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ROOTING OF MINICUTTINGS OF *Castanea sativa* Mill. HYBRID CLONES[1]

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ABSTRACT – The objective of this study was to evaluate the effect of the growth regulator indole butyric acid (IBA) on the rooting of mini-cuttings of *Castanea crenata* x *C. sativa* hybrid clones. Minicuttings were left to root for 60 days in an acclimatized greenhouse and then transferred to a shade house for a further 30 days. The experiment was a random block design with a double factorial arrangement consisting of five IBA concentrations (0, 2,500, 5,000, 7,500 and 10,000 mg L^{-1}) and five clones, with three repetitions, composed of eight minicuttings per repetition. The use of IBA significantly affected the rooting and survival of the mini-cuttings, and good rates were achieved. However, at each IBA concentration we found significant differences between clones, thus suggesting that the conditions of the process of mini-cutting propagation should be specifically adapted to each particular clone.

Keywords: IBA; Vegetative propagation; Chestnut.

ENRAIZAMENTO DE MINIESTACAS DE CLONES HÍBRIDOS DE Castanea sativa Mill.

*RESUMO – O objetivo deste estudo foi avaliar o efeito do regulador de crescimento ácido indolbutírico (AIB) no enraizamento de miniestacas de clones híbridos de **Castanea crenata** x **C. sativa**. O enraizamento das miniestacas foi conduzido durante 60 dias em casa de vegetação climatizada e, em seguida, as mudas foram transferidas para casa de sombra, permanecendo por 30 dias. O delineamento experimental utilizado foi em blocos ao acaso, em arranjo fatorial duplo, constituído de cinco concentrações de AIB (0, 2.500, 5.000, 7.500 e 10.000 mg L^{-1}) e cinco clones, com três repetições, compostas de parcelas com oito miniestacas por repetição. O uso de AIB afeta significativamente ao enraizamento e sobrevivência das miniestacas de castanheiro alcançando índices satisfatórios, entretanto se observa que existem diferenças significativas no comportamento dos clones para a mesma dose de AIB, pelo qual se sugerem modificações no processo da propagação adaptando a técnica para cada clone em particular.*

Palavras-chave: AIB; Propagação vegetativa; Castanheiro.

1. INTRODUCTION

The chestnut genus, *Castanea*, is widespread throughout China, Japan and Europe. In Asia the most common species are *Castanea mollissima* Blume and *Castanea crenata* Sieb. et Zucc., while in Europe it is *Castanea sativa* Mill.. In recent years in Europe there has been an expansion in the use of interspecific hybrids with Asian species which are tolerant to American canker (*Chryphonectria parasitica* (Murrill) Bar) and chestnut ink disease (*Phytophthora* spp.) (MILLER et al., 1996), diseases which have hitherto been responsible for the decline of the species.

In Spain, there are a number of research programs in Galicia, such as those as carried out by CIFL (Centro de Investigaciones Forestales de Lourizan, Lourizan Forestry Investigation Center), where, since 1989, the combined study of interspecific hybrids and local chestnut cultivars has been undertaken. The aim is to try to give a comprehensive overview of the problems associated with chestnut management both in terms of forest (vigor and straightness of stem), agronomic (quality of the fruit) and mixed production. Thus they have been able to combine the qualities of the European chestnut with the resistance of Asian species to ink disease and canker, and develop clones specifically for grafting, for fruit production and for forestry (PEREIRA-LORENZO et al, 1996a,b,c; PEREIRA-LORENZO; FERNANDEZ-LOPEZ, 1997), and then observe their behavior in the field (PEREIRA-LORENZO et al., 2000). More recently, in 2003, 2004 and 2005, TRAGSA (Tecnologías y Servicios Agrarios, SA), has been carrying out a selection of adult chestnut plants which are resistant to ink disease (*Phytophthora cinnamomi*), cloning them through micro-propagation and then testing them in the field (CUENCA et al., 2009). This illustrates to some extent the importance of this species in the development of regions such as Galicia.

However, in parallel with these genetic breeding programs, vegetative propagation programs are also needed as these are an important tool in increasing the competitiveness of forest-based industries, where potential is maximized through the establishment of clonal forests with higher productivity and better quality wood (BORGES et al., 2011), as has been the case for eucalyptus.

In the case of mini-cuttings, Wendling and Xavier (2001) and Titon et al. (2002) stated that the use of juvenile material is crucial to the success of rooting mainly because of its higher rooting rates and the reduction in the time required to produce the plants, depending on the age of the material and the characteristics of the clone (WENDLING; XAVIER, 2003).

However, when a new species is studied in order to establish a method of vegetative propagation, it is necessary to use substances to promote rooting, protocols which are already established for many species. Among them, auxin is worthy of note as it shows significant effects on the rooting of many tree species (HARTMANN et al., 2002).

Application of auxins is usually based on synthetic compounds due to their greater stability, in addition to low toxicity and a more localized action compared to other products (HARTMANN; KESTER, 1998). Among the auxins, indole butyric acid (IBA) is the most widely used, and its validity in this respect has been corroborated by various authors working with both pine and eucalyptus (HIGASHI; GONÇALVES., 2000; WENDLING et al., 2000; TITON et al., 2003; ALCÂNTARA et al., 2008;. BORGES et al., 2011; MAJADA et al., 2011; MARTÍNEZ-ALONSO et al., 2012). However, there can be problems with detailing specific recommendations since the concentrations of IBA necessary are dependent on the physical, morphological and genetic characteristics of the species (HARTMANN, KESTER, 1998), as well as on environmental conditions (HARTMANN, KESTER, 1998; TITON et al, 2003), and according to Assisi and Teixeira (1999), genotype is one of the factors that influences rooting.

