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STORAGE TIME EFFECT ON MINI-CUTTINGS ROOTING IN *Tectona grandis* LINN F. CLONES¹

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ABSTRACT – The study aimed to evaluate the influence of storage length on *Tectona grandis* mini-cuttings survival and rooting. A factorial arrangement (4 x 7) was utilized, based on four clones (Carapá, Ipê, GU5 and TB7) and seven time intervals from mini-cuttings harvesting until final sowing (0, 1, 2, 4, 8, 12 and 16 hours). A randomized block design with three replicates and 16 mini-cuttings per experimental unit was utilized. Survival and rooting rates were evaluated after greenhouse culture (30 days after sowing) and after shadow house culture (40 days after sowing); as well as height, collar diameter, aerial and root biomass 55 days after sowing. No significant differences were observed in survival and rooting rates among time intervals in teak mini-cuttings preparation from these four clones. However differences among clones were registered for rooting rate, suggesting a genotypic effect. Survival and rooting rates were very high after greenhouse culture (93% and 90% respectively), as well as survival after culture in a shadow-house (88%).

Keywords: vegetative propagation, clonal forestry, teak.

INFLUÊNCIA DO TEMPO DE ARMAZENAMENTO NO ENRAIZAMENTO DE MINIESTACAS DE CLONES DE *Tectona grandis* LINN F.

RESUMO – Este estudo teve como objetivo avaliar a influência do período entre coleta/preparo e estaqueamento na sobrevivência e enraizamento de miniestacas de clones de *Tectona grandis*. O delineamento experimental foi em arranjo fatorial (4 x 7), considerando quatro clones (Carapá, Ipê, GU5 e TB7) que são parte do programa de melhoramento genético da empresa Agrícola VerdeNovo, selecionados em Mato Grosso, Brasil. Com sete períodos de tempo (tratamentos) entre a coleta/preparado e o estaqueamento das miniestacas (0, 1, 2, 4, 8, 12 e 16 horas), em delineamento estatístico de blocos ao acaso, com três repetições e parcelas de 16 miniestacas. Avaliaram-se a sobrevivência e o enraizamento de miniestacas na saída da casa de vegetação (30 dias após estaqueamento) e da casa de sombra (40 dias após estaqueamento), o crescimento em altura e diâmetro do colo, a biomassa da parte aérea e do sistema radicular aos 55 dias após estaqueamento. Os resultados evidenciam que não há influência significativa do intervalo de tempo, entre coleta/preparo e estaqueamento de miniestacas de teca, na sobrevivência e no enraizamento dos quatro clones estudados. Foi constatada a existência de efeito genotípico no enraizamento dos quatro clones avaliados, os quais obtiveram altas percentagens de sobrevivência e de enraizamento na saída da casa de vegetação (93% e 90% respectivamente), assim como sobrevivência na saída da casa de sombra (88%).

Palavras-Chave: propagação vegetativa, silvicultura clonal, teca.

1. INTRODUCTION

Teak (*Tectona grandis*) belongs to Lamiaceae botanical family and it is a pioneer heliophytes, deciduous tree species of India, Myanmar, Thailand and Laos forests. It was introduced and planted commercially in tropical Africa, Australia, Pacific islands, Central and South America (White, 1991; Pandey and Brown, 2000).

Teak wood is one of the most important in the world and its economic value depends on the stem diameter and wood color (CTF, 1990). Its main wood characteristics are durability, stability, pre-treatment facility and natural resistance. It is used for luxury furniture, naval building and interior and exterior housing decoration (Goh and Galiana, 2000). The main consumer markets are England, United States, Nederland, Denmark, France, South Africa and China, beyond some Middle East countries (OLIVEIRA, 2003).

The teak plantings are traditionally based on seedlings, though some work has already been conducted by aiming to evaluate the capacity of these tree species concerning the vegetative propagation using mini-cuttings techniques, grafting and micro propagation (MASCARENHAS; MURALIDHARAN, 1993; MONTEUUIS et al., 1995). Since the beginning of 1990 has been developed in Costa Rica protocols for clonal production of materials at large scale using mini-cuttings technique (MURILLO et al. 2013).

