting position where they were collected (apical or basal) and presence or absence of leaves with entire or half reduced area. The experiments were installed in randomized blocks with four blocks and six minicuttings per parcel. The evaluation of minicuttings rooting was done after 45 days. We conclude that also basal and apical minicuttings with entire or half reduced leaves exhibit higher percentage of rooting, as well as 14 cm minicuttings. Besides that, rooting response also varies according to the clone genotype.

Keywords: *Acacia mearnsii*, apical minicutting, basal minicutting, leaves in minicutting, minicutting size

INTRODUCTION

Considering the rising interest in using clones to develop forest projects, a great technological advance has been observed in processes of cloning and selection of superior genotypes. The use of vegetative propagation of forest species associated to improvement programs intents to accelerate growth and generate homogeneous and wood of quality (Valeri et al., 2012).

In Brazil, the species *Acacia mearnsii* De Wildeman, known as black wattle, is economically important in forest sector mainly for tannin extraction. Thus, vegetative propagation is an alternative to obtain genetically superior individuals. It is common that some species display limitations in seedling

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production via cutting mostly because to the complexity of rooting process of cuttings under natural conditions (Hartmann et al., 2011).

The quality, the survival and the percentage of rooting in a minicutting which will became a seedling in field, depend on several factors. In a mini-clonal hedge, the stem cutting position of which the minicutting will be prepared is one of this factors. According to Hartmann et al. (2011), woody basal minicuttings are more lignified and thus, more resistant to handling and transport. However, herbaceous apical juvenile minicuttings perform higher rooting capacity, with better percentage and speed of roots development (Wendling et al., 2002).

The maintenance of leaves in stem minicuttings is another factor that can benefit the rooting by providing carbohydrates and hormones, such as auxins. This, ensures better physiological conditions in rooting process (Xavier et al., 2013). Leaves maintenance is related to water adjustment and to the beginning of roots development, once the metabolites synthesized in the matrix plant could be transported to the rooting region in the minicuttings (Oliveira et al., 2001).

Another factor associated to the rooting success is the size of the minicutting. Small minicuttings may not have enough quantity of reserves required for adventitious rooting. Long minicuttings may be more susceptive to dehydration, once they demand more water to supply all the tissue exposed to the environment (Lima et al., 2006).

Therefore, in order to improve vegetative propagation techniques of black wattle, the objective was to determine the ideal size to induce rooting in minicuttings; if the presence, absence or leaves size influences in the rooting process and which stem cutting position is more effective to produce seedlings of black wattle commercial clones.

MATERIAL AND METHODS

Rooting experiments were installed in the forest nursery at TANAGRO S.A. Company, located in Montenegro city, state of Rio Grande do Sul, Brazil, 29º41'19" South latitude and 51º27'40" West longitude (Instituto Brasileiro de Geografia e Estatística [IBGE], 2014). Minicuttings were collected from outdoor mini-clonal hedges in summer time. The mini-clonal hedges were established with sand in a semi-hydroponic system and flood-irrigated by an automated system in which just the roots were in contact with nutritional solution (Bolle-Jones, 1954).

Mini-clonal hedges were composed by three commercial clones of black wattle nominated as A, B and C. From each one, they were collected juvenile branches and minicuttings were prepared with straight cut at the top and bevel cut at the base in adequate amount for an experiment considering type and leaves condition, and another experiment considering minicuttings size. Both experiments were installed in randomized blocks with four blocks and six minicuttings per parcel.

Evaluation for minicutting type considered: the three clones; two types of minicuttings according to stem cutting position (apical or basal) and three leaves condition (leafless, one pair of leaves with half reduced area, one pair of leaves entire area), totaling 432 minicuttings. In the experiment to evaluate the effect of size, they were considered the three clones and five sizes: 5, 8, 11, 14 and 17 centimeters long, totaling 360 minicuttings.

Minicuttings were treated with indole butyric acid (IBA) 4000 mg L^{-1} and placed in tubes containing substrate prepared with rice husks and vermiculite medium grain size (1:1) with application of 3 Kg/m^3 of slow releasing fertilizer Osmocote® (NPK 15-03-12). Tubes containing the minicuttings were disposed in greenhouse with temperature (25 to 30 °C) and humidity (90 to 95%) controlled for 45 days.