Furthermore, there is great variation between species, cultivars and clones in relation to their greater or lesser natural ability to form roots, which is also affected by the degree of maturity of propagules (GOMES, 1987; KAMLESH et al., 1995). Higashi and Gonçalves (2000) stated that the genotype, the age of the donor plant, the time of harvesting, the type of substrate, environmental conditions, rejuvenation and the size of the cutting, all influence rooting success. Assis et al. (2004) add that the type, concentration and method of application of auxin, are also have an effect.

Among the various techniques of asexual multiplication available, taking cuttings presents certain difficulties with respect to the sweet chestnut as it is considered a highly recalcitrant species, and it is thus difficult to root cuttings (MILLER-ROETHER et al., 1999; SIERRA, 2001). However, the establishment

of programs to obtain different varieties and clones for specific purposes (forest, fruit or mixed farming production), as mentioned above, has enabled the establishment of mass propagation by micro-cutting (GONÇALVES et al., 1993; SANCHES et al., 1997; GONÇALVES et al, 1998, 1999; CUENCA et al, 2009; HASBÚN et al., 2005; RÍOS et al., 2005). More recently, with the expansion of cloned forest cultivation the mini-cutting technique has become popular. It is defined as plants propagated through conventional vegetative propagation in mini-gardens which have not previously been rejuvenated "*in vitro*" (XAVIER; WENDLING, 1998; WENDLING, 1999), and has substantially improved the rooting success of woody species.

Although there do exist traditional chestnut propagation programs via micro- and mini-stumps, the objective of this work was to find a faster, more economic and shorter system of multiplication based on the mini-cutting technique and the use of different doses of IBA.

2. MATERIALS AND METHODS

2.1. Study location

The trial was carried out in the experimental facilities of SERIDA (Servicio Regional de Investigacion y Desarrollo Agroalimentario, Agri-food Research and Development Regional Service) located at the Experimental Station "La Mata" in Grado, Asturias, Spain, (43º 23'N and 6º 4'W, 60 m asl), during the summer months of 2010. Data collection was carried out 60 days after the initial setting up of the experiment.

2.2. Plant material used

The plant material used comprised five chestnut hybrid clones (*C. crenata* x *C. sativa*) from donor plants multiplied asexually at a commercial nursery by the method of stooling: plants grown under normal conditions are pruned to 10-15 cm above groundlevel to stimulate the production of shoots. When the shoots have grown considerably but are still in a herbaceous state, the leaves from the first 20 cm of each stem are removed, they are brushed with auxin and then each has its base ringed with fine wire, taking care not to damage regrowth, but in such a way that the principal stem will be progressively strangulated as it grows and its diameter increases. After this, the basal area of the donor plant is covered with soil, in order for root formation to occur in the shoots in autumn-winter, following leaf fall. This method is traditionally used in the vegetative propagation of chestnut. The clones for this trial were acquired from a commercial nursery and were later treated as mother plants (see below) and placed in a glasshouse where they were decapitated at a height of 20-30 cm from the ground, leaving 3 to 4 lateral buds to stimulate the growth of the shoots to be used in the assay.

The five hybrid clones used in the test were: C_3, C_7810, C_2671, and C_111 C_90025.

2.3. Maintenance of mother plants

Mother plants were transferred to 2L containers and maintained under fertigation system, based on the protocol for the application of N-P-K-Mg and micronutrients developed for the cultivation of ministumps of *Pinus pinaster* Ait. by Majada et al. (2011) and Martinez-Alonso et al. (2012). The fertigation was performed weekly using the CLIMAGRO v program 2.1xp - July 03 (SYSTEMS INKOA) throughout the study period.

2.4. Harvesting and preparation of mini-cuttings

Mini-cuttings were harvested from mother plants by removing those stems with more than four shoots, but always leaving at least one shoot on the mother plant, to enable the production of new cuttings.

The mini-cuttings were prepared for rooting by trimming to just below the bottom bud, eliminating the basal leaf and, in order to decrease surface evaporation and prevent the dehydration of the mini-cuttings, two thirds of the surface of upper leaves were also removed. Mini-cuttings ranged from 5 to 8 cm and included 3 to 4 buds, depending on the intermodal distance of the cuts.

After pruning, the mini-cuttings were treated with a liquid fungicide to prevent contamination from the substrate [propamocarb (60.5%), Previcur - Bayer], and dried with paper towels. Next, the bases (bottom 1 cm) of all prepared mini-cuttings were dipped (for 10 s) into one of a number of different solutions of IBA (Duchefa Biochemie) concentrations (T1 - 0, T2 – 2,500, T3 – 5,000, T4 – 7,500 and T5 - 10,000 mg L^{-1}) dissolved in 50% ethanol and water. After that, they were immediately planted into the substrate

medium, comprised of peat / perlite (2:1; v:v) in black polypropylene trays, model CETAP 40A (40 wells 110 ml volume).

