

Clones production of *Tectona grandis*

Anne Francis Agostini Santos¹ Bruna Cristina Almeida¹ Fernando Hérique Gava¹ Hérique Guimarães Favare¹
Joamir Barbosa Filho¹ Reginaldo Brito Costa¹ Gilvano Ebling Brondani^{1*}

¹ College of Forestry Engineering, Federal University of Mato Grosso, Av. Fernando Corrêa da Costa, 2367, Boa Esperança, 78060-900, Cuiabá-MT, Brazil.

* Author for correspondence: gebrondani@yahoo.com.br

Received: 16 January 2014 / Accepted: 02 April 2014 / Published: 29 June 2014

Abstract

Tectona grandis is a woody forest species of commercial interest that presents extensive plantations in the world, mainly due to the wood to be destined for several purposes, including noble applications which elevate their price in the market. Nevertheless, despite the importance, there is a lack of research regarding the clone multiplication in a large scale of selected genotypes, limiting progress in the improvement of the species. This review aims to perform a literature review of the main scientific methods used to obtain clones of species, such as the processes of macropropagation and micropropagation, and present perspectives and future trends for the application of new cloning techniques aiming for large scale for clones production. According to the literature, some techniques such as minicutting, autotrophic cultivation and synthetic seeds may be applied to *T. grandis* for clones production. Further scientific studies are however needed in order to prove the viability to produce clones. The improvement aiming the disseminating such techniques can minimize costs, shorten production stages and consequently, reduce the cultivation time in the laboratory.

Key words: Cutting technique; Minicutting technique; Grafting; Micropropagation; Epicormic shoots.

Produção de mudas clonais de *Tectona grandis*

Resumo

A *Tectona grandis* é uma espécie florestal de interesse comercial que apresenta extensos plantios no mundo. Sua madeira pode ser destinada para diversas finalidades, incluindo aplicações nobres que elevam o seu preço no mercado. Contudo, apesar da importância, há uma carência de pesquisas em relação à multiplicação clonal em larga escala de genótipos previamente selecionados, o que limita avanços no melhoramento da espécie. A presente revisão tem por objetivo realizar um levantamento dos principais métodos científicos utilizados para a obtenção de mudas clonais da espécie, abordando tanto os processos de macropropagação quanto os de micropropagação, além de apresentar perspectivas e tendências futuras para a aplicação de novas tecnologias de clonagem que visem a produção de mudas em larga escala. Algumas técnicas como miniestaquia, cultivo fotoautotrófico e sementes sintéticas poderão ser aplicadas à produção de mudas clonais de *T. grandis*, porém há necessidade de novos estudos científicos visando comprovar a viabilidade. O aprimoramento visando a difusão de tais técnicas pode minimizar custos, encurtar etapas de produção e consequentemente, reduzir o tempo de cultivo em laboratório.

Palavras-chave: Estaquia; Miniestaquia; Enxertia; Micropropagação; Brotações epicórmicas.

Introduction

Teak (*Tectona grandis* L. f.) is a wood forest species from Asia that has noble use and high international value.

This species, from the Verbanaceae family, naturally occurs in Myanmar, the center of Thailand and the south of India (Tiwari et al. 2002; Gyves et al. 2007; Feroz et al. 2013). This wood has noble destinations as the production of luxury furniture, watercrafts and decoration (Akram and Aftab 2009).

The cultivation of teak is widespread in the tropics, as the majority of planted forests are provided from seedlings (Nautiyal et al. 1991). Sexual propagation, however, has disadvantages such as non-uniform plantation (caused by genetic variability) and low germination rate (it is often between 20 to 25%) due to tegumentary dormancy, in which there is the need to adopt costly techniques for sowing, besides the dependency on seed availability, which only happens in periods of fruiting (Gyves et al. 2007; Slator et al. 2013).

Due to the difficulties involved in production of teak seedlings, the methods of vegetative propagation can become a suitable alternative, because it promotes constant availability of clones with superior genetic characteristics (due to the selection of better phenotypes) and plantation uniformity. Vegetative propagation can also be used when the seed is considered as limiting and input is costly, causing difficulties on its attainment, beyond low germination and/or production of unviable seeds (Inoue and Putton 2007; Wendling et al. 2010; Hartmann et al. 2011).

Based on the economic importance of *Tectona grandis*, the development of studies on forest tree improvement through the use of cloning techniques are needed for large-scale production, and some models similar to those developed with *Eucalyptus* culture such as minicutting, micropropagation and microcutting (Titon et al. 2003; Brondani et al. 2012; Ruedell et al. 2013) would be useful. The viability to produce these clones on a commercial scale is however dependent on other aspects such as those related to juvenility of the tissues, which is directly associated to the ability to adventitious rooting. Vegetable maturity can be manipulated using appropriate cloning techniques, which able to reversing the juvenility of the tissues, greatly benefiting roots induction. The correct induction of biochemistry stimuli to tissues and organs results a differentiated range of organogenic responses, being necessary to develop specific protocols for each situation.

There is, therefore, the need to know such clonal propagation techniques and their potential application, aiming to establish of commercial planting with high production of *Tectona grandis*, as well as the description of scientific studies that promote advances in species improvement, pointing out new tendencies and perspectives to meet quality and quantity requirements of clones production.

Cutting

Cutting is a vegetative propagation method that consists of organs extraction followed by the respective use of the parts of the donor plant to form a whole plant, in which the

tissues have the ability to regenerate and produce adventitious roots and viable sprouts (Hartmann et al. 2011).

