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Effects of substrates and exogenous hormones on rooting of rubber seedling cuttings

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***Abstract*— *An orthogonal experiment (three factors × three levels) was designed to establish a cutting propagation technique for Hevea brasiliensis rootstock seedlings. The effects of substrate, exogenous hormone type, and concentration on rooting were evaluated across nine treatments. Rooting rate, four root traits, and mineral element contents were measured, and rooting performance was comprehensively assessed using the membership function method. Substrate was found to be the dominant factor, significantly affecting rooting rate, total root length, primary root number, and K, Ca, Mg, Fe, and Zn contents. Neither hormone type (IAA, IBA, NAA) nor concentration (100–500 mg/L) exhibited significant effects on rooting. The soil yielded the highest mean membership value, with the soil + 500 mg/L IAA treatment ranking first (0.984). Overall, the highest rooting rate and root traits were obtained with soil and 500 mg/L IAA, which is recommended as the optimal protocol for rooting of rubber tree seedling cuttings.*

Keywords*— *Hevea brasiliensis; cutting propagation; substrate; orthogonal design; membership function

I. INTRODUCTION

Pará rubber tree (*Hevea brasiliensis*) is native to the Amazon rainforest of South America. As a typical tropical arbor, it is primarily cultivated in tropical rainforest climate zones. The rubber tree is not only the world's most important source of natural rubber, but its wood is also a valuable raw material for furniture, granting it significant economic importance in tropical regions.

Currently, commercially cultivated rubber trees rely mainly on grafting, with rootstocks predominantly derived from the sexual progeny of specific varieties such as GT1 and RRIM600. An insufficient supply of rootstock seeds has become a critical bottleneck restricting the development of the rubber tree grafting and nursery industry. This shortage arises from two factors: firstly, the cultivation area of rubber varieties used for rootstock seed production has been shrinking annually, leading to a sharp decline in seed sources; and secondly, rubber tree seeds are recalcitrant, and their natural, dispersed seedfall is difficult to collect, further limiting the large-scale production of rootstock seedlings. Therefore, there is an urgent need to conduct research on the clonal propagation of rubber tree rootstocks and to explore alternative propagation pathways that are not dependent on seeds.

As a simple and economical method of asexual propagation, cutting propagation offers advantages such as rapid multiplication, ease of operation, and the ability to preserve desirable maternal traits. It has been widely applied in the seedling propagation of various woody plants, including poplar (Duan et al. 2024), mulberry (Yuan et al. 2025), Manchurian ash (Peng et al. 2024) and India rubber fig (Chen et al. 2017). However, systematic research on the cutting propagation of rubber trees remains relatively scarce. Masson et al. (2013) induced adventitious roots using tissue-cultured regenerated plants as cutting sources. Vallejos-Torres et al. (2021) preliminarily explored the effects of leaf number retained on cuttings and IBA concentration on rooting rates. While these studies confirmed the feasibility of rubber tree cutting propagation, the cutting materials were predominantly tissue-cultured regenerants, and the research indicators were often limited, primarily using rooting rate as the sole criterion. They failed to systematically evaluate the combined effects of multiple factors such as cutting substrate, hormone type, and concentration, and lacked a comprehensive assessment of root development quality and mineral nutrient uptake.

Cutting propagation is a complex physiological, ecological, and anatomical process, regulated by multiple factors including the plant's genetic characteristics, the physiological age and lignification degree of the cuttings, the season of cutting, environmental conditions, and exogenous hormones(Wang et al. 2015). Among these, the cutting substrate and exogenous hormones are two critical external factors influencing rooting and root system development. An appropriate substrate provides adequate moisture, aeration, and physical support for the cuttings, while exogenous hormones promote the induction and elongation of adventitious root primordia by modulating endogenous hormone levels. Previous studies have demonstrated that substrate type and hormone treatment significantly affect the cutting propagation of species such as *Malpighia emarginat*a (Xiong et al. 2021), *Zelkova sinica* (Chen et al. 2021) and *Melliodendron xylocarpum* (Dai et al. 2026); however, these effects are species-specific and vary depending on the treatment combinations, and thus cannot be generalized.

In commercial rubber tree cultivation, rootstock seedlings are primarily derived from seedlings. Therefore, this study utilizes young rubber tree seedlings as the source of cuttings. Employing an orthogonal experimental design, we systematically investigate the effects of three factors (cutting substrate, exogenous hormone type, and hormone concentration), each at three levels, on the rooting rate, root morphological parameters, and mineral element content of the cuttings. A membership function analysis will be employed for multi-index comprehensive evaluation. The objective is to identify the optimal cutting propagation protocol, thereby providing a theoretical basis and technical guidance for the large-scale clonal propagation of rubber tree rootstock seedlings.

