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Research Article

Propagation of *Theobroma cacao* by Rooted Cuttings in Mini-Tunnels

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Cacao is an economically important crop that is currently planted in Asia, Africa, and America. Cuttings is a technique of vegetative propagation suitable for the conservation of selected desirable characteristics in cacao trees. The objective of this study was to evaluate the rooting of cacao cuttings in mini-tunnels with different doses of indole-3-butyric acid (IBA) to obtain a simple and viable vegetative propagation protocol. The experiment was carried out under a completely randomized design (CRD). Cuttings 5 to 7 cm long were collected from the middle area of cacao tree crowns and treated with doses of 0, 1000, 2000, and 3000 ppm of IBA; then they were placed in Jiffy© pellets and set to root in plastic-covered mini-tunnels with fog irrigation. The results indicate that treatments with 0 and 1000 ppm of IBA produced the highest values in survival (100%, both treatments) and rooting percentage (87.7 and 90.0%, respectively) as well as number and length of roots (4.3 roots-4.21 cm in length and 4.5 roots-5.32 cm in length, respectively); likewise, cuttings treated with 0 ppm rooted after 24–40 days, followed by doses of 1000 ppm (24–46 days). All rooted cuttings without IBA (0 ppm) achieved 100% acclimatization in nursery. In general, the best results were obtained without IBA application (Control 0 ppm) in all the parameters evaluated, similar to those obtained with the application of 1000 ppm IBA; these results indicate the success and efficiency of the vegetative propagation protocol proposed in this study.

1. Introduction

Theobroma cacao L. “cacao” (Malvaceae) is an important crop that grows in tropical areas and it is currently planted in three continents: Asia, Africa, and America; the main exporting countries are Côte d’Ivoire, Ghana, Ecuador, Cameroon, Nigeria, Indonesia, Brazil, Peru, the Dominican Republic, and Colombia [1]. Cocoa is grown by approximately 6 million farmers worldwide and is the livelihood of more than 40 million people who depend on cocoa [2]. Cacao beans are used as a fundamental ingredient in the production of cocoa bars and chocolate, which are demanded worldwide for their pleasant taste and nutritional value [3]. Additionally, cocoa beans have a number of other nonfood uses in the pharmaceutical and cosmetic industries [4]. According to the latest published data from the International Cocoa Organization (ICCO), the total world production in 2020–2021 was 5.2 million metric tons of cocoa beans [5].

Cacao plants can be propagated by botanical seed (sexual propagation) and vegetative methods (asexual propagation); the latter includes conventional vegetative propagation techniques such as grafting, cuttings, and air layering, as well as tissue culture [6, 7]. Propagation through botanical seed presents certain difficulties to preserve superior characteristics of selected cacao trees, mainly due to cross-pollination or allogamy of the plants, which does not allow the desirable agronomic characteristics of some selected individuals to remain constant in successive generations [8]. In this sense, asexual vegetative propagation has become a convenient alternative to solve this problem, because the new plants will be genetically identical to the source material and will retain their desirable characteristics, including high yield and resistance to pests and diseases [9]. Many countries such as Brazil, Colombia, Ecuador, and Costa Rica have used vegetative propagation techniques on a large scale, mainly grafting, and reported an increase in the yield of their cultivation areas [10, 11]. Despite this, there is a need to optimize alternative methodologies that guarantee the successful propagation of selected plants. Regarding rooting of cuttings, these challenges are mainly related to rooting substances, the propagation system used, and the control of conditions during propagation [12].

Rooting of cuttings can be most effective under a controlled environment; due to this, it is important to choose a propagation system that allows, partially at least, controlling and maintaining such stable conditions. In that respect, the mini-tunnels system has proven to fulfill that purpose, offering better control capacity against restrictive weather conditions, preventing the cuttings from drying out and overheating before they develop roots, and maintaining a favorable environment for the rooting and growth of plants [13]. In the Amazon region, the successful use of mini-tunnels has been previously reported in different plant species [14–17] using IBA (indole butyric acid). However, there is no information about the use of mini-tunnels for large-scale propagation of cacao, since vegetative propagation of cacao traditionally takes place under a sub-irrigation and other systems [18–23].

Auxins such as IAA (indoleacetic acid), IBA (indole butyric acid), and NAA (naphthaleneacetic acid) have an important role in different processes related to the growth and development of plants [24, 25]. In the context of plant propagation, the role of auxins has been reported to stimulate the formation of adventitious roots of a large number of species, not only by increasing the percentage of rooting but also by accelerating the initiation of roots, increasing the number and quality of roots and promoting uniformity of rooting [26–28]. In general, indole-3-butyric acid (IBA) is considered to be more stable and efficient than IAA [24].