Many factors can influence mini-cuttings rooting, among them, the occurrence of injuries, hormonal balance, genetic constitution of the mother plant, endogenous levels of regulator inhibitors, reaction of oxidation, rate of maturation/juvenility of propagule, nutritional and hydric conditions of the plant donor of propagules, time spent from harvesting/preparation to the propagation stocking and, environmental conditions (WENDLING, 2002; PAIVA; GOMES, 2005; GOULART; XAVIER, 2008; ALFENAS et al., 2009; XAVIER et al., 2013).

For the success of **mini-cuttings** production it is important to take in account strategies related to culture practices of the clonal garden (phytosanitary control, nutrition, environment conditions), just as well as factors related to the production scale, such as distance between the clonal garden and the rooting facilities, time demanded for the mini-cuttings preparation, as well as the operational need of mini-cuttings storage before field dispatch (ASSIS et al., 1992; ALFENAS

et al. 2009).

According to Xavier et al. (2013) and Goulart and Xavier (2008) storage time of mini-cuttings and rooting success depends on the following conditions: relative humidity, temperature, species, pathogen, plant growth conditions given of propagule and the harvesting season of growth destined to mini-cuttings process. The mini-cuttings should be harvested in its maximum vegetative vigor and turgidity, due to its vulnerability to bear the hydric stress, in front of the difficulty of the tissue without having yet a root system.

Generally, among the practices to make possible a longer storage time of mini-cuttings, it is highlighted temperature reduction, increase of relative humidity, light reduction, and antiperspirant application. These conditions intend to keep vigor, turgidity and to minimize the growth physiological activities, looking for to guarantee the maximum potential of rooting of mini-cuttings (XAVIER et al., 2013). Ferrari et al. (2014), recommend time intervals below 15 minutes, however, in situation of long distances of growth harvesting and a big number of plants to be produced, it is necessary to store mini-cuttings for longer periods (ALFENAS et al., 2009).

Thereby, this work intended to evaluate the effect of different time intervals between harvesting and mini-cuttings preparation and, their final sowing for rooting, based on four *Tectona grandis* clones.

2. MATERIAL AND METHODOLOGY

This research was developed from October 2013 to January 2014, in the forest greenhouse of the agricultural company Verde Novo Ltda, located in Colider, Mato Grosso, Brazil. Mato Grosso has the *Aw* climate, classified by Köppen, characterized as rainy tropical with a clear dry season of two months and annual average temperature about 25° C, with annual average precipitation about 2.200 mm, latitude of 10°57'2" S, longitude 55°32'55" W and average altitude of 256 m (MATO GROSSO, 2008).

The mini-cuttings were harvested from four clones of *Tectona grandis* (Carapá, Ipê, GU5 e TB7) in the mini-clonal hedge which are part of the breeding program of Verde Novo. These genotypes were selected in plantations throughout Mato Grosso Brazil.

2.1. Management of clonal mini-clonal hudge

In accordance to teak mini-cuttings propagation protocols adopted by Verde Novo Ltda, the mini-clonal hudge was established in a greenhouse, covered by transparent polyethylene and under shading of 60% on the walls and roof for luminosity reduction, by aiming to keep temperature below 35° C and relative humidity above 85%.

The mini-clonal hudge was developed based on mini-cuttings rooted and planted at a spacement of 10x10 cm, in asbestos cement channel, with RCSW (Reinforced Cement with Synthetic Wires), filled with gravel at the bottom and washed sand in the upper.

The mineral fertilization of the mini-clonal hudge was executed with a nutritive solution composed by calcium nitrate, (0,5 g L⁻¹), potassium nitrate, (0,5 g L⁻¹), ammonium phosphate (0,15 g L⁻¹), boric acid (2,5 mg L⁻¹), sodium molibden (2,5 mg L⁻¹), copper chelate (0,0015 mL L⁻¹), zinc chelate (0,0005 mL L⁻¹), manganese chelate (0,0005 mL L⁻¹), iron chelate (0,0005 mL L⁻¹), applied through an automatized drip irrigation system, activated once a day, in the afternoon. The nutrition solution excess was dried out in the channel bottom and discarded. The irrigation was accomplished by an sprinkler system, activated two or five times a day, directing to keep the temperature below 35 °C and relative humidity above 85% inside the greenhouse.