This method has potential as a means of propagation and of rescuing selected genetic material from *Tectona grandis*, as it was used for cloning many species and hybrids of *Eucalyptus*. This technique is able to be applied by farmers for clones production (predominantly when the rescue of selected adult material is considered), aside from the maintenance of interesting genotypic characteristics, which assists the formation of homogenous stands or supports other techniques applied to forest tree improvement, such as minicutting and micropropagation.

The etiolation of *Tectona grandis* (dark environment) was studied by Husen (2011) who observed an increase in rooting percentage of single-node leafy cuttings from etiolated stock-plants in all ages of donor plants selected (in a range of 1 to 5 years old). In this study, etiolated and non-etiolated cuttings were compared in which the latter ones did not present a satisfactory rooting percentage. The author suggested that the etiolation process caused a higher mobility of carbohydrate from starch, stimulating adventitious rooting. In another study with teak, Husen (2008) analyzed the effect of total soluble carbohydrate accumulation on rooting of etiolated and non-etiolated cuttings and suggested that the etiolation process can be considered as a rejuvenation factor due to total soluble carbohydrate accumulation allowing starch mobilization, favoring the rooting of cuttings.

Husen and Pal (2007a) study about the effects of cutting at branch position (basal, middle and apical) while collecting cuttings, indicates that cuttings from middle position followed by the application of indol-3-butyric acid (IBA) at the concentration of 4,000 mg L⁻¹ favor a higher

rooting percentage. Husen and Pal (2007b) evaluated rooting response and the changes of the metabolism in the rooting zone of teak coppice shoots during the development of primordial adventitious roots in tissues from different ages (2 months, 15 years and 30 years) and observed that the increase of the age of donor plants was followed by the reduction in tissue response to adventitious root formation. A larger percentage of rooting was observed for coppice shoots of 2 months, and the use of auxin (i.e., concentration of 4,000 mg L⁻¹ IBA) favored root induction.

Husen and Pal (2006) reported that the interaction effect between donor plant age followed by the treatment with auxin (4,000 mg L⁻¹ IBA) results in a significant increase of rooting percentage. Besides age and the concentration of plant growth regulator, Singh et al. (2006) reported that the effect harvesting regimes for the collection of single node stem cuttings (once, twice or three times per year) during strain handlings interfered in root induction processes and better results were related to the collection carried out twice a year prior to IBA application.

According to protocols developed for cutting *Tectona grandis* (Table 1) can be observed some limitations to its application on commercial scale, predominantly because the variation on rooting percentage. This technique of clonal propagation is therefore not the most suitable large-scale clones production method for this species, although the rescue of selected plants may be applied to aim the constitution of germoplasm data banks, under controlled conditions (*in vitro*, *ex vitro* and *in situ*), such as miniclinal gardens for the use of propagules in minicutting and micropropagation.

Table 1. Cutting of *Tectona grandis* considering donor plant age (DPA), type of cutting (TC), plant growth regulator (PGR) and percentage of rooting (R) according to the literature.

DPA	TC	PGR (mg L ⁻¹)	R (%)	Source	
1 year			78.16	Husen (2011)	
2 years	Cutting with leaf and one leaf node	-	73.33		
3 years			70.00		
4 years			63.30		
5 years			53.33		
2 months				NAA ¹ (2,000)	66.67
	NAA (4,000)	53.33			
	IBA ² (2,000)	93.33			
	IBA (4,000)	86.67			
15 years	Cutting with leaf and one leaf node	NAA (2,000)	60.00		
		NAA (4,000)	66.67		
		IBA (2,000)	66.67		
		IBA (4,000)	73.33		
30 years		NAA (2,000)	53.33		
		NAA (4,000)	63.33		
		IBA (2,000)	20.00		
1 year	Cutting with leaf	NAA (2,000)	36.64	Husen (2008)	
		NAA (3,000)	33.33		
2 months	Cutting with leaf and one leaf node	IBA (2,000)	53.33	Husen and Pal (2007a)	
		IBA (4,000)	67.67		
5 years	Woody cutting with leaf and one leaf node	IBA + Tiamin (500 + 400)	36.70	Singh et al. (2006)	
		IBA + Tiamin (1,000 + 800)	38.30		
3 years	Cutting with leaf and one leaf node (basal)	IBA (2,000)	35.76	Husen and Pal (2007b)	
		IBA (4,000)	44.55		
	Cutting with leaf and one leaf node (middle)	IBA (2,000)	73.49		
		IBA (4,000)	75.17		
	Cutting with leaf and one leaf node (apical)	IBA (2,000)	45.62		
IBA (4,000)		53.49			

¹ NAA – α -naphthalene acetic acid; ² IBA – indol-3-butyric acid.

Minicutting

A promising alternative for clones production refers to the minicutting technique, which favors considerably adventitious rooting compared to traditional cutting (Hartmann et al. 2011). This technique can be summarized in the following phases: (i) production of minicutting in miniclonal gardens; (ii) rooting induction in a greenhouse under intermittent nebulization and temperature control; (iii) acclimation in shade house and (iv) external growing and hardening under sun (Wendling et al. 2009; Brondani et al. 2010; Wendling et al. 2010). Propagule rooting is also a characteristic which indicates tissue rejuvenation (George et al. 2008; Hartmann et al. 2011).