2.5. Experiment installation

Finally, the trays were transferred to a glasshouse with a computer controlled environment (CLIMAGRO v.2.1xp 2003 / INKOA SYSTEMS). Temperature was maintained at 25 ± 2 °C, relative humidity at 90% and irradiance at 450 Wm^{-2}, automatically regulated by a cooling system, fogging, and shade cloths, respectively. The cuttings remained under these controlled conditions for 60 days and then were transferred to the uncontrolled environment of a shade house for another 30 days.

2.6. Data collection and analysis

The experiment was a totally randomized design with a double factorial arrangement, consisting of five concentrations of IBA and five clones with three replications of eight minicuttings each.

Rooting, survival and callus formation were all calculated at the end of the trial period in the glasshouse (60 days), as a percentage of the number of initial cuttings. In addition, the number of primary roots and root morphological parameters of rooted cuttings were determined using an image analysis program from the scanner WinRhizo 2003 software (REGENT INSTRUMENTS, CANADA, WINPRO 2003b version). The root morphology parameters recorded were: total length (cm), projected area (cm^2), surface area (cm^2), diameter (mm), volume (cm^3) and the number of root apices.

At 90 days of culture, i.e. 30 days after the transfer to the shade house, the percentage of final survival of rooted cuttings was determined.

Data were subjected to the relevant statistical analysis using SPSS Inc.® Win TM, vs 12.0.

For the percentage data, we performed a chi-squared analysis and data were transformed with the arcsine function before applying an analysis of variance (ANOVA) to the square root of x/100, where x is the percentage of each variable. The original percentage data are presented in Tables 1 and 2. An ANOVA was used to detect differences and an *a posteriori* Tukey test was used to check significance ($\alpha < 0.05$).

3. RESULTS

The results obtained demonstrate significant differences ($P < 0.05$) between treatments with regard to survival, rooting and callus formation. The clones, however, only differed between themselves with respect to rooting and callus formation. An interaction effect between treatment and clone was also observed with respect to rooting. There was no difference in survival rate in the shade house (Table 1).

Rooting percentage was significantly higher in all treatment conditions compared to the control, but no significant differences between concentrations of IBA in the different treatment conditions were found. Callus formation was found to be lower with increasing concentration of IBA. Furthermore, at the highest IBA concentration, survival at 60 days was reduced although this effect disappeared after the shade house period, i.e., at 90 days of cultivation.

With regard to clones, the highest rooting percentage was for clone C_2671 (52.5%), although this difference was not significant compared to clone C_7810 (45.8%). Clone C_111 had the lowest rooting (6.7%). Survival, both at 60 and 90 days of culture, showed no significant differences and callus formation was inversely proportional to rooting percentage, in other words, the higher the rooting percentage, the lower the callus formation, and differences between clones were statistically significant.

The different clones showed different responses to the different concentrations of IBA with respect to rooting. The results in Table 2 show that C_3 and C_90025 clones present increased rooting percentage as IBA concentration increases. Clone C_2671 also followed this pattern, but the highest IBA concentration appears to have had an inhibitory effect. Differences in rooting percentage for different IBA concentrations for clones C_7810 and C_111 were not significant and indeed, for C_7810, the most suitable IBA concentration seems to have been the lowest, and for clone C_111, increasing concentration of IBA appears to have had no effect on rooting.

Thus these varying responses of the different clones suggest that, in general, each clone responds differently to IBA.

With respect to survival at 90 days (i.e, after 30 days in the shade house), analysis of variance (ANOVA) found no significant differences, when data for each

Table 1 – Survival, rooting and callus formation average of minicuttings of chestnut hybrids (C. crenata x C. sativa), on leaving the greenhouse, and final survival, on leaving the shade house, in response to IBA application with five clones.

Tabela 1 – *Médias da sobrevivência, do enraizamento e da formação de calo das miniestacas de híbridos de castanheiro (**C. crenata** x **C. sativa**), na saída da casa de vegetação, e da sobrevivência final, na saída da casa de sombra, de cinco clones em resposta a aplicação de AIB.*

Tratamentos		Sobrevivência	Enraizamento	Calo	Sobrevivência
AIB	mg.L^{-1}	60 dias (%)	60 dias (%)	60 dias (%)	90 dias (%)
T1	0	96,7 a	12,5 b	62,5 a	66,7 a
T2	2500	93,3 ab	33,3 a	46,7 ab	60,0 a
T3	5000	95,8 a	38,3 a	44,2 ab	69,6 a
T4	7500	90,8 ab	39,2 a	34,2 bc	68,1 a
T5	10000	80,0 b	40,8 a	20,8 c	73,5 a
Qui quadrado		<0,0001*	<0,0001*	<0,0001*	0,751 ns
Clones	C_3	94,2 a	26,7 cd	54,2 a	68,8 a
	C_7810	86,7 a	45,8 ab	26,7 b	72,7 a
	C_2671	96,7 a	52,5 a	20,8 b	74,6 a
	C_111	88,3 a	6,7 d	55,8 a	25,0 a
	C_90.025	90,8 a	32,5 bc	50,8 a	59,0 a
Qui quadrado		0,037*	<0,0001*	<0,0001*	0,039*
ANOVA	Tratamento	0,005*	0,001*	<0,0001*	0,263 ns
	Clone	0,142 ns	<0,0001*	<0,0001*	0,391 ns
	Trat. x Clone	0,394 ns	0,010*	0,244 ns	0,277 ns

ANOVA performed on data transformed using square root of x/100. * indicates significant differences, and ns indicates non-significance (P<0,05). Averages followed by the same letter do not differ according to a Tukey test ($\alpha < 0,05$).