2.2. Harvesting, mini-cuttings preparation, sowing and rooting

The sprouts were harvested in the clonal mini-clonal hudge, chopped to 4-6 cm length and leaving two pairs of leaves, that were reduced by scissors to a quarter of its original area. To maintain the turgidity conditions of the vegetative material, the mini-cuttings were placed inside a cooler box. The mini-cuttings were previously sprayed manually every five minutes until storage into the cooler box. Once the mini-cuttings were harvested and prepared, they received the following treatments: T0 – s immediately after the preparation (0 hours); T1, T2, T3, T4 and T5 regarding the mini-cuttings time storage, like 1, 2, 4, 8, 12 and 16 hours respectively. The mini-cuttings were stored in sealed cooler boxes and covered. Inside the cooler box was placed at the bottom a layer of humid substrate (composed by sphagnum peat, expanded vermiculite, carbonized rice shell) in order to keep humidity. In the

storage time, the boxes were placed inside the greenhouse under temperature controlled conditions (25-35 °C) and relative humidity above 85%.

For rooting, it was applied AIB according to the company protocol (1000 mg L⁻¹) in the mini-cuttings basis, subsequently it was sowed into the Ellepot papers band ELLEGAARD (6 cm height and 3,5 cm of diameter) filled with the commercial substrate CAROLINA II BR (composed by sphagnum peat (40%), expanded vermiculite (34,5%), carbonized rice shell (24%), dolomitic limestone (1%), gypsum (0,5%), fertilizer NPK (trace), pH 5,5 and electric conductivity 0,7 mS cm⁻¹, and placed inside the greenhouse.

Once the mini-cuttings were sowed in the Ellepot, they were transferred into the shade house, in order to promote rooting. This area was covered by transparent polyethylene and polyethylene shade at 60% in the walls and roof area, in order to reduce luminosity and keeping the temperature under 35°C and relative humidity above 95%. An irrigation frequency of 30 seconds each 20 minutes was established and the mini-cuttings remained for 15 days in these conditions. After rooting stage, they were kept in acclimatization area under same shade house conditions, but with an irrigation frequency of 30 seconds each 60 minutes, staying there for 15 more days. Next, they were transferred to the shade house with polyethylene and shading only in the roof where remained for 10 days and finally, rooted mini-cuttings were transferred to the growth yard in full sunlight, for the final evaluations at day 55 after sowing.

The factorial arrangement (4 x 7) was adopted, considering the four clones in study (Carapá, Ipê, GU5 e TB7) and the seven storage hours (0, 1, 2, 4, 8, 12 e 16 hours) of the mini-cuttings before planting, in a statistical factorial randomized block design, with three repetitions and parcels of 16 mini-cuttings.

2.3. Experimental evaluations

Mini-cuttings survival percentage (SCV) and rooting rate (ENR) were assessed in the greenhouse (after 30 days of sowing) and shade house (SCS) (after 40 days of sowing). In the full sunlight area (55 days after sowing), survival, height (h), collar diameter (ld), weight of dry aerial biomass (WAP) and root biomass (WRS) of all plants were evaluated. In the WAP and WRS determination there were taken four mini-cuttings per repetition as sample for the biomass assessment.

From the data collected for the characteristics evaluated, it was performed an analysis of variance and, a Tukey test at 95% probability, using the SAS program System for Windows (Statistical Analysis System), version 6.12.

3. RESULTS

Regarding the time between collection/preparation of mini-cuttings and sowing them, it was analyzed survival (SCV) and rooting characteristics (ENR) at the greenhouse stage and, survival at the shade house outcome (SCS) (Table 1). Significant differences on rooting capacity among clones were detected, indicating the existence of genetic effects on this trait. Nevertheless, no significant differences were detected between all time storage of mini-cuttings tested, neither the interaction “clone x treatment – storage time” interaction, by the F test ($P < 0.05$) for the evaluated characteristics.