In tests to evaluate *Piptocarpha angustifolia* rooting in different periods, Ferriani et al. (2011) observed that minicutting collected in the summer showed leaf loss and generalized oxidation, even under controlled humidity and temperature. During spring and winter a similar rooting percentage was observed. These results show the strong influence of seasonal effects on minicutting rootings. When *Cedrela fissilis* minicuttings survivals of 60 and 90 days were compared, Xavier et al. (2003) observed 100% mortality for foliar minicuttings, which indicated that this vegetable tissue was not able to develop new shoot meristems. The opposite effect occurred when stem and apical stem minicutting were compared, presenting survival superior to 85%. In *Calophyllum brasiliense*, minicutting presents itself as an efficient strategy for clonal propagation due to its rooting percentage superior to 80% (Silva et al. 2010). Positive results were also reported in *Toona ciliata* (Ferreira et al. 2012), *Eucalyptus benthamii* (Brondani et al. 2012), *Corymbia citriodora* and *Eucalyptus dunnii* (Trueman et al. 2013), *Erythrina falcata* (Cunha et al. 2008) and *Ilex paraguaiensis* (Brondani et al. 2008), highlighting the fact that this is an efficient technique to clone a variety of species and with potential to be used for the large-scale propagation of *Tectona grandis*.

Using the minicutting technique for the clonal propagation of *Tectona grandis*, Gatti (2002) observed minicutting survival in the greenhouse superior to 80% and the highest rooting was observed without the application of plant growth regulators, with means of 88.4%. According to the results, it was observed that this genetic material presented a high rooting capacity, which justified the absence of plant growth regulators.

Considering the results, the minicutting technique may present a potential application for *Tectona grandis* production on a commercial scale, contributing to increase of clones production, facilitate handling, and reduce or eliminate the application of plant growth regulators to promote root induction. Since it is a sought and prized species in a noble wood market, minicutting becomes important in clones production. Further studies, however, remain to be completed to obtain information about the influential factors that affect the whole process and validate its application on a commercial scale.

Grafting

This technique is defined as the art of uniting parts of vegetable tissues from different sources so that both parts establish the communication between the tissues and form one plant, but genetically maintain their own individualities (Monteuuis 1995; Sanou et al. 2004).

One of the main difficulties in vegetative propagation of mature material from woody species is associated with the increase in maturation level, which occurs with its ontogenetic development, reflecting its difficulty in or even loss of rooting capacity (Hartmann et al. 2011; Wendling et al. 2014).

According to Palanisamy and Subramanian (2001), the level of physiologic maturity related to the nutritional state of the vegetable material of *Tectona grandis* is the main limiting factor for macropropagation success. *Tectona grandis* was successfully propagated by grafting (Nautiyal et al. 1991; Husen and Pal 2003), however, the effect of maturation level on cutting rooting was not described (Andrade 2010). In other study, Husen and Pal (2003) observed 20% rooting in cuttings from regrafted plants (from 2nd cultivation) while cuttings from the first graft presented 1.67% rooting. Andrade (2010) evaluated serial grafting propagation through the budding method with three different genetic materials of *Tectona grandis*: one from seminal source of 6 months and two others from clones of 35 years. Excised shoots were grafted onto rootstocks of seminal sources, aiming to perform serial grafting until the 5th subculture. According to the author, a satisfactory result of serial grafting of genetic material from seminal seedlings is related to the level of juvenility, with the emission of shoots observed 15 days after the grafting process.

Grafting technique can be used on *Tectona grandis* propagation for genetic material rescue aiming for genetic improvement, or when other propagation techniques are not successfully applied for tissue rejuvenation or rooting. For cellular competence recovery, the tissues are preferably multiplied using quicker techniques of cloning that allow for large-scale production, such as micropropagation and minicutting.

Epicormic shoots

The clearcutting (coppicing) is the most used method for rescuing matrices of previously selected plants, as applied for *Eucalyptus*. Some woody species can, however, have regrowth difficulties. Considering this situation, the use of epicormic shoots is an alternative to rescue mature vegetable material (Wendling et al. 2009; Wendling et al. 2013) that can be used as a source of propagules for adventitious rooting. The collection of pruned branches to induce epicormic shoots is a viable alternative to recover the vegetative potential of trees (without the need to shoot down the mother plant), due to the fact that when the shoots reach an appropriate size they can be used in cutting or grafting methods for clones production (Wendling et al. 2009) and can be used for *in vitro* culture through micropropagation.

Considering the potential for the application of forest species of interest, the pruning of branches 20 to 50 cm from the stem, for *Araucaria angustifolia* was an efficient technique for shoot production, highlighting those with an orthotropic growth habit (Wendling et al. 2009). Bachelard (1969) observed that different factors such as plant growth regulators, substances with nitrogen, mineral nutrients and water have influence on epicormic shoots production. Beyond these factors, the shoot production was also dependent on the season. In the winter, higher development was observed compared to summer, due to the competition between cambium activity and epicormic shoots production, in which shoot production is favored when cambium is in a dormant state. Almeida et al. (2007) used pruned branches from *Eucalyptus cloenziana* (20 years old) and observed an elevated potential for epicormic shooting technique. The adventitious rooting, however, was not observed when cuttings from these shoots were used, because the branches were ontogenetically adults, with a lower adventitious rooting capacity compared to younger ones. Therefore, further studies need to be carried out in this area.

Clonal multiplication is an interesting method to promote quicker productivity, but one of the difficulties is the attainment of a genetic material that presents adequate

rooting capacity (i.e., superior to 85%). Using this technique the rejuvenation of the material from epicormic shoots can be promoted, maintaining the characteristics of the selected individual, and obtaining shoots that are physiologically able for the rooting process. In the literature there are no reports from the use of this technique in *Tectona grandis* aiming at the attainment of shoots for cloning, making it an excellent field to conduct further studies.