II. MATERIALS AND METHODS

Experimental materials

Seedlings from GT1 variety with approximately four months old were used as the source of cuttings.

Experimental design

An L9 (3^3) orthogonal experimental design was adopted, comprising three factors: cutting substrate (soil, sand and soil-less mix), exogenous hormone type (IAA, IBA, NAA), and hormone concentration (100, 250, 500 mg/L). Each factor was set at three levels, resulting in a total of nine treatment combinations. Each treatment consisted of 32 cuttings, and the experiment was repeated three times.

Cutting and post management

The test substrates were filled into 32-cell plug trays respectively, and thoroughly watered. The cuttings were approximately 5 cm in length, with each cutting retaining one fully expanded leaf. The basal ends of the cuttings were immersed in the respective growth hormone solutions for 60 minutes, and then inserted into the substrate. After insertion, the plug trays were placed in an intermittent-mist propagation chamber. During the first 15 days after cutting, mist was applied once every 2 hours during the daytime, with each application lasting 4 minutes; after 15 days, the regime was adjusted to once every 3 hours, with each application lasting 3 minutes.

Assessment of rooting indices

Roots was assessed after four months later. Rooting rate (%) = (number of rooted cuttings / total number of test cuttings) × 100%. After the root systems were rinsed clean, they were scanned using a digital scanner (STD1600 Epson), and a LA-S root analysis system was employed to quantitatively analyze four root morphological parameters: total root length, number of primary roots, number of secondary roots, and root surface area.

Determination of mineral element contents in cutting seedlings

Cutting seedlings from the same substrate were pooled into one sample group, yielding three groups of samples: the sand treatment group, the soil-less mix treatment group, and the soil treatment group. The samples were oven-dried at 110 °C for 30 min to inactivate enzymes, then dried at 60 °C to constant weight (approximately 24 h), ground, and sieved before use. Total nitrogen content was determined by Nessler's colorimetric method, total phosphorus content by the molybdenum-antimony-ascorbic acid colorimetric method, and the contents of potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) by flame atomic absorption spectrometry (Bao, 2000).

Data analysis

To comprehensively evaluate the rooting performance of each treatment, the membership function method was employed to assign integrated scores to multiple rooting indicators for each treatment. The membership function value for a given indicator in a given treatment was calculated using the following formula:

$$U(X_j) = (X_j - Xmin) / (Xmax - Xmin)$$

where X_j is the measured value of a given indicator for a given treatment, and Xmax and Xmin are the maximum and minimum values of that indicator among all treatments, respectively. The average of the membership function values across all measured indicators for each treatment was used as the comprehensive evaluation criterion for that treatment; a larger average value indicates superior rooting performance.

Data processing was performed using Microsoft Excel 2010. Analysis of variance (ANOVA) and multiple comparisons (Duncan's new multiple range test, $P < 0.05$) were conducted using SPSS 16.0.

III. RESULTS

Variance analysis of the orthogonal experiment

Variance analysis of the orthogonal experiment was conducted on five rooting indicators of cuttings taken from rubber tree seedlings, and the results are shown in Table 1. As indicated in Table 1, the effects of different factors on each rooting indicator varied considerably. Cutting substrate had a significant effect on rooting rate, total root length, and the number of primary roots ($P < 0.05$), but its effect on the number of secondary roots and root surface area did not reach statistical significance ($P > 0.05$). Neither exogenous hormone type nor hormone concentration had significant effects on any of the five rooting indicators ($P > 0.05$).

In terms of the contribution of each factor, based on the ranking of F-values in Table 1, the overall influence of the three factors on the five rooting indicators followed the order: cutting substrate > exogenous hormone type > hormone concentration. This indicates that cutting substrate was the dominant factor affecting the rooting of rubber tree seedling cuttings, while hormone concentration exerted the least influence on the rooting indicators.

Effect of cutting substrate on rooting traits of rubber tree cuttings

The above variance analysis results indicated that cutting substrate significantly influenced multiple indices of rooting in rubber tree seedling cuttings. To further clarify the differences among the different substrate levels, multiple comparisons of each rooting indicator were performed using the LSD method, and the results are presented in Table 2.