Table 2 – Rooting average of minicuttings of chestnut hybrids (*C. crenata* x *C. sativa*), on leaving the greenhouse, and final survival on leaving the shade house, in response to IBA application with five clones, comparing the treatments within each clone (column), and the clones in each treatment (row).

Tabela 2 – *Enraizamento médio das miniestacas de híbridos de castanheiro (**C. crenata** x **C. sativa**), na saída da casa de vegetação, e da sobrevivência final, na saída da casa de sombra, de cinco clones em resposta a aplicação de AIB, comparando os tratamentos dentro de cada clone (coluna), e os clones dentro de cada tratamento (fila).*

Tratamentos			Enraizamento médio (%)		
AIB (mg.L^{-1}) / **Clone**	**C_3**	**C_7810**	**C_2671**	**C_111**	**C_90.025**
T1 – 0	4,2 b A	29,2 a A	16,7 b A	4,2 a A	8,3 b A
T2 – 2500	16,7 ab B	70,8 a A	45,8 ab AB	8,3 a B	25,0 b AB
T3 – 5000	29,2 ab AB	50,0 a AB	70,8 a A	8,3 a B	33,3 ab AB
T4 – 7500	29,2 ab BC	45,8 a B	83,3 a A	4,2 a C	33,3 ab BC
T5 – 10.000	54,2 a A	33,3 a A	45,8 ab A	8,3 a A	62,5 a A
Tratamentos			Sobrevivência media (%)		
T1 – 0	100,0 a A	71,4 a A	50,0 a A	100,0 a A	50,0 a A
T2 – 2500	50,0 a A	64,7 a A	72,7 a A	0,0 a A	50,0 a A
T3 – 5000	42,9 a BC	91,7 a A	82,4 a AB	0,0 a C	50,0 a BC
T4 – 7500	71,4 a A	63,6 a A	80,0 a A	0,0 a A	50,0 a A
T5 – 10.000	84,6 a A	75,0 a A	63,6 a A	50,0 a A	73,3 a A

ANOVA performed on data transformed using square root of x/100. *indicates significant differences, and ns indicates non-significance (P<0,05). Averages followed by the same letter do not differ according to a Tukey test (α<0,05). For each variable, the lower case letters compare treatments for a specific clone (column), while capitals compare the different clones within each treatment (row).

clone was analyzed individually at the different concentrations of IBA. Considering the results between clones for each concentration level, significant difference were only found in treatment T3, where C_7812 and C_2671 clones performed differently.

For the average number of roots (Table 3), no differences were observed between treatments, but there were differences between clones, though the interaction between them was not significant. On average, clone 7810 had the greatest number of principal roots

Table 3 – Average number of primary roots of minicuttings of chestnut hybrids (*C. crenata* x *C. sativa*), on leaving the greenhouse, in response to IBA application to five clones.

Tabela 3 – *Número médio de raízes primaria das miniestacas de híbridos de castanheiro (**C. crenata** x **C. sativa**), na saída da casa de vegetação, de cinco clones em resposta a aplicação de AIB.*

Tratamentos	Nº médio de raízes primaria						ANOVA
AIB (mg.L^{-1}) **/Clone**	**C_3**	**C_7810**	**C_2671**	**C_111**	**C_90.025**	**Media/Trat**	**Clone**
T1 – 0	2,00 — —	2,29 a A	2,00 a A	2,00 — —	1,50 a A	**2,07 a**	**0,927 ns**
T2 – 2500	1,50 a A	2,71 a A	2,27 a A	1,00 a A	2,00 a A	**2,28 a**	**0,443 ns**
T3 – 5000	3,29 a A	3,42 a A	2,76 a A	1,50 a A	2,25 a A	**2,87 a**	**0,469 ns**
T4 – 7500	2,29 a A	5,18 a A	3,80 a A	1,00 — —	2,00 a A	**3,53 a**	**0,154 ns**
T5 – 10.000	2,23 a A	4,75 a A	2,36 a A	2,00 a A	3,67 a A	**3,10 a**	**0,22 ns**
Media/Clone	2,38 AB	3,60 A	2,89 AB	1,50 B	2,67 AB		
ANOVA							
Tratamento	**0,544 ns**	**0,177 ns**	**0,187 ns**	**0,772 ns**	**0,397 ns**		**0,478 ns**
Clone							**0,037***
Trat x Clone							**0,844 ns**

*Indicates significant differences and ns indicates non-significance (P<0,05). Averages followed by the same letter do not differ according to a Tukey test (α< 0,05). For each variable, the lower case letters compare treatments for a specific clone (column), while capitals compare the different clones within each treatment (row). - indicates data that was not analysed due to there being less than two cases.

(3.60 units) and the clone with the lowest number was 111 (1.50 units). Analyzing each clone separately, differences between treatments are not significant.