Variation coefficients ranged from 7.56% to 9.05% highlighting experimental precision concerning the studied characteristics, in agreement with reported in literature (XAVIER; COMÉRIO, 1996; Zuffellato-Ribas, 1997; WENDLING et al., 2000; TITON, 2001; WEDLING, 2002).

The results presents a high survival index (SCV) and rooting rate (ENR) of mini-cuttings in the greenhouse and survival (SCS) in the shade house. Ipê clone registered higher values, followed by Carapá, Gu5 and TB7 clones. It was observed a reduction in mini-cuttings survival rates, as well as in the shade house, in relation to survival inside greenhouse conditions, notwithstanding, keeping the same trend (Figure 1)

With regard to the results obtained for the characteristics height (h) and collar diameter (ld), evaluated at full sunlight stage (55 days after sowing), it was observed significant different between clones tested (table 2). Nonetheless, it was not observed significance differences among the application of different storage time, neither in the interaction “clone x storage time”, by F test ($P < 0.05$).

Experimental variation coefficients for the characteristics total height and collar diameter, were 14.36% and 7.06% respectively, presenting good levels of experimental precision, conform to the values found in other studies RIBAS, 1997; WENDLING et al., 2000, TITON. 2003).

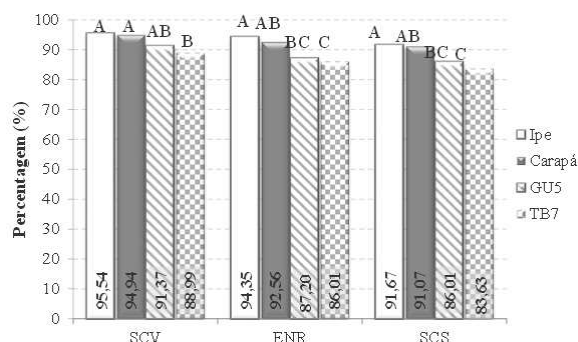


Figure 1 – Minicuttings survival percentage (SCV) and rooting (ENR) after greenhouse, as well as survival after shadowhouse (SCS) from four *Tectona grandis* clones. Means under same letter are non-significantly different at $p > 95\%$ probability based on Tukey’s test.

Figura 1 – Percentual de sobrevivência (SCV) e de enraizamento (ENR) de miniestacas na saída da casa de vegetação e de sobrevivência na saída da casa de sombra (SCS), dos quatro clones de *Tectona grandis*. Médias seguidas da mesma letra não diferem entre si, pelo teste de Tukey, a 95% de probabilidade.

The results of height (h) and collar diameter (ld) on 55-days-cuttings among treatments (Figure 2), indicated significant difference between the clones Carapá and Ipê, in comparison to clones GU5 e TB7.

About the results obtained from aerial and root biomass (55 days after sowing), it was observed by analysis of variance (Table 3), statistics differences between clones, but not regarding time between collection/preparation and sowing, as well as for interaction “clone x storage term”, by F test ($P < 0.05$).

When Tukey test was applied, to 95% of probability for dry biomass data (g), of aerial part of seeding to 55 days of sowing, it was determined that there is not significant differences according to the storage time of mini-cuttings. However, clones Carapá and Ipê (1.21 e 1.20 g) differ statistically from GU5 e TB7 (1.00 e 0.98 g).

In terms of dry biomass of rooting system, for the four clones studies under the conditions tested, significant differences were not registered (Tukey test, at level of 95 % of probability) on storage times assessed.

4. DISCUSSION

The differences found in this study, concerning the survival and rooting in greenhouse happen more

Table 1 – Summary of variance analyses on survival (SCV) and rooting (ENR) after greenhouse period, as well as survival after shadowhouse period (SCS), as a response to storage of minicuttings after harvest/preparation, from four *Tectona grandis* clones.

Tabela 1 – Resumo da análise de variância de sobrevivência (SCV) e enraizamento (ENR) na saída da casa de vegetação e de sobrevivência na saída da casa de sombra (SCS) em função da resposta ao armazenamento entre coleta/preparo e estaqueamento de miniestacas de quatro clones de *Tectona grandis*.