Micropropagation

The micropropagation technique consists of the *in vitro* cultivation of propagules in aseptic conditions, controlling lighting, temperature, photoperiod and culture medium (formulated with different concentrations of macro and micronutrients, carbohydrate, vitamins, aminoacids and plant growth regulators) (George et al. 2008).

Micropropagation of teak has often been used due to the difficulty via seminal reproduction because these seeds have hard tegument and low quality and its germination is irregular (Gyves et al. 2007; Kozgar and Shahzad 2012). The use of this technique for teak, however, presents some limitations due to exudation of phenolic compounds by the tissues, causing explants to brown, promoting oxidation and hamper growth and development (Akram and Aftab 2009). Another difficulty refers to shoot production to be used in the phase of multiplication, due to susceptibility to vitrification and low rooting capacity (Gyvesa et al. 2007).

The combinations of more efficient culture media for node multiplication of teak are: 0.5 μM ($\approx 0.093 \text{ mg L}^{-1}$) NAA + 4.4 μM ($\approx 0.99 \text{ mg L}^{-1}$) BAP (6-benzylaminopurine) for apical nodes and 0.5 μM ($\approx 0.093 \text{ mg L}^{-1}$) NAA + 2.2 μM ($\approx 0.49 \text{ mg L}^{-1}$) BAP for nodal segments (Fermino Junior et al. 2009), 22.2 μM ($\approx 2.25 \text{ mg L}^{-1}$) BAP with 0.0 or 0.57 μM ($\approx 0.099 \text{ mg L}^{-1}$) IAA – indol-3-acetic acid) (Tiwari et al. 2002) and 2.5 μM ($\approx 0.55 \text{ mg L}^{-1}$) TDZ (thidiazuron) (Kozgar and Shahzad 2012).

The rooting phase of propagules obtained of the *in vitro* multiplication and/or elongation aim to induce adventitious root initiation for *in vitro* or *ex vitro* experiments (Dutra et al. 2009; Brondani et al. 2012). In an experiment conducted by Kozgar and Shahzad (2012), with teak, testing three plant growth regulators: NAA (1 to 5 μM / ≈ 0.186 to $\approx 0.931 \text{ mg L}^{-1}$), IBA (1 to 5 μM / ≈ 0.264 to $\approx 1.32 \text{ mg L}^{-1}$) and IAA (1 to 5 μM / ≈ 0.175 to $\approx 0.875 \text{ mg L}^{-1}$), and the better result was observed for *ex vitro* rooting using NAA at a concentration of 2.5 μM ($\approx 0.465 \text{ mg L}^{-1}$).

Numerous micropropagation protocols have been developed for *Tectona grandis* culture, which shows the efficiency of the technique when applied in order to obtain superior genetic material on a large scale. Further studies, however, are necessary when the reduction of costs during clones production and the genetically modified plants are considered, in which the protocols are normally specific and genotype-dependent.

Photoautotrophic cultivation

This technique is based on chemical alterations of culture media, such as a reduction or absence of carbohydrate, allow gas exchange between *in vitro* and external environments, and an increase in irradiance by better use of natural or artificial lights. This promotes an environment to the propagules which stimulates photosynthetic competence since its multiplication, improving hydric relations of the explants, favors more rustic growth of the plants, reducing or even eliminating the *ex vitro* acclimatization phase (Ziv 1991).

Autotrophic cultivation is highlighted as a promising alternative when there is a need to reduce costs and problems related to the physiological quality of clones.

Despite the advantages of this method, it is not widespread (Eriget al. 2005).

According to Kozai and Kubota (2001), many factors that affect this technique have been studied, such as: the lack of carbohydrate in the culture media that stimulates photosynthetic competence and absorption of inorganic nutrients; carbon dioxide and oxygen concentrations in the *in vitro* environment; different models and dimensions of recipients with diverse types of membranes which favor gas exchange through forced or natural ventilation; use of alternative substrates to agar to promote more aeration, and the use of different light sources (amount and quality). Furthermore, the reduction of carbohydrate source in the culture media can reduce the manifestation of microorganisms and favor photosynthesis, which is a factor that prepares the clones for the acclimatization process (George et al. 2008).

Studies related to photoautotrophic cultivation in woody species are few in literature, and the study of Zobayed et al. (2000) can be highlighted, which compared the use of different cultivation recipients (20 to 3.4 L) for *Eucalyptus camaldulensis* cultivation, obtaining satisfactory results in both recipients. Tanaka et al. (2005) evaluated an alternative material to FPF (fluorocarbon polymer film) in the production of disposable recipients for photoautotrophic propagation of hybrid *Eucalyptus urophylla* \times *Eucalyptus grandis*, observing satisfactory results for propagule development with reduced costs.

Photoautotrophic cultivation technique may be applied for *Tectona grandis* propagation, as this species can be propagated on a commercial scale through the method of micropropagation. The use of this technique can result in cost reduction and optimization of the large-scale cloning process. Despite all the potential for photoautotrophic cultivation found for woody species, this technique has not been tested in *Tectona grandis*, and it therefore represents an excellent field to be explored in further studies.

Bioreactors

Bioreactors are equipment especially dimensioned for explant cultivation, and are composed by a set of flasks made from stainless steel, polycarbonate, polypropylene, or autoclaving support material (Paek et al. 2001; Curtis 2005). Bioreactors can be classified according to the immersion time, constituting two main types: temporary immersion or permanent immersion (Takayama and Akita 1994; Cid et al. 2002). For the temporary immersion system, the propagule remains temporarily immersed, while for the permanent immersion system, the vegetable material remains continuously immersed in a liquid or semi-liquid culture media (Cid et al. 2002; Teixeira 2002).