The study of root morphological parameters (see Table 4), in general, showed no significant differences between the various morphological indicators analyzed, either between treatments or between clones, although the interaction between the parameters projected area, surface area and average diameter was significant. However, treatment with 5,000 mg L^{-1} of IBA appears to result in higher values for nearly all indicators, although these are only significant in the cases of total length and number of root apices compared to the control.

Table 4 – Average root morphology of minicuttings of chestnut hybrids (*C. crenata* x *C. sativa*), on leaving the greenhouse, in response to IBA application to five clones.

Tabela 4 – *Morfologia média das raízes das miniestacas de híbridos de castanheiro (**C. crenata** x **C. sativa**), na saída da casa de vegetação, de cinco clones em resposta a aplicação de AIB.*

Tratamentos	Morfologia					
AIB (mg.L^{-1})	Longitude total (cm)	Área projetada (cm^2)	Área superficial (cm^2)	Diâmetro médio (mm)	Volume radicular (cm^3)	Nº de ápices de ápices radiculares
T1_0	17,15 b	4,16 a	13,07 a	2,02 a	0,285 a	26,7 b
T2_2500	22,36 ab	4,83 a	15,16 a	2,19 a	0,335 a	40,8 ab
T3_5000	27,19 a	5,49 a	17,24 a	2,07 a	0,327 a	44,8 a
T4_7500	22,05 ab	5,25 a	16,49 a	2,32 a	0,372 a	34,0 ab
T5_10000	20,41 ab	4,18 a	13,13 a	2,01 a	0,310 a	35,7 ab
Clones_Nº						
C _3	18,36 a	4,39 a	13,78 a	2,26 a	0,255 a	30,5 a
C_7810	25,97 a	5,75 a	18,08 a	2,24 a	0,365 a	40,1 a
C_2671	22,62 a	4,63 a	14,55 a	2,02 a	0,336 a	39,4 a
C_111	15,61 a	3,68 a	11,55 a	1,95 a	0,214 a	28,1 a
C_90025	22,43 a	4,58 a	14,39 a	2,06 a	0,364 a	40,3 a
ANOVA						
Tratamento	0,31 ns	0,551 ns	0,551 ns	0,307 ns	0,478 ns	0,334 ns
Clone	0,053 ns	0,069 ns	0,069 ns	0,434 ns	0,103 ns	0,213 ns
Trat x Clone	0,458 ns	0,041 *	0,041 *	0,020 *	0,680 ns	0,485 ns

Indicates significant differences, and ns indicates non-significance (P<0,05). Averages followed by the same letter do not differ according to a Tukey test (α < 0,05).

4. DISCUSSION

The results indicate that the application of IBA to the rooting of chestnut mini-cuttings originating from clones multiplied by the traditional stooling system of vegetative propagation for this species has a significant effect. Azcón-Bieto and Talon (1993) claimed that the increased capacity of cuttings subjected to different IBA concentrations to form adventitious roots is related to the stimulatory effect on root differentiation of auxins. This view was corroborated by Wigmore and Woods (2000), who recommended using plant growth regulators or not depending on the species in question: for example, 10,000 mg L^{-1} of IBA for *Tsuga heterophylla* and between 5,000 and 10,000 mg L^{-1} for *Pseudotsuga menziesii*, the lower amount being appropriate for semi-lignified cuttings and the higher for completely lignified cuttings.

Similar results were found by Zuffellato-Ribas and Rodrigues (2001), working with *Eucalyptus grandis*, where they observed that the best results for rooting cuttings was treatment with 6,000 or 8,000 mg L^{-1} of IBA, both of which resulted in rooting success of 64%.

In relation to survival in this experiment, increase in IBA concentration had no influence, and in fact could have even reduced survival since the cuttings subjected to the highest IBA concentration had the lowest survival rate after 60 days. This corroborates the work of Wendling and Xavier (2005), who found that IBA application at concentrations of 0, 500, 1,500 and 3,000 mg L^{-1}, had no effect on the rooting or survival of four clones of *Eucalyptus grandis*, while noting that for certain clones, general plant vigor was negatively affected by concentrations of over 500 mg L^{-1}.

The high survival rates of the mini-cuttings of all clones at the end of the glasshouse period (60 days) may be related, as Wendling and Xavier (2005) suggest, to the fact that the control of the ambiental conditions in the glasshouse favors the survival of vegetative propagules.

However, survival at the end of the period in the shade house (90 days) was not influenced by IBA application, confirming the view of Wendling and Xavier (2005). These same authors observed that the vegetative propagation of clones with lower degrees of rejuvenation was more efficient, corroborated to a certain extent by the results of this work, where we used material from the first productive pruning of mother plants produced in the traditional manner from woody donor plants which therefore produce semi-woody cuttings with a degree of lignification. The results of this work are comparable with those obtained by Gonçalves et al. (1999) who, propagating hybrid chestnut (*Castanea sativa* x *C. crenata*) using adult material, obtained good results in terms of rooting and survival, in *in vitro* induction using 3 mg L^{-1} IBA (97% rooting and 50% survival) or in rapid *ex vitro* induction using 1 g L^{-1} IBA (77% and 100% respectively) (GONÇALVES et al.,1998), confirming the role of rejuvenation.