In terms of rooting rate and root morphological parameters, significant inter-group differences were observed among the different substrate treatments (Table 2). Specifically, the rooting rates for the soil treatment and sand treatment were 56.60% and 47.22%, respectively, with no significant difference between them; however, both were significantly higher than that of the soil-less mix treatment (22.92%). Regarding total root length, cuttings in the soil treatment exhibited a total root length (26.82 cm) significantly greater than those in the sand treatment (14.53 cm) and the soil-less mix treatment (18.00 cm), while the latter two did not differ significantly. In terms of the number of primary roots, cuttings in the soil treatment had an average of 3.50 primary roots, which was significantly higher than that in the sand treatment (2.02) and the soil-less mix treatment (1.76); no significant difference was detected between the sand and soil-less mix treatments. For the number of secondary roots and root surface area, no significant differences were detected among the three substrate treatments.

Effect of cutting substrate on mineral element contents of rubber tree cuttings

The foregoing results indicated that the three substrates significantly affected the rooting traits of rubber tree cuttings. Therefore, the mineral element contents of cuttings under different substrate treatments were further analyzed, and the results are shown in Table 3. As presented in Table 3, no significant differences were detected among the different substrate treatments in the contents of the macronutrients N and P, nor in the micronutrients Mn and Cu. However, significant differences among substrate treatments were observed in the contents of the macronutrients K, Ca, and Mg, as well as the micronutrients Fe and Zn. Notably, for the soil treatment, which exhibited the best rooting performance, the contents of Ca, Mg, and Zn in the cuttings were significantly lower than those in the soil-less mix or sand treatments. In contrast, cuttings in the soil-less mix treatment had significantly higher contents of K, Ca, Mg, and Zn than those in the sand or soil treatments.

Effect of exogenous hormones on rooting indices of rubber tree cuttings

This study also analyzed the effects of three exogenous hormones (IAA, IBA, NAA) and their three concentrations (100, 250, 500 mg/L) on each rooting indices of rubber tree cuttings. The variance analysis results of the orthogonal experiment (Table 1) indicated that neither exogenous hormone type nor hormone concentration had significant effects on any of the rooting indicators (rooting rate, total root length, number of primary roots, number of secondary roots, and root surface area) of rubber tree cuttings ($P > 0.05$).

Comprehensive evaluation of treatment combinations on rooting of rubber tree cuttings

Given that the nine treatment combinations exhibited inconsistent differences across the five indicators (rooting rate, total root length, number of primary roots, number of secondary roots, and root surface area) (Table 4), the fuzzy mathematics membership function method was employed for multi-indices comprehensive evaluation of the rooting performance of each treatment. The membership function value for each index in each treatment was calculated according to the membership function formula, and the average of the membership function values across the five indices for each treatment was used as the criterion for comprehensive rooting performance; a larger average value indicates superior rooting performance. As shown in Table 4, among all treatment combinations, the soil_IAA_500 treatment had the largest average membership function value, and its rooting rate (64.58%), total root length (30.39 cm), number of primary roots (3.57), number of secondary roots (1.65), and root surface area (10.32 cm^2) were all the best among all treatments, indicating that cuttings treated with soil as the substrate combined with 500 mg/L IAA exhibited the optimal rooting performance. With respect to the substrate factor, the three treatments with soil substrate (soil_IAA_100, soil_IBA_250, soil_IAA_500) ranked the top three in average membership function values, further demonstrating that the comprehensive rooting performance of soil substrate was superior to that of sand and soil-less mix. In contrast, the three treatments of sand_NAA_500, soil-less mix_IBA_500, and soil-less mix_NAA_100 had the lowest membership function values among the nine treatments, indicating relatively poor rooting performance.

IV. DISCUSSION

Plant cutting propagation is a complex process regulated by the integrated effects of internal physiological factors and external environmental conditions. Mature rubber trees are considered difficult-to-root by cuttings, which is mainly attributed to their internal developmental phase (the maturation effect). Exogenous hormones are one of the important external factors influencing cutting rooting. Numerous studies have demonstrated that the type and concentration of exogenous hormones significantly affect the rooting of plant cuttings (Zhou et al, 2022, Wang et al, 2023, Zhao et al, 2020). For instance, Chen et al.(2021) found that IBA was more effective than NAA for *Taxodium distichum* rooting, and Zhai et al. (2021) also reported that IBA outperformed NAA and IAA in rooting efficacy. Regarding concentration effects, it is generally considered that low concentrations promote callus induction, medium to high concentrations facilitate the initiation and elongation of adventitious roots, while excessively high concentrations may cause damage to cutting tissues (Zhang et al, 2014). However, in the present study, neither the three exogenous hormones (IAA, IBA, NAA) nor the three concentration levels (100, 250, 500 mg/L) had significant effects on any rooting indicator of rubber tree cuttings. This result may be related to the developmental stage of the cuttings. The cuttings used in this study were derived from 4-month-old seedlings, which are still in the juvenile phase, characterized by strong meristematic capacity and high endogenous hormone levels, resulting in low dependence on exogenous hormones and, consequently, an inconsequential supplementary effect of exogenous hormone application. Therefore, it is suggested that for cutting propagation using rubber tree seedlings as material, the type and concentration of exogenous hormones are not priority technical parameters to consider.

