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RESEARCH ARTICLE

Optimizing *Tectona grandis* L.F. (teak) Tree Clonal Propagation via Regrowth and the Application of Plant Growth Regulators

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Abstract: *Tectona grandis* L.F., known as teak, is a species of high commercial and ecological value for which the development of efficient clonal propagation strategies is being sought. The availability of clonally propagated materials from plus trees is scarce. The main objective of the research was to evaluate the root induction capacity of *Tectona grandis* L.F. plus tree sprouts using different hormone concentrations of indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) selected at 30 and 60 days. A completely randomized design (CRD) with seven treatments, four replicates, and three experimental units per replicate was used to determine statistical differences between treatments. The best results for rooting of teak shoots were observed in treatment T5 (500 mg kg−1 of IBA) with an average of three roots per plant, T1 (control), and T7 (1500 mg kg−1 of IBA) with an average of four leaves per plant. The highest survival rates were observed in T2 (500 mg kg−1 of NAA) and T7 (1500 mg kg−1 of IBA) with 58.33% and in T1 (control), T2 (500 mg kg−1 of NAA) 50% rooting was obtained after 60 days. Although no differences were observed between treatments, the numerical differences allowed to observe that the use of auxins promotes rooting and survival of the shoots of adult trees of *T. grandis* and opens the possibility of propagation and cloning strategies for this species. This study provides valuable knowledge on vegetative propagation, breeding and conservation of forest species, which is relevant for academia, public and private sectors.

Palabras claves: Asexual propagation; rooting; substrates; regrowth; plant growth substances

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1. Introduction

Tectona grandis L.F. (teak) is probably the world's most widely planted high-value timber, cultivated in Africa, the Pacific, South America and throughout Asia[1-3]. The global area of teak planted is approaching 7 million hectares, which is used for

shipbuilding, architecture, residential and commercial spaces, furniture manufacturing (solid and veneer), and the manufacture of other decorative and symbolic wood products a. Teak is a member of the *Lamiaceae* family and has gained a worldwide reputation due to its high quality and durability, high resistance to fungal and insect attack, as well as abiotic conditions[4,5]. Due to its excellent timber characteristics, teaks are considered one of the most valuable woods in the world [1,6]. The remarkable adaptation to the physical and chemical characteristics of the soil, biomass accumulation, and wood properties have legitimized it to become a forest species of economic importance [7].

Increasing demand and deforestation have caused a decline in natural teak forests, making future supply increasingly dependent on teak plantations [8]. Traditional methods of teak propagation fail to meet the demand for the crop or ensure the availability of disease-free plants [9]. The use of unselected and untested teak seeds has led to supply shortages. However, by using improved and tested plant materials, significant advantages in terms of growth and quality can be obtained. These materials effectively address the previously unfulfilled demand and guarantee a sufficient supply of high-quality wood, meeting the productivity and quality requirements associated with teak [10]. In this context, vegetative propagation in forest species has become a strategy to establish homogeneous plantations with desirable phenotypic characteristics in a reduced time. Cloning serves as an initial measure within a genetic enhancement initiative, facilitating the acquisition of enhanced clones that manifest the primary physical and phenotypic attributes inherent to the species [11,12]. In order to clone, the technique of rejuvenation or reinvigoration of adult plants is frequently employed, which has been commonly used in native tree species to induce juvenile propagules more suitable for vegetative propagation, especially for adventitious rooting [13,14].

Plant growth regulators (PGRs), or phytohormones, are organic substances produced naturally in plants. PGRs play a crucial role in the control of growth and other functions in plants. Regulators act at sites distant from their production site, and they do so in minute amounts [15]. The application of growth regulators promotes vegetative propagation, which can be carried out through several techniques that are currently proven and efficient; for example, the induction of epicormic shoots; with this method, it is possible to recover the rejuvenation of the adult material of the timber species that are carried out clonally [16]. Auxin, a crucial phytohormone, exerts regulatory control over various facets of plant development, physiology, and environmental adaptability[17,18]. However, it is essential to note that the effects of auxin are not solely attributable to its independent action[19]. The pivotal involvement of the phytohormone auxin in orchestrating various crucial developmental processes renders it a central regulatory entity[20–22]. The phenomena above encompass the restraint of axillary bud proliferation, lateral root elongation, control over embryonic polarity, modulation of shoot meristem activity and arrangement, and facilitation of vascular tissue formation[23].

The present study elucidates the pivotal role of coordinated mechanisms in actively contributing to the process of organ formation in plants [12–15]. The primary objective of the current study is to establish the adequate concentration of the phytohormones naphthaleneacetic acid (NAA) and indolbutyric acid (IBA) in the induction of roots in *T. grandis* shoots.