In addition, the best rooting response of three of the five clones used in this experiment (C_3, C_2671 and C_ 90025) was obtained with different IBA treatments. This support the hypothesis of Assis and Teixeira (1999) that genotype is one of the factors that influence rooting and that there is wide variation between species, cultivars and clones in relation to the greater or lesser natural ability to form roots. Similar results to those of the present study were found by Cuenca et al. (2009), in the micro-propagation of *Castanea sativa* Mill. hybrids, who found that the percentage of rooting varies widely, depending on the genotype.

According to Van Staden and Harty (1987), a high auxin/cytokinin ratio, characteristic of juvenile plants, determines root primordia formation while an intermediate ratio induces callus formation and a low ratio promotes leaf bud formation. This work provides support for this hypothesis in that it demonstrates an inverse relationship between callus formation and IBA concentration.

With respect to number of primary roots, Stumpf et al. (2001), working with *Chamaecyparis lawsoniana* Parl., demonstrated that the number of roots increased when using concentrations from zero up to 10,000 mg L^{-1}, where it peaked with an average of 3.31 roots, confirming the beneficial effect of IBA for this type of cutting. MacDonald (1995) found that auxins increase the number and quality of roots and promote uniform rooting. However, it must be noted that the effects of these compounds varies considerably between genus, species and, in many cases, cultivars. In addition, Hartmann and Kester (1998) describe that treating cuttings with auxin-type growth regulation substances increases the percentage of cuttings which form roots and accelerates their initiation, increases the number and quality of roots produced by individual cuttings and increases uniformity of roots. These assertions are

supported by the present work where, while cuttings were not uniformly affected by the use of IBA, there was a beneficial effect of IBA treatment in certain clones.

Root morphological indices appear to be positively affected by high concentrations of IBA, which, according to Foster et al. (2000), results in a greater number of roots and the improved functioning of the plant in the field. In addition, the root system is the main factor involved in successful survival and good early growth after transplant into the field (DURYEA, 1984). Rose et al. (1990) state that a well-developed root system can increase water and nutrient absorption potential, which results in an increased growth potential, and thus plant survival. However, this can also influence the rate of transpiration and gas exchange, since, according to Royo et al. (1998), having more branched roots means increased plant stability and a greater ability to exploit the top layers of the soil profile. Primary lateral roots also constitute the basic branching for the production of new roots, which are not only important in the absorption of water and mineral nutrients, but also in mycorrhizal associations (THOMPSON; SCHULTZ, 1995).

Finally, high concentrations of IBA, such as 5,000 and 10,000 mg $L^{-1,}$ have also been indicated for other forest species, such as in the works carried out by Higashi and Gonçalves (2000) and Wigmore and Woods (2000).

5. CONCLUSIONS

In general, the vegetative propagation by minicuttings of hybrid clones of *Castanea sativa* Mill. appears to be a viable alternative for the production of this interesting forest species.

The use of IBA significantly favors rooting and survival of chestnut mini-cuttings, reaching good levels for this species which is generally considered recalcitrant.

The different responses between the clones studied in terms of rooting and survival suggest the value of making adjustments to the mini-propagation process to adapt the technique to the particular material in question.

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7. REFERENCES

ALCANTARA, G. B.; RIBAS, L. L. F.; HIGA, A. R.; RIBAS, K. C. Z. Efeitos do ácido indolbutírico (AIB) e da coleta de brotações em diferentes estações do ano no enraizamento de miniestacas de *Pinus taeda* L. **Scientia Forestalis**, v.36, n.78, p.151-156, 2008.

ASSIS, T.F.; TEIXEIRA, S.L. Enraizamento de plantas lenhosas. In: TORRES, A.C.; CALDAS, L.S.; BUSO, J.A. **Cultura de tecidos e transformação genética de plantas**. Brasília: Embrapa-SPI/Embrapa-CNPH, 1999. p.261-296.

ASSIS, T.F.; FETT-NETO, A.G.; ALFENAS, A.C. Current techniques and prospects for the clonal propagation of hardwoods with emphasis on *Eucalyptus*. In: WALTER, C.; ARSON, M. (Ed.). **Plantation forest biotechnology for the 21th century**. Kerala: Research Signpots, 2004. p.303-333.

AZCON-BIETO, J.; TALON, M. **Fisiología y bioquímica vegetal**. Interamericana. Madrid: 1993. 581p.

BORGES, S. R.; XAVIER, A.; OLIVEIRA, L. S.; MELO, L. A.; ROSADO, A. M. Enraizamento de miniestacas de clones híbridos de *Eucalyptus globulus*. **Revista Árvore**, v.35, n.3, p.425-434, 2011.

CUENCA, B.; GONZÁLEZ, L.; FERNÁNDEZ, M. R. Micropropagación de genotipos adultos de *Castanea sativa* Mill. seleccionados por resistencia a *Phytophthora cinnamomi*. En: CONGRESO FORESTAL ESPAÑOL,5., 2009. **Libro de Resúmenes.** Ávila. España. 21-25 Sept 2009. 10p.

DURYEA, M. Nursery cultural practices. Impacts on seedling quality. In: DURYEA, M. (Ed**.). Evaluating seedling quality: principles, procedures and predictive abilities of mayor test.** 1984. Corvallis: Oregon State University, Forest Research

Laboratory, 1984. 143p.