Source of Variation	DF	SCV (%)			ENR (%)			SCS (%)		
		Mean square	F	P	Mean square	F	P	Mean square	F	P
Clone (C)	3	200.27	4.91	0.0115**	344.12	5.86	0.0057***	321.18	3.91	0.0259**
Treat. (T)	5	105.25	2.58	0.0554 ^{ns}	148.03	2.52	0.0599 ^{ns}	117.65	1.43	0.2564 ^{ns}
(C) * (T)	15	40.76	0.83	0.6582 ^{ns}	58.748	0.88	0.5991 ^{ns}	82.10	1.45	0.1463 ^{ns}
Mean			92.71			90.03			88.09	
CV _{exp} (%)			7.56			9.05			8.53	

^{ns}, *, ** and *** : non significant at 0.05; significative at 0.05; 0.01 and 0.001 probability (P), respectively for the F test.

Table 2 – Summary of variance analyses for heigth (h) and collar diameter (dc), as a response function of minicuttings storage after harvest/preparation, from four *Tectona grandis* clones after 55 days-age.

Tabela 2 – Resumo da análise de variância para altura (h) e diâmetro de colo (dc), em função da resposta ao armazenamento entre coleta/preparo e estaqueamento de miniestacas de quatro clones de *Tectona grandis*, após 55 dias do estaqueamento.

Source of variation	DF	h (cm)			dc (mm)		
		Mean square	F	P	Mean square	F	P
Clone (C)	3	68.119	21.21	<.0001**	0.956	12.31	0.0001**
Treat (T)	6	0.658	0.20	0.9707 ^{ns}	0.03	0.5	0.8016 ^{ns}
(C)* (T)	18	3.210	1.31	0.2196 ^{ns}	0.077	1.15	0.335 ^{ns}
Mean		10.9			3.68		
CV (%) exp.		14.36			7.06		

^{ns}, *, ** and *** : non significant at 0.05; significative at 0.05; 0.01 and 0.001 probability (P), respectively for the F test.

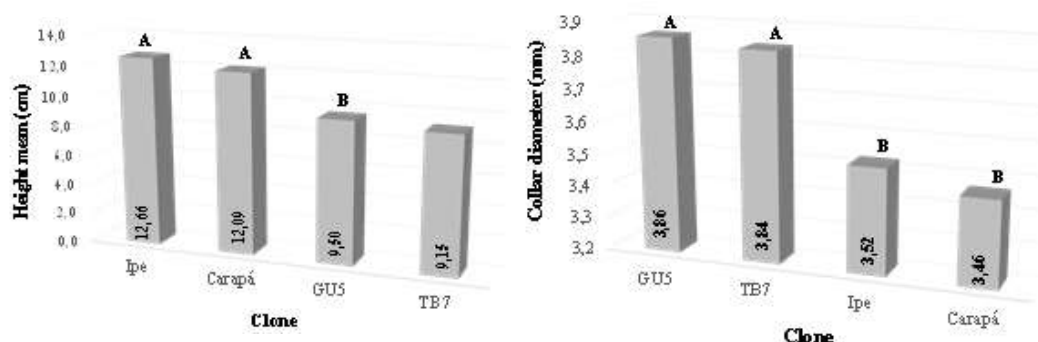


Figure 2 – Total height mean values (cm) and collar diameter (mm), on minicuttings of four *Tectona grandis* clones, after 55 days-age. Means under same letter are non significantly different at $p > 95\%$ probability based on Tukey's test.

Figura 2 – Valores médios de altura total (cm) e diâmetro de colo (mm) de miniestacas de quatro clones de *Tectona grandis*, após 55 dias de estaqueamento. Médias seguidas da mesma letra não diferem entre si, em nível de 95% de probabilidade pelo teste Tukey.

because the genotypes effects of clones evaluated than the effects of storage time which the mini-cuttings were submitted, indicating that the rooting arising from clones are result from gene expression and

environment conditions of rooting structure used. The same results were reported by XAVIER et al., (2013) for eucalypt. The absence of mini-cuttings answer to the storage time can be attributed to the fact that

Table 3 – Summary of variance analyses on biomass (PPA) and root system (PSR), as a response function of minicuttings storage after harvest/preparation, from four *Tectona grandis* clones after 55 days-age.