2. Material and methods

2.1 Study area

The research was conducted at the teak plantation in the experimental farm La Represa, affiliated with the State Technical University of Quevedo. The farm is located at Km. 7 ½ of the Quevedo-San Carlos road in the province of Los Rios. The research was conducted at a location with an elevation of 73 m a.s.l. This location is situated at a longitude of 79° 25` 24`` west longitude and 1° 03` 18`` south latitude).

2.2 Process of selection, collection and disinfection of basal branches

The plus trees were selected from a 15-year-old stand for their superior morphological characteristics, such as diameter at breast height DBH, form factor, trunk cylindricality, straight

stem, and disease-free trees. Then the cuttings were collected in the morning from basal branches 10 cm in diameter and two to four buds, immediately placed in a wet newspaper in a thermal box to avoid dehydration, before the establishment in a humid chamber were disinfected with vitavax solution at 0.1% (1g L^{-1}) diluted in water for 15 min.

2.3 Preparation of the wet chamber

The sand-based substrate was disinfected with vitavax 0.1% seven days before establishment. A transparent plastic cover was placed over the substrate, creating a tunnel two meters long by one meter wide and 80 cm high. A shade net (saran 70%) was placed over this structure, providing a favorable shoot induction environment.

2.4 Rooting powder preparation

The rooting powder preparation protocol was used according to Del Valle [28]. The hormones with a purity of 99.9% were mainly used to prepare different concentrations of 500, 1000, and 1500 mg Kg^{-1} of NAA and IBA. The different hormone concentrations were dissolved in 70% alcohol, mixed with talc $Mg_3Si_4O_{10}(OH)_2$ (1 Kg^{-1}), and left to dry at room temperature for 24 h, after which they were stored in properly labeled bottles.

2.4 Harvesting and planting of shoots from basal branches

Once the branches had sprouted (three weeks), they were collected with pruning shears previously disinfected with 70% alcohol. The shoots were submerged in water with 1% vitavax solution to disinfect and prevent dehydration. Then, the hormone was placed at the base of each shoot and then they were placed in the substrate according to each treatment. Every eight days, the mineral salts (macro and micronutrients) were applied at half the concentration of the salts proposed by Murashige & Skoog [29].

2.5 Statistical NAAlysis and *evaluation process of the different variables*

A completely randomized design (CRD) was applied to evaluate the rooting capacity of *T. grandis* plus tree shoots with the use of NAA and IBA phytohormones in each of the seven treatments, which in turn consisted of four replicates and three observations per experimental unit, randomly distributed [30]. The data obtained were subjected to Duncan's test with a significance level of 95% ($P \geq 0.05$). The variables evaluated were: root number, root length, number of leaves, number of shoots, shoot length, percentage of survival, and rooting

3. Result

3.1. Induction of shoots from basal branches

In the present experimental study, shoot development from basal branches of adult trees was examined. Lengths of 1 to 4 cm appeared between 7 and 15 days after harvesting. There were no statistically significant differences between the various hormone concentrations and the studied variables in relation to the treatments, except for the number of roots at 30 days and the number of leaves at 30 and 60 days

3.2. Effect of auxin levels on root number and root length

The results indicate a significant effect at $p \leq 0.05$ of the number of roots at 30 days. The highest average was when using 500 mg kg^{-1} of IBA with an average of 2.5 roots per stake. At 60 days, the 500 mg kg^{-1} IBA concentration obtained an average of 2.5 roots per stake. Greater root length was observed at 30 days when using 500 mg kg^{-1} of IBA, giving an average of 1 cm root length per stake. At 60 days, the 500 mg kg^{-1} concentration of NAA showed the highest average of 2.73 cm root length per stake (Table 1).

Table 1. Number and length of roots in clonal propagation from *T. grandis* plus trees using growth regulators.

N°	Treatment	Number of roots		Root length	
		30 days	60 days	30 days	60 days
T1	Without hormones (Control)	0.17 a b	1.00 a	0.45 a	2.12 a
T2	500 mg kg^{-1} of NAA	0.67 a b	1.67 a	0.50 a	2.72 a
T3	1000 mg kg^{-1} of NAA	0.33 a b	0.83 a	0.75 a	1.75 a
T4	1500 mg kg^{-1} of NAA	0.08 b	0.75 a	0.08 a	0.60 a
T5	500 mg kg^{-1} of IBA	2.00 a	2.50 a	1.11 a	1.62 a
T6	1000 mg kg^{-1} of IBA	0.25 a b	0.83 a	0.82 a	1.58 a
T7	1500 mg kg^{-1} of IBA	0.17 a b	0.75 a	0.38 a	1.92 a
	CV%	61.46	65.10	57.26	66.55

Note: Values under the same letter are significantly similar according to Tukey's test at p > 0.95.