After this period, they were evaluated: percentage of rooting (R%); number of roots per minicutting (NR) and the length of the three largest roots per minicutting (LR). Variances homogeneity was verified by Bartlett test, the homogeneous variances were evaluated by F test and the non-homogenous by Friedman test. Quantitative factors were submitted to regression analyses and treatments averages compared by Scott-Knott test. Statistical analyses were performed in Assistat 7.7 software (Silva & Azevedo, 2016).

RESULTS AND DISCUSSION

Considering the minicuttings type according to stem cutting position, apical or basal, there was no difference in rooting percentage for any clone. In clone A, leaves condition was significant for rooting percentage (R%), number of roots per minicutting (NR) and the length of the three largest roots per minicutting (LR). In clone B, it was observe interaction between the factors type and leaves condition just for NR. In clone C, leaves condition was significant to R% and LR.

In clone A, minicuttings with one pair of leaves with half reduced area presented the best results for rooting (75.0%), followed by minicuttings with one pair of leaves entire (45.8%) and leafless only 4.2%. In clone C, leafless minicuttings were the same (4.2%), minicuttings with one pair of leaves with half reduced area (66.7%) and with entire leaves (72.9%) of rooting. In clone B, it was not observed rooting differences according to leaves condition (Table 1).

Regarding the number of roots per minicutting, there was no difference to any clone considering apical or basal minicuttings. In clone A, it was observed difference in roots number according to leaves condition, minicuttings with one pair of leaves with half reduced area presented in average 22.4 roots/minicutting, with leaves entire (13.9) and leafless (0.9) (Table 2).

Length of the three largest roots per minicutting varied according to leaves condition in clones A and C. Leafless minicuttings presented the lowest mean of length, 1.35 cm to clone A and 0.92 cm to clone C. The highest roots length were observed in minicutting with one pair of leaves with half reduced area, 8.12 cm in average for the three clones, followed by minicutting with one pair of leaves entire area (6.90 cm) (Table 3).

Table 1 – Rooting percentage of three clones of black wattle considering two types of minicuttings according to stem cutting position (apical or basal) and three leaves conditions

		Leaves condition			
	Minicutting type	Leafless	One pair of leaves with half reduced area	One pair of leaves entire area	Mean
Clone A	Apical	4.17	66.67	54.17	41.67 [a]
	Basal	4.17	83.33	37.50	41.67 [a]
	Means	**4.17 C**[1]	**75.00 A**	**45.83 B**	**41.67**
Clone B	Apical	50.00	50.00	50.00	50.00 [a]
	Basal	25.00	62.50	70.83	52.78 [a]
	Means	**37.50 A**	**56.25 A**	**60.42 A**	**51.38**
Clone C	Apical	4.17	54.17	70.83	43.06 [a]
	Basal	4.17	79.17	75.00	52.78 [a]
	Mean	**4.7 B**	**66.67 A**	**72.92 A**	**47.92**

[1]Mean followed by the same lowercase letter in column and capital letter in line do not differ statistically by Scott-Knott test at 5% and 1%

Table 2 – Number of roots per minicutting of three clones of black wattle considering two types of minicuttings according to stem cutting position (apical or basal) and three leaves conditions

		Leaves condition			
	Minicutting type	Leafless	One pair of leaves with half reduced area	One pair of leaves entire area	Mean
Clone A	Apical	0.5	20.9	15.3	12.24 [a]
	Basal	1.3	23.9	12.5	12.54 [a]
	Means	**0.87 C**[1]	**22.39 A**	**13.90 B**	**12.39**
Clone B	Apical	5.49	11.52	7.70	8.24 [a]
	Basal	4.17	9.31	6.93	6.80 [a]
	Means	**4.83 A**	**10.41 A**	**7.31 A**	**7.52**
Clone C	Apical	4.50	6.01	9.96	6.82 [a]
	Basal	1.25	8.66	6.94	5.62 [a]
	Mean	**2.87 A**	**7.33 A**	**8.45 A**	**6.22**

[1]Mean followed by the same lowercase letter in column and capital letter in line do not differ statistically by Scott-Knott test at 5% and 1%

Table 3 – Length of the three largest roots per minicutting (cm) of three clones of black wattle considering two types of minicuttings according to stem cutting position (apical or basal) and three leaves conditions