In relation to its use for woody species, bioreactor systems presented success when the cultivation was performed in temporary immersion, such as *Alocasia amazonica* (Jo et al. 2008), *Gypsophila paniculata* (Wang et al. 2013), *Theobroma cacao* (Niemenak et al. 2008), *Kalopanax septemlobus* (Kim et al. 2011), as well as for forest species with economic importance such as *Eucalyptus grandis* \times *Eucalyptus urophylla* (Oliveira et al. 2011), *Eucalyptus grandis* (Castro and González 2002), *Coffee* spp. (Etienne et al. 2006) and for *Hevea brasiliensis* (Martre et al. 2001), showing its potential to be applied for large scale clones production, which is a desirable characteristic in biofactories.

Studies related to the use of bioreactors aiming at the clonal multiplication of *Tectona grandis* are few in the literature. Quiala et al. (2012) studied proliferation and growth responses of the apical node of *Tectona grandis* in temporary immersion bioreactors with a diversity of BA

(benzyladenine) concentrations, reporting a better response of apical node development in culture media without cytokinin (100% rooting in culture media). The authors concluded that the use of a temporary immersion bioreactor system of *Tectona grandis* when compared to the control treatment (conventional method of micropropagation in culture media of semisolid MS - Murashige and Skoog 1962), presented greater explant growth and development, showing the potential use of this technique for this species. Further studies, however, should be performed to confirm the application of this technique on a commercial scale, considering contamination control and *ex vitro* rooting.

Somatic embryogenesis and synthetic seeds

Somatic embryogenesis is the development of somatic or haploid cells without gametes fusion in various embryogenic stages, forming a new plant (Jiménez 2001). Somatic embryogenesis is an important technique for plant propagation, however, many factors can influence its applicability such as the type of explant, culture media, and plant growth regulators (Jiménez 2001; Ipekci and Gozukirmizi 2003; Kumar and Chandra 2014; Sridevi and Giridhar 2014). The explants used in somatic embryogenesis can also originate from all parts of the plant, which facilitate attainment of propagules.

For *Tectona grandis*, many authors have reported the need and importance to develop protocols that allow the transformation, because the processes that are known today are not satisfactory for complete genetic transformation (Tambarussi 2009).

Nathalang (2012) used explants from new leaves of teak to analyze somatic embryo induction and observed that a culture media with 50% salt concentration form the MS culture medium (Murashige and Skoog 1962) added 1 mg L⁻¹ NAA and 1 mg L⁻¹ BAP, resulting in better somatic embryo induction and greater callus growth. Kushalkar and Sharon (1996) observed the induction of somatic embryos in *Tectona grandis* from apical node callus in MS media supplemented with 0.1 mg L⁻¹ BAP and 0.01 mg L⁻¹ NAA. Callus originating from axillary buds formed somatic embryos in MS media with half of the concentration of BAP and NAA, being that both direct and indirect somatic embryogenesis were more competent with axillary buds, although complete regeneration did not occur.

Somatic embryogenesis and synthetic seeds are considered interesting tools with a possible trend in forestry, especially for high demand species, considering practice and time gaining (Asmah et al. 2011). Multiplication of *Tectona grandis* via somatic embryogenesis and other meristematic tissues, combined with the use of synthetic seed technology (Ara et al. 2000) may enable a constant clones production in any period of the year. With the advances in forest biotechnology, new strategies for higher quality clones production will be developed and can impact on the increase of productivity and profitability in the timber industry.

Final considerations

This study composes an approach of grafting, cutting, minicutting, micropropagation and the tendencies of the use of bioreactors, autotrophic cultivation, somatic embryogenesis and synthetic seeds for *Tectona grandis*.

Some techniques such as minicutting, micropropagation, autotrophic cultivation and synthetic seeds may be applied to *Tectona grandis*. Further scientific studies are however needed in order to prove the viability to produce clones. The improvement aiming the at disseminating such techniques can minimize costs, shorten production stages and consequently, reduce the cultivation time in the laboratory.

Not all techniques of clonal propagation aim towards large scale plants production, but also consist of promoting the rejuvenation of tissues, conservation of germplasm banks, clonal minigarden production, genetic transformation and field material rescue. Due to this fact, the deepening in scientific knowledge about propagation techniques of *Tectona grandis* is important, because only through this way is it possible to identify the areas of knowledge that need to be strengthened and then make viable techniques that have not been tested or standardized for the species.

Acknowledgment

The authors are grateful to CAPES and CNPq.