3.3. Effect of auxin levels on shoot number and length

The best result in the variable number of shoots at 30 days was observed when using 500 mg kg−1 of NAA and 1500 mg kg−1 of IBA with an average of 0.58 shoots per stake. At 60 days, the best average was obtained when using 500 mg kg−1 of NAA, 500 mg kg−1 of IBA and 1500 mg kg−1 of IBA with an average of 0.67 number of shoots. Shoot length at 30 days was more significant when using 500 mg kg−1 of IBA; however, at 60 days, shoot length was more significant when using 500 mg kg−1 of NAA (Table 2)

Table 2. Number and length of shoots in clonal propagation from *T. grandis* plus trees using growth regulators

No.	Treatment	Number of shoots		Shoot length	
		30 days	60 days	30 days	60 days
T1	Without hormones (control)	0.50 a	0.50 a	0.45 a	2.12 a
T2	500 mg kg^{-1} of NAA	0.58 a	0.67 a	0.50 a	2.72 a
T3	1000 mg kg^{-1} of NAA	0.33 a	0.42 a	0.75 a	1.75 a
T4	1500 mg kg^{-1} of NAA	0.25 a	0.33 a	0.08 a	0.60 a
T5	500 mg kg^{-1} of IBA	0.50 a	0.67 a	1.11 a	1.62 a
T6	1000 mg kg^{-1} of IBA	0.42 a	0.58 a	0.82 a	1.58 a
T7	1500 mg kg^{-1} of IBA	0.58 a	0.67 a	0.38 a	1.92 a
	CV%	27.61	26.13	60.08	74.76

Note: Values under the same letter are significantly similar according to Tukey's test at p > 0.95.

3.4. Effect of auxin levels on leaf number, survival and rooting percentage

At 30 and 60 days, differences were found at p ≤ 0.05. At 30 days, it used the 1500 mg kg−1 concentration of IBA with 2.83 leaves per stake. At 60 days, the highest average was observed when using 1500 mg kg−1 of IBA with 3.83 average leaves per stake and in control with 4.33 average number of leaves per stake. Numerically, the best survival percentage values were observed when using 500 mg kg−1 of NAA, 500 mg kg−1 of IBA and 1500 mg kg−1 of IBA with

an average 67 % of seedling survival at 30 days. At 60 days, the numerically highest percentage was observed in the concentration 500 mg kg−1 of NAA and 1500 mg kg−1 of IBA with 58.33% of live plants. The rooting percentage was higher when using 500 mg kg−1 of IBA, with an average of 33% rooting. At 60 days, it was higher when using 1500 mg kg−1 of IBA with an average of 42% rooting (Table 3, Figure 1).

Table 3. Number of shoots, percentage survival and rooting of shoots in clonal propagation from *T. grandis* plus trees using growth regulators

No.	Treatment	Number of sheets		Survival		Rooting	
		30 days	60 days	30 days	60 days	30 days	60 days
T1	Without hormones (Control)	1.42 abc	4.33 to	fifty to	fifty a	16.67 a	50.00 a
T2	500 mg kg^{-1} of NAA	0.92 ab	2.33 ab	66.67 to	58.33 a	25.00 a	50.00 a
T3	1000 mg kg^{-1} of NAA	0.67 ab	1.42 ab	41.67 to	33.33 a	25.00 a	25.00 a
T4	1500 mg kg^{-1} of NAA	0.5 c	0.58 b	33.33 to	25 a	8.33 a	16.67 a
T5	500 mg kg^{-1} of IBA	2.25 bc	2.58 ab	66.67 to	41.67 a	33.33 a	33.33 a
T6	1000 mg kg^{-1} of IBA	2 abc	0.92 ab	58.33 to	41.67 a	8.33 a	33.33 a
T7	1500 mg kg^{-1} of IBA	2.83 to	3.83 to	66.67 to	58.33 a	16.67 a	41.67 a
	CV%	49.63	66.4	58.47	66.02	58.47	66.02

Note: Values under the same letter are significantly similar according to Tukey's test at $p > 0.95$

(A) (B)

Figure 1. Root induction from *T. grandis* plus trees with the use of growth regulators. (A) treatment 2 at 30 days (B) treatment 2 at 60 days after the trial was established

4. Discussion

Careful handling of the harvested material and its establishment in the substrate proved to have a significant impact on shoot growth. The emission days are similar to the results reported by Meza et al. [12] seven to 10 days, and are complemented by other relevant references in the field [31]. The results for root number are higher than those reported for the same species in the work developed by Del Valle [28] who obtained in T3 (1000 mg kg^{-1} of NAA + 1000 mg kg^{-1} of IBA) 1.13 average roots. They were also similar to those Husen (2013) obtained in different teak clones with 2.67 roots per plant at a concentration of 500 mg kg^{-1} of IBA. Similarly, Meza et al. [12] also obtained a similar average of 2.55 roots per plant when using higher doses of 5000 mg

kg^{-1} of IBA combined with 4000 mg kg^{-1} of PVP Polyvinylpyrrolidone. Finally, Sawitri et al. (2020) obtained an average of 2 to 3.5 roots with no difference between the treatments used with the AIN hormone (0 ppm, 50 ppm, 100 ppm, 150 ppm) on two substrates.