Tabela 3 – Resumo de análise de variância da biomassa (PPA) e do sistema radicial (PSR), em função da resposta ao armazenamento entre coleta/preparo e estaqueamento de miniestacas de quatro clones de *Tectona grandis*, após 55 dias do estaqueamento.

Source of variation	DF	PPA (g)			PSR (g)		
		Mean square	F	P<0.05	Mean square	F	P
Clone (C)	3	0.318	6.74	0.0031**	0.015	1.43	0.267 ^{ns}
Trat (T)	6	0.085	1.81	0.1531 ^{ns}	0.006	0.63	0.7076 ^{ns}
(C) * (T)	18	0.047	1.18	0.3133 ^{ns}	0.01	0.6	0.8835 ^{ns}
Média	1.09				1.04		
CV _{exp.} (%)	18.25				12.73		

^{ns}, *, ** and *** : non significant at 0.05; significative at 0.05; 0.01 and 0.001 probability (P), respectively for the F test.

teak is a species which keeps vigor and turgidity for long terms in the tested conditions (MURILLO; BADILLA, 2005). Other fact that can be attributed the non-observation of significant differences in these characteristics, regarding to the treatment of storage submitted, is the maintenance of these appropriate environmental conditions for mini mini-cuttings storage, minimizing the hydric stress, agreeing with the recommend one by Goulart e Xavier (2008).

The high survival index obtained in the greenhouse output (92,71%) confirmed the results of the studies accomplished by some authors with many species *Eucalyptus*, referring to the appropriate manage of environmental conditions of greenhouse (ZUFFELLATO-RIBAS; RODRIGUES, 2001; TITON et al., 2003; WENDLING; XAVIER, 2005; GOULART; XAVIER, 2008; BORGES et al., 2011; SOUZA et al., 2013). Gatti (2002) got average of 90,1 % of mini-cuttings survival of *Tectona grandis* of seminal origin, attributing the mortality occurred to the irrigation excess in the environment of root.

It was found that the survival of shade house output was low, indicating that the species adapts well to the environment changes carried out along the rooting process, once the adventitious root occurred, the same result reported by Mascarenhas; Muralidharan (1993) with other forest species. The mortality occurs because of the mini-cuttings transference non-rooted or with a root system a little vigorous for a more open place, with greater temperature and humidity variation, beyond the reserves exhaustion of the mini-cuttings (FERREIRA et al., 2004; FREITAS et al., 2006; MELO et al., 2011b).

For hybrids of *Eucalyptus urophylla* x *Eucalyptus grandis*, Melo et al. (2011a) found that the time interval between

harvesting/preparation and mini-cuttings sowing stored in polystyrene boxes provided with a hole in the bottom and without a lid, for periods longer than two hours, it tended to decrease the percentage of plants rooting and growth. The author recommends avoiding the storage of mini-cuttings, for later sowing should be avoided, for most cases. However, it is possible to note that this factor may interfere positively in the rooting process for certain genetic materials, which indicates genotypic effect. This fact confirms by Hartmann et al. (2011) report, who affirm that it is frequent to find differentiated behaviors in the vegetative propagation, depending on the genetic material evaluated.

In this work it was possible to determine that the survival and rooting index decreased at shade house output, as the storage time of the mini-cuttings increased. The same results were obtained by Goulart and Xavier (2008), when studying the effect of storage time (days) on the mini-cuttings rooting of four *Eucalyptus grandis* x *E. urophylla* clones. Is important because it contributes to the planning of the production activities of the teak clonal plants, and can extend the sowing schedules.

5. CONCLUSION

The results showed that there are not significant influence of treatments of 0, 1, 2, 4, 8, 12 and 16 hours evaluated between harvesting / preparation and sowing of teak mini-cuttings in their survival and rooting of the four clones studied.

Significant differences were found among clones evaluated, in the percentages of survival and rooting at the exit of the greenhouse, as well as in the survival of the clones at the exit of the shade house.

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