References

- Akram M, Aftab F (2009) An efficient method for clonal propagation and *in vitro* establishment of softwood shoots from epicormic buds of teak (*Tectona grandis* L.). *Forestry Studies in China*, 11(2):105-110. doi: 10.1007/s11632-009-0018-1
- Almeida FD, Xavier A, Dias JMM (2007) Propagação vegetativa de árvores selecionadas de *Eucalyptus cloeziana* F. Muell. por estaquia. *Revista Árvore*, 31(3):445-453. doi: 10.1590/S0100-67622007000300010
- Andrade WF (2010) *Indução de rejuvenescimento de teca (Tectona grandis L. f.) através da enxertia seriada e micropropagação*. Tese. Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo. 75p.
- Ara H, Jaiswal U, Jaiswal VS (2000) Synthetic seed: prospects and limitations. *Current Science*, 78(12):1438-1444.
- Asmah HN, Hasnida HN, Zaimah NAN, Noraliza A, Salmi NN (2011) Synthetic seed technology for encapsulation and regrowth of *in vitro*-derived *Acacia* hybrid shoot and axillary buds. *African Journal Biotechnology*, 10(40):7820-7824. 2011. doi: 10.5897/AJB11.492
- Bachelard EP (1969) Studies on the formation of epicormic shoots on eucalypt stem segments. *Australian Journal of Biological Sciences*, 22(5):1291-1296. doi: 10.1071/BI9691291
- Brondani GE, Araujo MA, Wendling I, Kratz D (2008) Enraizamento de miniestacas de erva-mate sob diferentes ambientes. *Pesquisa Florestal Brasileira*, (57):29-38.
- Brondani GE, Baccarin FJB, Wit Ondas HW, Stape JL, Gonçalves AN, Almeida M (2012) Low temperature, IBA concentrations and optimal time for adventitious rooting of *Eucalyptus benthamii* mini-cuttings. *Journal of Forestry Research*, 23(4):583-592. doi: 10.1007/s11676-012-0298-5
- Brondani GE, Grossi F, Wendling I, Dutra LF, Araujo MA (2010) Aplicação de IBA para o enraizamento de miniestacas de *Eucalyptus benthamii* Maiden & Cabbage × *Eucalyptus dunnii* Maiden. *Acta Scientiarum. Agronomy*, 32(4):667-674. doi: 10.4025/actasciagron.v32i4.4879
- Castro D, González J (2002) Micropropagación de eucalipto (*Eucalyptus grandis* Hill ex. Maiden) en el sistema de inmersión temporal. *Agricultura Técnica*, 6(1):68-78. doi: 10.4067/S0365-28072002000100007

- Cid LPB, Cruz ARR, Teixeira JM (2002) Biorreatores de imersão permanente. *Biotecnologia Ciência e Desenvolvimento*, (25):50-53.
- Cunha ACMCM, Wendling I, Souza Júnior L (2008) Miniestaquia em sistema de hidroponia e em tubetes de corticeira-do-mato. *Ciência Florestal*, 18(1):85-92.
- Curtis WR (2005) Application of bioreactor design principles to plant micropropagation. *Plant Cell, Tissue and Organ Culture*, 81(3):255-264. doi: 10.1007/s11240-004-6646-1
- Dutra LF, Wendling I, Brondani GE (2009) A micropropagação de eucalipto. *Pesquisa Florestal Brasileira*, (58):49-59. doi: 10.4336/2009.pfb.58.49
- Erig AC, Schuch MW (2005) Micropropagação fotoautotrófica e uso da luz natural. *Ciência Rural*, 35(4):961-965. doi: 10.1590/S0103-84782005000400039
- Etienne H, Dechamp E, Barry-Etienne D, Bertrand B (2006) Bioreactors in coffee micropropagation. *Brazilian Journal of Plant Physiology*, 18(1):45-54. doi: 10.1590/S1677-04202006000100005
- Fermino Junior PCP, Nagão EO, Pereira JES (2009) Estabelecimento, germinação e multiplicação *in vitro* de teca (*Tectona grandis* L. f.) a partir de genótipos da Amazônia Sul-Occidental. *Scientia Florestalis*, 37(84):427-435.
- Feroz SM, Alam Md R, Das P, Mamun AA (2013) Community ecology and spatial distribution of trees in a tropical wet evergreen forest in Kaptai national park in Chittagong Hill Tracts, Bangladesh. *Journal of Forestry Research*. doi: 10.1007/s11676-013-0423-0
- Ferreira DA, Barroso DG, Silva MPS, Souza JS, Freitas TAS, Carneiro JGA (2012) Influência da posição das miniestacas na qualidade de mudas de cedro australiano e no seu desempenho inicial no pós-plantio. *Ciência Florestal*, 22(4):715-723. doi: 10.5902/198050987553