The root lengths obtained in this research are lower than those found by Husen [32], who obtained a 3.91cm root length when using 500 mg kg^{-1} of IBA. Similar results were reported by [33,34] suggests that the age of the material to be propagated significantly affects the propagation capacity of the plant. Therefore, the techniques to maintain or induce juvenility are critical in vegetative propagation. For the initiation of adventitious roots, the hormonal action of compounds naturally present in plants is favorable [35,36]. Of these, auxins are the hormones that have the most significant effect on root formation in cuttings. The presence of auxins in *T grandis* seems sufficient to induce roots; however, applying auxins at low concentrations can stimulate an increase in the number of roots.

The average number of shoots obtained was higher than those reported by Del Valle [28], who obtained an average of 0.31 shoots with the combination of (1000 mg kg^{-1} of NAA + 1000 mg kg^{-1} of IBA). These results allow inferring that the application of auxins without combination positively influences shoot induction. Regarding shoot length, the results we similar reported by Carranza et al.[16], who worked on vegetative propagation of teak using combined NAA and IBA hormones. According to Balzan et al. [37] one of the most outstanding characteristics of auxins is that it is differentially distributed between cells and tissues; in some cases, it accumulates locally in a cell or a group of cells; in others, it changes their distribution between cells and, finally, it can also have a differential distribution in plant tissues.

An increase in IBA favored the number of leaves. These results exceed those obtained by Del valle [28], whose average number of live plants in all treatments was lower when using auxins NAA and IBA combined at concentrations of: (0, 500, 1000, 1500 and 2000 mg kg −1). Leaves influence the rooting of cuttings [35], because they are a source of photoassimilates, auxins and other substances vital for rooting, however leaves provide a large surface area through which the cuttings lose water by transpiration [38].

The survival percentage exceeded those reported by Del valle [28], who, after 60 days of vegetative propagation of *T. grandis*, used a concentration of (1000 mg kg^{-1} of NAA + 1000 mg kg^{-1} of IBA) and obtained a 25% survival rate. Auxins are characterized by their ability to cause one or more biological phenomena, such as: inducing stem elongation in bioassays, promoting cell division in callus cultures in the presence of cytokinins, and forming adventitious roots in leaves and cut stems [39]. Regarding the percentage of rooting of *T. grandis* shoots in the presence of the auxins (NAA and IBA) used, low concentrations of hormones showed better results, indicating that low doses without combinations are sufficient to induce roots up to 50%. In contrast to the results reported, Meza et al. [12] affirm that the increase in the concentration of hormones causes an increase in the rooting percentage. On the other hand, Sawitri et al. [10] report that producing superior teak seedlings through the shoot–cutting method depends on the rooting medium, IBA concentration and the specific teak clone. These results corroborate what Arroyo [40] described, who states that the different responses will be determined by auxin concentration, perception, transport, the sensitivity of the cell type and tissue and the developmental stage of the plants.

5. Conclusions

Even though statistically, the treatments applied did not differ except for the number of roots at 30 days and the number of leaves at 30 and 60 days, it should be noted that in general terms, low concentrations of NAA (500 mg kg^{-1}) and high concentrations of IBA (1500 mg kg^{-1}) showed up to 58.33 % survival at 60 days after the trial was established. However, from the null statistical difference in most of the evaluated variables, it is highlighted that the conditions under which the trial was established, such as plant material, substrate, irrigation, application of mineral nutrients, and humid chamber, allowed the survival of shoots between 25 to 58.33 %.

These results directly relate to the profitability obtained in 500 mg kg^{-1} of NAA and 1500 mg kg^{-1} of IBA. The percentage of rooting at 60 days suggests that the auxin content without auxin application or the application of low doses without combinations (NAA plus IBA) is sufficient to induce roots up to 50% in teak shoots obtained from adult trees.

Future studies should focus on optimizing auxin concentrations and combinations for root induction in teak shoots from adult trees, as well as exploring alternative propagation techniques. Long-term assessments of growth and survival are necessary to evaluate the effectiveness of rooting treatments and contribute to the development of sustainable teak propagation practices in forestry.

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