As the immediate environmental medium for cutting rooting, the substrate's physicochemical properties are closely related to rooting performance. An ideal cutting substrate generally possesses appropriate water-holding capacity, permeability, aeration, and a certain nutrient supply capacity. When substrate permeability is favorable, sufficient air porosity and oxygen diffusion rate can meet the respiratory demands of the root system, which is beneficial to adventitious root development (Akwatulira et al, 2011, Wang et al, 2020); a good water-air balance also facilitates adventitious root primordium induction and enhances root vitality (Zhou et al, 2022). Wang et al. (2023) found that substrates significantly affected rooting rate, maximum root length, and root number, and Xue et al. (2022) also reported significant effects of substrate on rooting rate and average root length. These findings were further validated in the present study: cutting substrate significantly affected the rooting rate, total root length, and number of primary roots of rubber tree cuttings, with the soil treatment exhibiting the best overall rooting performance.

Notably, a somewhat "anomalous" result of this study is that soil significantly outperformed sand and soil-less mix, which are generally considered to have better aeration and permeability. Some studies have pointed out that soil often yields inferior rooting performance compared to sand or other artificial substrates due to its relatively poor water permeability and aeration (Zhao et al, 2019). However, under the experimental conditions of this study (intermittent misting, with irrigation once every 2 hours during the early stage), the sand and soil-less mix maintained relatively high moisture content over prolonged periods, and their aeration advantages were not fully realized. In contrast, the moderate water-holding capacity of soil may have provided a more stable water–air balance in this high-humidity environment. On the other hand, water permeability and aeration may not be the sole determinants of substrate quality for rubber tree cutting propagation. Mineral element content measurements showed that cuttings cultivated in the three substrates exhibited varying levels of macro- and micronutrient contents, but the overall differences were not substantial, indicating that during the short-term rooting phase, substrate type had not yet induced fundamental changes in the nutritional status of the cuttings. Therefore, the superior performance of soil in this study may be more attributable to its comprehensive advantages in physical structure (such as aggregate stability and appropriate solid/liquid/gas phase ratios) and rhizosphere microenvironment (such as microbial activity), rather than purely to nutrient supply or aeration capacity.

Based on the nine treatment combinations from the three-factor, three-level orthogonal experimental design, the membership function method was employed to rank the comprehensive rooting performance using five rooting indicators as evaluation criteria. The results showed that the Soil + 500 mg/L IAA combination had the highest average membership function value, representing the optimal comprehensive rooting performance.

In summary, the cutting substrate played a dominant role in the rooting of rubber tree seedlings, with its effect far exceeding those of exogenous hormone type and concentration. Therefore, substrate type should be prioritized as the primary optimization factor in future studies. It should be noted that this study only examined three single substrates (sand, soil-less mix, and soil), and the overall rooting rates remained relatively low (the highest being 64.58%). Moreover, certain mixed substrates have been shown to be superior to single substrates in other tree species (Xue et al, 2022, Zhao et al, 2019). Consequently, further studies are warranted to explore the blending ratios of different single substrates and the rooting effects of mixed substrates, with the aim of further improving the cutting rooting rate of rubber tree seedlings.

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Table1 Variance analysis (F-values) of orthogonal experiment for rooting indices of rubber tree seedling cuttings

Index	Substrate	Hormone type	Hormone concentration
Rooting rate	14.59*	0.57	0.20
Total root length	11.53*	0.45	0.15
Primary root number	49.43*	0.10	0.01
Secondary root number	1.11	2.34	0.52
Root surface area	4.23	1.40	0.03

Note： * indicates significant difference at $p < 0.05$.