In this context, the present research aimed to evaluate the rooting of cacao cuttings in plastic mini-tunnels using different doses of indole-3-butyric acid (IBA 0, 1,000, 2,000, and 3,000 ppm) and to propose an efficient protocol for acclimatizing rooted cacao cuttings.

2. Materials and Methods

2.1. Site and Material Collection. The study was conducted in the greenhouse of the Faculty of Agricultural Sciences, San Martin National University (UNSM-T), San Martín region, Peru, between the months of May-July 2019. The University campus is located at 278 m.a.s.l. and has a temperature of 24.6°C on average, a relative humidity of 45% on average, and a monthly rainfall of 63.1 mm.

Shoots were collected from only 01 cacao tree (Clone IMC67) established in the field of UNSM-T campus. To obtain the highest quantity and quality of shoots, selected trees were fertilized with a mixture of nitrogen, phosphorus, and potassium (20 : 20 : 20) 30 days before cutting collection.

2.2. Collection and Preparation of Cuttings. Shoots were collected in the morning (before 8 a.m.) from the middle area of cacao tree crowns (Figure 1(a)), using sterilized pruning shears. All collected shoots had active axillary buds (Figure 1(b)) and were transported to the propagation greenhouse in an icebox, to minimize the physiological stress of the tissues. Once in the propagation area, 2 to 3 cuttings, 5–7 cm long, were obtained from each shoot; each cutting had 3 axillary buds, and leaflets were trimmed to 30% of their area (75–100 cm^2/leaflet). Before the application of IBA, cuttings were immersed in a fungicide solution (Carbendazim 50 g/L) for 1 minute. Immediately, they were placed on a sterile tray to allow the evaporation of excess water.

2.3. Treatments and Experimental Design. A completely randomized design with 30 repetitions (cuttings) per treatment was used. The cuttings bases were treated with one of four concentrations of chemically pure IBA (Sigma-Aldrich) dissolved in 96% alcohol: 0, 1000, 2000, and 3000 ppm, applied by immersing the base of the cuttings for 5 seconds in the different concentrations of the hormone. Cuttings under the control treatment were immersed in pure alcohol. Immediately after the application, the alcohol was quickly evaporated by keeping the cutting base in a stream of cold air for 30 seconds.

Cuttings were placed in Jiffy© pellets N°50F (50 × 95 mm), inserted at a depth of 2 cm approximately, and slightly pressing the substrate to displace the air around the cutting base. Subsequently, the Jiffy© pellets with cuttings were placed in sterile trays of 45 cm × 55 cm sides × 3 cm tall and transferred to plastic-covered mini-tunnels for the rooting phase (Figure 1(c)).

Mini-tunnels were made of a galvanized metal frame with dimensions 3.0 × 1.0 × 0.6 m (length × width × height), with support legs 0.9 m long, and covered with white translucent plastic (200 microns) which allowed light transmission of 86% (Figures 1(d) and 1(e)). Mini-tunnels had 3 internal nebulizers connected to a timer-controlled system which generated nebulization as follows: 10 s each 15 min during the first 2 weeks, 10 s each 30 min during the following 3 weeks, and finally 15 s each 60 min for the rest of the propagation period. This irrigation frequency maintained a relative humidity above 80% and temperatures of 26–34°C during the day throughout the rooting phase. Once cuttings sprouted, after 24–62 days, depending on the dose of IBA, they were extracted to record the number of cuttings rooted and the number and length of roots (Figure 1(f)).

For the acclimatization process, 30 cuttings were propagated again with the best dose of hormone. Rooted cuttings were placed in a greenhouse (humidity: 70–90%,