	Minicutting type	Leaves condition			
		Leafless	One pair of leaves with half reduced area	One pair of leaves entire area	Mean
Clone A	Apical	1.6	6.9	7.2	5.23 [a]
	Basal	1.1	8.9	6.3	5.42 [a]
	Means	**1.35 B[1]**	**7.87 A**	**6.75 A**	**5.32**
Clone B	Apical	4.46	7.84	4.96	5.75 [a]
	Basal	3.94	7.32	6.57	5.95 [a]
	Means	**4.20 A**	**7.58 A**	**5.77 A**	**5.85**
Clone C	Apical	0.92	8.03	8.09	5.68 [a]
	Basal	0.92	9.77	8.26	6.31 [a]
	Mean	**0.92 B**	**8.90 A**	**8.17 A**	**5.99**

[1]Mean followed by the same lowercase letter in column and capital letter in line do not differ statistically by Scott-Knott test at 5% and 1%

According to Hartmann et al. (2011) maintaining leaves half reduced or entire area is an important condition to produce auxin, rooting co-factors and photosynthesis products, which are translocated and benefit the rhizogenic process. This can be applied to the black wattle minicuttings considering that singular genetic materials have different rooting response according to the presence of complete or partial leaves. In our study, clone B was the genotype that showed the best disposition to adventitious rooting, once achieved 51.4% even in leafless minicutting (37.5%) (Table 1).

In vegetative propagation of forest species, apical cuttings are typically superior for rooting, because the presence of leaves and auxin offers better rhizogenic potential (Ferreira et al., 2012). In the other hand, Câmara et al. (2017) observed in mulberry that intermediate and basal cuttings present significant increase in aerial part better than apical cuttings. Besides that, IBA application in rising doses until 2000 mg L^{-1} were related to decrease of sprout and rooting.

Black wattle did not present significant difference to rooting according to the stem cutting position, because mean of rooting for apical minicuttings was around 45% and basal 49%. The found rooting percentage can be influenced by IBA application interacting with endogenous auxin which is produced in the tissues and can act in rhizogenesis process independent to genetic constitution of each clone.

According to Santana et al. (2010) the reduction of foliar area helps to minimize pathogens occurrence, increase irrigation efficiency, prevent drought triggered by transpiration and reduce cutting bending caused by water deposition over leaves. Also, adventitious rooting in leafless minicuttings is not satisfactory to ensure survival and quality of seedling in the field. The condition of root system considering number and length of roots associated to leaves condition, should contribute to satisfactory absorption of nutrients to provide seedling growth.

In the experiment for different sizes of minicuttings, the results for rooting; number of roots per minicutting and length of the three largest roots per minicutting were significant. Minicuttings 14 cm long presented higher percentage of rooting and 5 cm long was the least efficient size (Table 4).

Table 4 – Rooting percentage of minicutting of three clones of black wattle considering four sizes

Treatments	**Rooting percentage**
Clone A	50.00 A
Clone B	56.25 A
Clone C	50.00 A
Friedman X^2	0.13^{ns}
DF	12
5 cm	5.56 D
8 cm	36.11 C
11 cm	72.22 B
14 cm	94.44 A
Friedman X^2	12**
DF	12

[1]Mean followed by the same letter in column do not differ statistically by Friedman test at 5% and 1%; DF = Degrees of Freedom

Number of roots per minicutting is also influenced by size, once minicuttings 14 cm long presented 10.41 roots/minicutting, whereas 5 and 8 cm long presented lower results (1.50 and 6.51, respectively). Minicuttings 5 cm long resulted in lower length of roots (2.29 cm) whereas 14 cm long reached 7.98 in average (Table 5).

Dias et al. (2015) and Souza (2012) found that cuttings 10 cm long of *Anadenanthera macrocarpa* (Benth.) and *Eucalyptus grandis* x *E. urophylla*, respectively, presented higher percentage of roots released when the cuttings are been removed from the greenhouse. As said by the work with *A. macrocarpa*, smaller cuttings presented larger mass and longer sprouts in aerial part. However, longer cuttings resulted in larger fresh mass and superior length of roots.