- Ferriani AP, Zuffellato-Ribas KC, Helm CV, Boza A, Wendling I, Koehler HS (2011) Produção de brotações e enraizamento de miniestacas de *Piptocarpha angustifolia*. *Pesquisa Florestal Brasileira*, 31(67):257-264. doi: 10.4336/2011.pfb.31.67.257
- Gatti KC (2002) Popagação vegetativa de pau mulato (*Calycophyllum spruceanum* (Benth) K. Schum.), jequitibá (*Cariniana estrellensis* (Raddi) Kuntze) e teca (*Tectona grandis* Linn. f.) por miniestaquia. Tese. Universidade Federal de Viçosa, 72p.
- George EF, Hall MH, De Klerk GJ (2008) *Plant propagation by tissue culture*. 3rd Edition. v.1. Berlin: Springer. 501p.
- Gyves EM, Royani JI, Rugini E (2007) Efficient method of micropropagation and *in vitro* rooting of teak (*Tectona grandis* L.) focusing on large-scale industrial plantations. *Annals of Forest Science*, 64(1):73-78. doi: 10.1051/forest:2006090
- Hartmann HT, Kester DE, Davies Jr FT, Geneve RL (2011) *Plant propagation: principles and practices*. 8th Edition. São Paulo: Prentice-Hall. 915p.
- Husen A (2008) Stock-plant etiolation causes drifts in total soluble sugars and anthraquinones, and promotes adventitious root formation in teak (*Tectona grandis* L. f.) coppice shoots. *Plant Growth Regulation*, 54(1):13-21. doi: 10.1007/s10725-007-9222-y
- Husen A (2011) Rejuvenation and adventitious rooting in coppice-shoot cutting of *Tectona grandis* as affected by stock plant etiolation. *American Journal of Plant Sciences*, 2(3):370-374. doi: 10.4236/ajps.2011.23042
- Husen A, Pal M (2003) Effect of serial bud grafting and etiolation on rejuvenation and rooting cuttings of mature trees of *Tectona grandis* Linn. f. *Silvae Genetica*, 52(2):84-88.
- Husen A, Pal M (2006) Variation in shoot anatomy and rooting behavior of stem cuttings relation to age of donor plants in teak (*Tectona grandis* L. f.). *New Forests*, 31(1):57-73. doi: 10.1007/s11056-004-6794-5
- Husen A, Pal M (2007a) Effect of branch position and auxin treatment on clonal propagation of *Tectona grandis* Linn. f. *New Forests*, 34(3):223-233. doi: 10.1007/s11056-007-9050-y
- Husen A, Pal M (2007b) Metabolic changes during adventitious root primordia development in *Tectona grandis* Linn. f. (teak) cuttings as affected by age of donor plants and auxin (IBA and NAA) treatment. *New Forests*, 33(3):309-323. doi: 10.1007/s11056-006-9030-7
- Inoue MT, Putton V (2007) Macropropagação de 12 espécies arbóreas da floresta ombrófila mista. *Revista Floresta*, 37(1):55-61.
- Ipekci Z, Gozukirmizi N (2003) Direct somatic embryogenesis and synthetic seed production from *Paulownia elongata*. *Plant Cell Reports*, 22(1):16-24. doi: 10.1007/s00299-003-0650-5
- Jiménez VM (2001) Regulation of *in vitro* somatic embryogenesis with emphasis on the role of endogenous hormones. *Revista Brasileira de Fisiologia Vegetal*, 13(2):196-223. doi: 10.1590/S0103-31312001000200008
- Jo UA, Murthy HN, Hahn EJ, Paek KY (2011) Micropropagation of *Alocasia amazonica* using semisolid and liquid cultures. *In Vitro Cellular & Developmental Biology - Plant*, 44(1):26-32. doi: 10.1007/s11627-007-9081-2
- Kim SJ, Dewir YS, Moon HK (2011) Large-scale plantlets conversion from cotyledonary somatic embryos of *Kalopanax septemlobus* tree using bioreactor cultures. *Journal of Plant Biochemistry and Biotechnology*, 20(2):241-248. doi: 10.1007/s13562-011-0052-7
- Kozai T, Kubota C (2001) Developing a photoautotrophic micropropagation system for woody plants. *Journal of Plant Research*, 114(4):525-537. doi: 10.1007/PL00014020
- Kozgar MI, Shahzad A (2012) An improved protocol for micropropagation of teak tree (*Tectona grandis* L.). *Rendiconti Lincei*, 23(2):195-202. doi: 10.1007/s12210-012-0176-2
- Kumar V, Chandra S (2014) High frequency somatic embryogenesis and synthetic seed production of the endangered species *Swertia chirayita*. *Biologia*, 69(2):186-192. doi: 10.2478/s11756-013-0305-0