Table2 Effects of different substrates on rooting indices of rubber tree seedling cuttings

Substrate	Rooting rate /(%)	Total root length /(cm)	Primary root number	Secondary root number	Root surface area /(cm^2)
Sand	47.22 ± 8.61^{a}	14.59 ± 3.44^{b}	2.02 ± 0.25^{b}	0.83 ± 0.56^{a}	4.44 ± 1.84^{a}
Soil-less mix	22.92 ± 6.50^{b}	18.00 ± 2.88^{b}	1.76 ± 0.30^{b}	0.67 ± 0.10^{a}	5.56 ± 1.12^{a}
Soil	56.60 ± 8.35^{a}	26.82 ± 3.30^{a}	3.50 ± 0.47^{a}	1.16 ± 0.43^{a}	8.10 ± 1.91^{a}

Note：Different letters within the same column indicate significant differences at $p<0.05$.

Table3 Effect of cutting substrates on mineral element contents of rubber tree cuttings

substrates	N/(%)	P/(%)	K/(%)	Ca/(%)	Mg/(%)	Fe/($mg \cdot kg^{-1}$)	Mn/($mg \cdot kg^{-1}$)	Cu/($mg \cdot kg^{-1}$)	Zn/($mg \cdot kg^{-1}$)
Sand	1.72 ± 0.16^{a}	0.307 ± 0.04^{a}	0.584 ± 0.07^{b}	0.161 ± 0.02^{ab}	0.552 ± 0.08^{a}	154.43 ± 28.7^{b}	88.84 ± 18.64^{a}	11.44 ± 1.28^{a}	92.15 ± 13.67^{b}
Soil-less mix	1.78 ± 0.18^{a}	0.344 ± 0.07^{a}	0.860 ± 0.13^{a}	0.181 ± 0.03^{a}	0.484 ± 0.10^{ab}	191.15 ± 27.5^{b}	100.43 ± 19.83^{a}	12.54 ± 1.40^{a}	121.82 ± 9.16^{a}
Soil	1.65 ± 0.10^{a}	0.293 ± 0.05^{a}	0.704 ± 0.14^{ab}	0.129 ± 0.02^{b}	0.346 ± 0.09^{b}	252.47 ± 18.9^{a}	111.07 ± 22.08^{a}	11.23 ± 1.06^{a}	93.47 ± 14.19^{b}

Note：Different letters in the same column indicate significant difference, $p<0.05$.

Table4 Rooting indices and membership function values of each treatment combination

Treatment	Rooting rate /%	Total root length /cm	Primary root number	Secondary root number	average root length /cm^2	Average membership function value
Sand_IAA_100	56.79 ± 7.54^{ac}	18.50 ± 1.71^{d}	2.31 ± 0.21^{b}	1.47 ± 0.12^{a}	6.46 ± 0.61^{b}	0.619
Sand_IBA_250	44.59 ± 4.77^{bce}	13.30 ± 1.93^{e}	1.91 ± 0.18^{bc}	0.64 ± 0.07^{c}	4.00 ± 0.64^{d}	0.306
Sand_NAA_500	40.08 ± 4.23^{de}	11.98 ± 1.76^{e}	1.84 ± 0.43^{bc}	0.39 ± 0.03^{d}	2.87 ± 0.31^{e}	0.203
Soil-less mix_IAA_250	28.13 ± 5.95^{f}	19.30 ± 2.59^{d}	1.80 ± 0.26^{c}	0.65 ± 0.06^{c}	6.12 ± 0.71^{b}	0.337
Soil-less mix_IBA_500	25.00 ± 4.37^{f}	14.70 ± 2.75^{de}	2.04 ± 0.58^{b}	0.58 ± 0.07^{c}	4.83 ± 0.52^{cd}	0.263
Soil-less mix_NAA_100	15.63 ± 3.83^{g}	20.00 ± 3.68^{cd}	1.44 ± 0.51^{c}	0.78 ± 0.09^{bc}	5.73 ± 0.98^{bc}	0.266
Soil_IAA_500	64.58 ± 7.41^{a}	30.39 ± 4.48^{a}	3.57 ± 0.31^{a}	1.65 ± 0.13^{a}	10.32 ± 1.92^{a}	1.000
Soil_IBA_100	57.29 ± 9.03^{ab}	26.20 ± 1.22^{ab}	3.50 ± 0.15^{a}	1.00 ± 0.11^{b}	7.08 ± 0.64^{b}	0.748
Soil_NAA_250	47.92 ± 6.44^{bcd}	23.87 ± 1.65^{bc}	3.43 ± 0.36^{a}	0.84 ± 0.07^{b}	6.99 ± 1.33^{b}	0.660

Note：Different letters in the same column indicate significant difference, $p<0.05$.

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