Table 5 – Number and length of the three largest roots per minicutting of three clones of black wattle considering four sizes

Variables	**Clone**	**Minicutting size**				
		5 cm	**8 cm**	**11 cm**	**14 cm**	**Mean**
Number of roots per minicutting	A	1.5	7.1	10.3	13.9	8.20 [a]
	B	1.5	5.8	7.1	10.3	6.16 [ab]
	C	1.5	6.6	5.8	7.1	5.23 [b]
	Mean	**1.50 C**	**6.51 B**	**7.71 AB**	**10.41 A**	**6.53**
Average length of roots per minicutting (%)	A	2.0	4.6	6.4	6.9	4.99 [a]
	B	4.5	6.6	6.8	8.5	6.60 [a]
	C	0.4	4.8	5.3	8.5	4.76 [a]
	Mean	**2.29 B**	**5.35 A**	**6.18 A**	**7.98 A**	**5.45**

[1]Mean followed by the same lowercase letter in column and capital letter in line do not differ statistically by Scott-Knott test at 5% and 1%

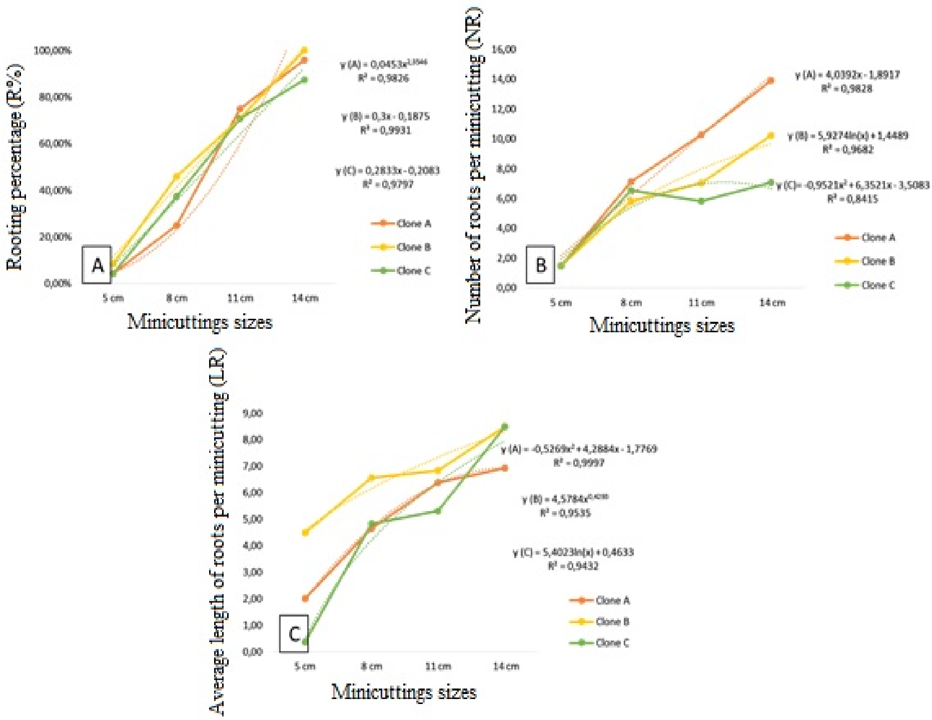

Figure 1 – Rooting of three clones of black wattle considering four minicutting sizes. A. rooting percentage; B. number of roots per minicutting and C. average of length of the three largest roots per minicutting

According to Santana et al. (2013) in hybrids of *E. urophylla,* the cutting size influenced directly the length of roots, once cuttings of 8 and 10 cm long reached 9.90 and 10.87 cm long and lower results were found with cuttings of 4 and 6 cm, similar what we found for black wattle. It should be owing to a better translocation of hormones and co-factors to form root system in longer minicuttings, which contributes to form a root system in a seedling better prepared to survival in field.

In regression analyses (Figure 1) we observed the clones related to minicutting size, which longer minicuttings size respond better to rooting. We found that there is an expressive increment in rooting results according to minicutting size increasing. All determinant model coefficients (R^2) are above 80% what is considered high and indicates that the model is highly adjusted to the equation of rooting estimative.

This behavior was observed to all the variables (R%, NR and LR) and for the three clones. The difference among the responses for rooting is low, but in general, clone B had the best performance in adventitious rooting. It indicates that in black wattle, root system development is influenced by genetic constitution.

CONCLUSIONS

Minicutting size influences in rooting response in black wattle and 14 cm long is the most efficient size. Leaves maintenance, entire or half reduced area promotes higher rooting taxes. The stem cutting position does not influence in rooting results to any clone tested. Also, clone B present higher rooting percentage, which indicate that genetic constitution provides different responses in vegetative propagation via minicutting in black wattle.

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