FOSTER, G.S.; STELZER, E.H.; McRAE, J.B. Loblolly pine cutting orphological traits: Effects on rooting and field performance**. New Forests**, v.19. n.3. p.291-306, 2000.

GONÇALVES, J.C.; AMÂNCIO, S.; PEREIRA, J.G. In vitro propagation comparative study of 7 chestnut hybrid clones *C. sativa x C. crenata*. In: **Proceeding International Congrress on Chestnut**. Spoleto, Italy, 1993. p.211-214.

GONÇALVES, J.C.; DIOGO, G.; AMÂNCIO, S. *In vitro* propagation of chestnut (*Castanea sativa* x *C. crenata*): Effects of rooting treatments on plant survival, peroxidase activity and anatomical changes during adventitious root formation. **Scientia Horticulturae,** v.72, n.3-4, p.265-275, 1998.

GONÇALVES, J. C.; DIOGO, G.; COELHO, M. T.; AMANCIO, S. Effect of rooting conditions on survival and growth during acclimatization of micropropagated chestnut plants (*Castanea sativa* x *Castanea crenata*). **Acta Hortic**ulturae, n.494. p.235-241, 1999.

GOMES, A.L. **Propagação clonal:** princípios e particularidades. Vila Real: Universidade de Trás-os-Montes e Alto Douro, 1987. 69p. (Série Didáctica, Ciências Aplicadas, 1).

HARTMAN, H.T.; KESTER, D. **Propagación de plantas**: principios y prácticas. Trad. A. Marino. 3ª. ed. México, DF: Continental, 1998. 760p.

HARTMANN H.T.; KESTER, D.E.; DAVIS, F.T.; GENEVE, R.L. **Plant propagation: principles and practices**. 6th. ed. New Jersey: Prentice Hall, 2002. 770p.

HASBÚN, R.; VALLEDOR, L.; BERDASCO, M.; SANTAMARIA, E.; CAÑAL, M. J.; RODRÍGUEZ, R.; SÁNCHEZ, M. *In vitro* proliferation and genome DNA methylation in adult chestnut. **Acta Hort**, n.693, p.333-340, 2005.

HIGASHI, E.N.; GONÇALVES, A.N. Uso de ácido indolbutírico no enraizamento de eucaliptos. **IPEF Notícias**, v.24, n.148, p.4-5, 2000.

KAMLESH, K.; SWAMY, S.; SEHGAL, R.; KHOSLA, P. Effects of auxins and carbendazim on rooting juvenile and mature stem cuttings of Grewia optiva. **Indian Journal of Forestry**, v.18, n.1, p.61-65, 1995.

MACDONALD, B. **Principles of vegetative propagation, clonal selection and stock (Mother)**. Practical woody plant propagation for nursery grower. Portland: Timber Press, 1995. 668p.

MAJADA, J.; MARTÍNEZ-ALONSO, C.; FEITO, I.; KIDELMAN, A.; ARANDA, I.; ALÍA, R. Minicuttings: an effective technique for the propagation of *Pinus pinaster* Ait. **New Forest**, v.41, n.3, p.399-412. 2011.

MARTÍNEZ-ALONSO, C.; KIDELMAN, A.; FEITO, I.; VELASCO, T.; ALÍA, R.; GASPAR, M. J.; MAJADA, J. Optimization of seasonality and mother plant nutrition for vegetative propagation of *Pinus pinaster* Ait. **New Forest**, v.43, n.5-6, p.651-663, 2012.

MILLER, G.; MILLER, D.D.; JAYNES, R.A. Chestnuts. In: Janick, J.; Moore, J.N. **Fruit breeding.** New York: John Wiley & Sons, 1996. v.3. p.99-124.

MILLER-ROETHER, V.; LEE, P.E.; SZENDRAK, E. Successful *in vitro* micropropagation of purported blight-resistant chestnuts (*Castanea* spp.). In: ANNUAL INTERNATIONAL CONFERENCE OF THE AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE, 96., 1999, Minneapolis, Minnesota USA; **Abstracts.** HortScience**,** v. 34, n. 3, p.460. 1999.

PEREIRA-LORENZO, S.; FERNÁNDEZ-LÓPEZ, J.; MORENO-GONZÁLEZ, J. Variabilidad morfológica en cultivares de castaño *(Castanea sativa* Mill.) en Galicia: Valores descriptivos. **Revista Investigación Agrária,** v.11, n.2, p.213-237, 1996a.

PEREIRA-LORENZO, S.; FERNÁNDEZ-LÓPEZ, J.; MORENO-GONZÁLEZ, J. Variability and grouping of northwestern spanish chesnut cultivars. I. morphological Traits. **Journal of the American Society for Horticultural Science**, v.121, n. 2, p.183-189, 1996b.

PEREIRA-LORENZO, S.; FERNÁNDEZ-LÓPEZ, J.; MORENO-GONZÁLEZ, J. Variability and Grouping of Northwestern Spanish Chesnut Cultivars. II. Isoenzyme Traits. **The Journal of the American Society for Horticultural Science**, v.121, n.2, p.190-197, 1996c.

PEREIRA-LORENZO, S.; FERNÁNDEZ-LÓPEZ, J. Description of 80 Cultivars and 36 Clonal Selections of Chesnut *(Castanea sativa* Mill.) from Northwestern Spain. **Fruit Varieties Journal, v.**51, n.1, p.13-27, 1997.