- Kushalkar R, Orlikowska T (1996) Direct and indirect somatic embryogenesis in teak (*Tectona grandis* L.). *Current Science*, 71(9):712-715.
- Martre P, Lacan D, Just D, Teisson C (2001) Physiological effects of temporary immersion on *Hevea brasiliensis* callus. *Plant Cell, Tissue and Organ Culture*, 67(1):25-35. doi: 10.1023/A:1011666531233
- Monteuuis O (1995) Influence of the grafting technique on meristem micrografting of Douglas-fir. *New Forests*, 10(3):267-273. doi: 10.1007/BF00027928
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with the tobacco tissue culture. *Physiologia Plantarum*, 15(3):493-497. doi: 10.1111/j.1399-3054.1962.tb08052.x
- Nathalang K (2012) Factors important for somatic embryo induction and proliferation from young leaf explant of the biggest gigantic teak. *The Journal of KMUTNB*, 22(1):24-30.
- Nautiyal S, Singh U, Gurumurti K (1991) Rooting response of branch cuttings of teak (*Tectona grandis*) as influenced by season and growth hormones. *Indian Forester*, 117(4):249-255.
- Niemenak N, Saare-Surminski K, Rohsius C, Ndoumou DO, Lieberei R (2008) Regeneration of somatic embryos in *Theobroma cacao* L. in temporary immersion bioreactor and analyses of free amino acids in different tissues. *Plant Cell Reports*, 27(4):667-676. doi: 10.1007/s00299-007-0497-2
- Oliveira ML, Xavier A, Penchel RM, Santos AF (2011) Multiplicação *in vitro* de *Eucalyptus grandis* x *E. urophylla* cultivado em meio semissólido e em biorreator de imersão temporária. *Scientia Forestalis*, 39(91):309-315.
- Paek KY, Hahn EJ, Son SH (2001) Application of bioreactors for large-scale micropropagation systems of plants. *In vitro Cellular & Developmental Biology - Plant*, 37(2):149-157. doi: 10.1007/s11627-001-0027-9
- Palanisamy K, Subramanian K (2001) Vegetative propagation of mature teak trees (*Tectona grandis*). *Silvae Genetica*, 50(5-6):188-191.
- Quijala E, Cañal MJ, Meijón M, Rodríguez R, Chavéz M, Valledon L, Feria M, Barbón R (2012) Morphological and physiological responses of proliferating shoots of teak to temporary immersion and BA treatments. *Plant Cell, Tissue and Organ Culture*, 109(2):223-234. doi: 10.1007/s11240-011-0088-3
- Ruedell CM, Almeida MR, Schwambach J, Posenato CF, Fett-Neto AG (2013) Pre and post-severance effects of light quality on carbohydrate dynamics and microcutting adventitious rooting of two *Eucalyptus* species of contrasting recalcitrance. *Plant Growth Regulation*, 69(3):235-245. doi: 10.1007/s10725-012-9766-3
- Sanou H, Kambou S, Teklehaimanot Z, Dembélé M, Yossi H, Sina S, Djingdia L, Bouvet JM (2004) Vegetative propagation of *Vitellaria paradoxa* by grafting. *Agroforestry Systems*, 60(1):93-99. doi: 10.1023/B:AGFO.0000009408.03728.46
- Silva RL, Oliveira ML, Monte MA, Xavier A (2010) Propagação clonal de guanandi (*Calophyllum brasiliense*) por miniestaquia. *Agronomía Costarricense*, 34(1):99-104.
- Singh S, Bhandari AS, Ansari AS (2006) Stockplant management for optimized rhizogenesis in *Tectona grandis* stem cuttings. *New Forests*, 31(1):91-96. doi: 10.1007/s11056-004-7361-9
- Slator NJ, Callister AN, Nichols JD (2013) Mechanical but not physical dormancy is a cause of poor germination in teak (*Tectona grandis* L.f.). *New Forests*, 44(1):39-49. doi: 10.1007/s11056-011-9298-0
- Sridevi V, Giridhar P (2014) *In vitro* shoot growth, direct organogenesis and somatic embryogenesis promoted by silver nitrate in *Coffea dewevrei*. *Journal of Plant Biochemistry and Biotechnology*, 23(1):112-118. doi: 10.1007/s13562-012-0186-2
- Takayama S, Akita M (1994) The types of bioreactors used for shoots and embryos. *Plant Cell, Tissue and Organ Culture*, 39(2):147-156. doi: 10.1007/BF00033922
- Tambarussi EV (2009) *Desenvolvimento de metodologias biotecnológicas para micropropagação, regeneração e transformação genética de teca (Tectona grandis L. f.) visando resistência a Hyblaea puera*. Dissertação. Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo. 122p.
- Tanaka M, Giang DTT, Murakami A (2005) Application of a novel disposable film culture system to photoautotrophic micropropagation of *Eucalyptus urophylla* (*Urophylla* x *grandis*). *In Vitro Cellular & Developmental Biology - Plant*, 41(2):173-180. doi: 10.1079/IVP2004622
- Teixeira JB (2002) Biorreatores. *Biotechnologia Ciência & Desenvolvimento*, (24):36-41.
- Titon M, Xavier A, Otoni WC, Reis GG (2003) Efeito do AIB no enraizamento de miniestacas e microestacas de clones de *Eucalyptus grandis* w. Hill ex Maiden. *Revista Árvore*, 27(1):1-7. doi: 10.1590/S0100-67622003000100001
- Tiwari SK, Siril EA (2002) An improved micropropagation protocol for teak. *Plant Cell, Tissue and Organ Culture*, 71(1):1-6. doi: 10.1023/A:1016570000846
- Trueman SJ, McMahon TV, Bristow M (2013) Production of cuttings in response to stock plant temperature in the subtropical eucalypts, *Corymbia citriodora* and *Eucalyptus dunnii*. *New Forests*, 44(2):265-279. doi: 10.1007/s11056-012-9315-y
- Wang SM, Piao XC, Park SY, Lian ML (2013) Improved micropropagation of *Gypsophila paniculata* with bioreactor and factors affecting *ex vitro* rooting in microponic system. *In Vitro Cellular & Developmental Biology - Plant*, 49(1):70-78. doi: 10.1007/s11627-012-9464-x
- Wendling I, Brondani GE, Biassio A, Dutra LF (2013) Vegetative propagation of adult *Ilex paraguariensis* trees through epicormic shoots. *Acta Scientiarum. Agronomy*, 35(1):117-125. doi: 10.4025/actasciagron.v35i1.15958
- Wendling I, Brondani GE, Dutra LF, Hansel FA (2010) Mini-cuttings technique: a new *ex vitro* method for clonal propagation of sweetgum. *New Forests*, 39(3):343-353. doi: 10.1007/s11056-009-9175-2
- Wendling I, Dutra LF, Hoffmann HA, Bettio G, Hansel F (2009) Indução de brotações epicórmicas ortotrópicas para a propagação vegetativa de árvores adultas de

- Araucaria angustifolia*. *Agronomía Costarricense*, 33(2):309-319.
- Wendling I, Trueman SJ, Xavier A (2014) Maturation and related aspects in clonal forestry – part II: reinvigoration, rejuvenation and juvenility maintenance. *New Forests*. doi: 10.1007/s11056-014-9415-y
- Xavier A, Santos, GA, Oliveira ML (2003) Enraizamento de miniestaca caulinar e foliar na propagação vegetativa de cedro rosa (*Cedrela fissilis*). *Revista Árvore*, 27(3):351-356. doi: 10.1590/S0100-67622003000300011
- Ziv M (1991) Quality of micropropagated plants - vitrification. *In Vitro Cellular & Developmental Biology - Plant*, 27(2):64-69. doi: 10.1007/BF02632130
- Zobayed SMA, Afreen F, Kozai T (2000) Quality biomass production via photoautotrophic micropropagation. *Acta Horticulturae*, 530:377-386.