PEREIRA-LORENZO, S.; FERNÁNDEZ-LÓPEZ, J.; VARELA ARIAS, I.; SAU, F. Comportamiento de patrones híbridos de castaño resistentes a la tinta en zonas de castañar en Galicia. **Investigación Agraria. Sistemas y Recursos Forestales,** v.9, n.1, p.89-101, 2000.

RÍOS, D., AVILÉS, F.; SÁNCHEZ-OLATE, M.; ESCOBAR, R.; PEREIRA, G. Variación de la tasa de enraizamiento asociada al número de subcultivo y diámetro de microtallos de castaño *Castanea sativa* MILL. **Agricultura Técnica,** v.65, n.3, p.258-264, 2005.

ROSE, R.; CARLSON, W; MORGAN, P. The targey seedling concept. In: ROSE, R.; CAMPBELL, J.; LANDIS, T. (Eds.) Tanget. Seedling Symposium. **Proceedings**. Combined meeting of the western forest nursery associations. Washington: USDA Forest Service, 1990. p.1-8. (General Technical Repert, 200)

ROYO, A.; BRICHLER, T.; ROSE, R. W.; MINGUEZ, M. P. La Plántula ideal: revisión del concepto, parámetros pp 1-8. definitorios e implementación práctica. **Investigación Agraria,** v.7, n.1, p.109-122, 1998.

SÁNCHEZ, M.C.; BALLESTER, A.; VIEITEZ, A.M. Reinvigoration treatments for the micropropagation of mature chestnut trees. **Annales des Sciences Forestières,** v.54, p.359-370, 1997.

SIERRA, C. **Propagación vegetativa de** *Castanea sativa* Mill. **y** *Juglans regia* L., a través de estacas. 2001. 48p. Tesis (Ingeniero Forestal) - Universidad de Concepción, Facultad de Ciencias Forestales, Concepción, Chile. 2001.

STUMPF, E.R.T.; GROLLI, P.R.; SCZEPANSKI, P.H.G. Efeito do ácido indolbutírico, substrato e tipo de estaca no enraizamiento de *Chamaecypris lawsoniana* Parl. **Revista Brasileira de Agrociência**, v.7, n.2, p.101-105, 2001.

THOMPSON, J.; SCHULTZ, R. Root System Morphology of *Quercus rubra* L. Planting stock and 3 year field performance in Lowwa. **New Forest,** v.9, n.3, p.225-236, 1995.

TITON, M.; XAVIER, A.; OTONI, W.C. Dinâmica do enraizamento de microestacas e miniestacas de clones de *Eucalyptus grandis.* **Revista Árvore**, v.26, n.6, p.665-673, 2002.

TITON, M.; XAVIER, A.; OTONI, W. C.; REIS, G. D. Efeito do AIB no enraizamento de miniestacas e microestacas de clones de *Eucalyptus grandis* W. Hill ex Maiden. **Revista Árvore**, v.27, n.1, p.1-7, 2003.

van STADEN, J.; HARTY, A.R. Cytokinins and adventitious root formation. In: DAVIES, T.D.; HAISSIG, B.E.; SANKHLA, N. **Adventitious root formation in cuttings**. Portland: Dioscorides Press, 1987. p.185-201. (Advances in Plant Sciences Series, 2).

WENDLING, I. Propagação clonal de híbridos de *Eucalyptus* spp. por miniestaquia. 1999. 70 f. Dissertação (Mestrado em Ciência Florestal) - Universidade Federal de Viçosa, Viçosa, 1999.

WENDLING, I.; XAVIER, A.; GOMES, J. M.; PIRES, I. E.; ANDRADE, H. B. Efeito do regulador de crescimento AIB na propagação de clones de *Eucalyptus* spp. por miniestaquia. **Revista Árvore**, v.24, n.2, p.187-192, 2000.

WENDLING, I.; XAVIER, A. Gradiente de maturação e rejuvenescimento aplicado em espécies florestais. **Floresta e Ambiente**, v.8, n.1, p.187-194, 2001.

WENDLING, I.; XAVIER, A. Miniestaquia seriada no rejuvenescimento de clones de *Eucalyptus*. **Pesquisa Agropecuária Brasileira**, v.38, n.4, p.475-480, 2003.

WENDLING, I.; XAVIER, A. Influência do ácido indolbutírico e da miniestaquia seriada no enraizamento e vigor de miniestacas de clones de

Eucalyptus grandis. **Revista Árvore**, v.29, n.6, p.921-930, 2005.

WIGMORE, B.G.; WOODS, J.H. **Cultural procedures for propagation of rooted cuttings of Sitka spruce, western hemlock, and Douglas-fir in British Columbia.** Working Paper - Ministry of Forests Research Program, British Columbia, n.46, 2000. 30 p.

XAVIER, A.; WENDLING, I. **Miniestaquia na clonagem de *Eucalyptus***. Viçosa, MG: SIF, 1998. 10p. (Informativo Técnico, 11).

ZUFFELLATO-RIBAS, K.C.; RODRIGUES, J.D. Relações entre época do ano e diferentes concentrações de ácido indolbutírico no enraizamiento de estacas de *Eucalyptus grandis*. **Boletim de Pesquisa Florestal**, n.42, p.71-80, 2001.