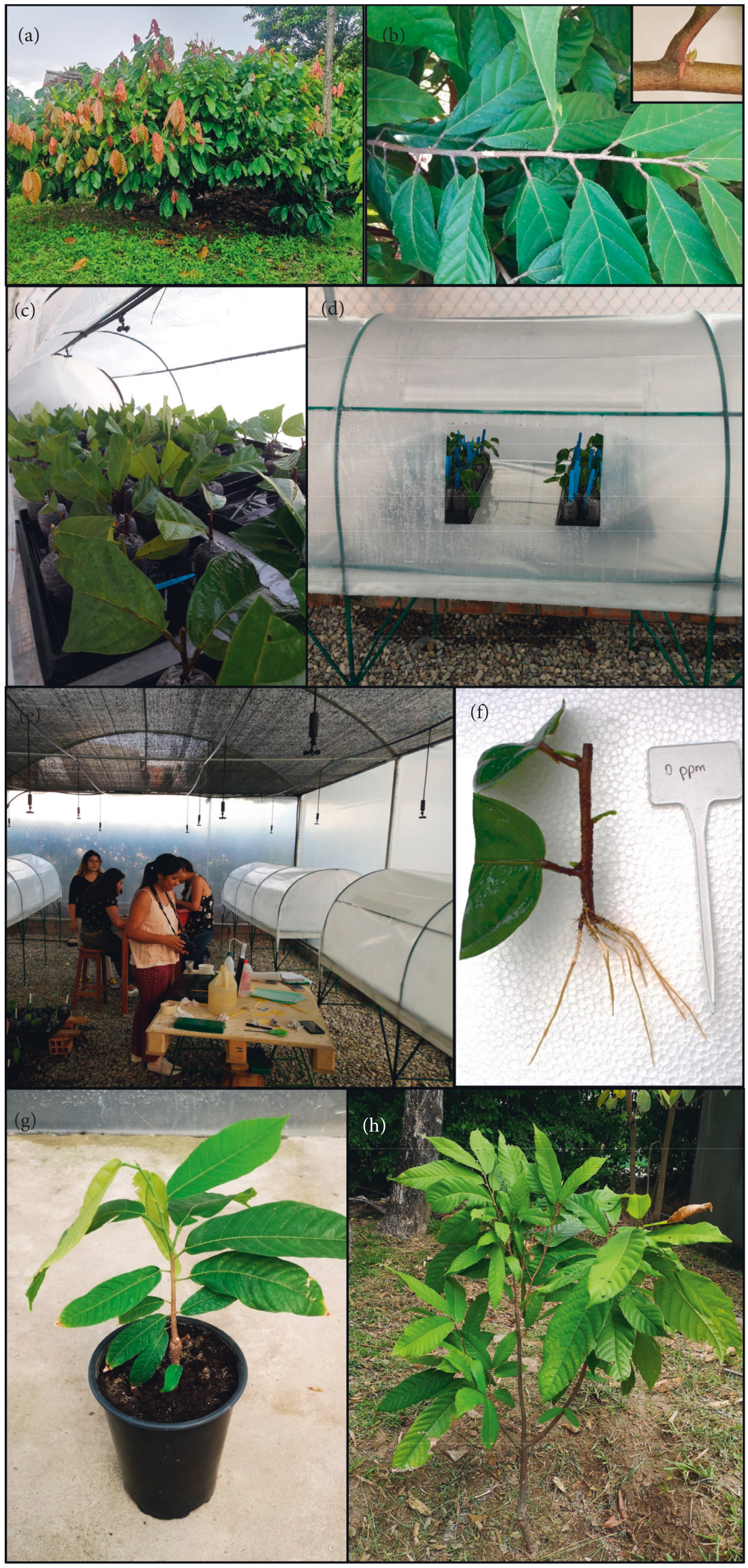

Figure 1: Process of rooting of *T. cacao* cuttings. (a) Selected tree. (b) *T. cacao* shoot with axillary buds (inner box). (c) Cuttings placed in Jiffy pellets with and without IBA. (d, e) External view of a mini-tunnels. (f) Root system in cutting after 40 days of treatment. (g) Potted *T. cacao* cutting, 90 days old. (h) *T. cacao* plant established in the field.

temperature: 24–32°C, and shade: 50%) for 7 days, and then the rooted cuttings were planted in 3 l pots filled with a 3 : 1: mixture by volume of agricultural soil: sand and transferred to a nursery for acclimatization. During the first 10 days, plants remained under a nebulization system with a frequency of 15 s every 60 min (6 : 00 am to 6 : 00 pm). The nursery had a Raschel mesh (4.0×6.0 mm) that allowed 50% light transmission during the first 10 days, and 80% during the rest of the nursery phase; plants were watered to field capacity once per day (Figure 1(g)). After the nursery phase, acclimatized plants were taken to field conditions, applying daily irrigation during the first 10 days and every other day during the following weeks until their adequate establishment was observed (approximately after 2 weeks, Figure 1(h)).

2.4. Statistical Analyses. Normality and homoscedasticity of variables were tested using Shapiro–Wilk and Levene tests, respectively. ANOVA followed by Tukey's HSD was used ($p < 0.05$) to test for significant differences among treatments. Prior to the analyses, data on survival percentage (number of successful cuttings turgid and without abscission of leaves) and rooting percentage (proportion of cuttings that generated roots) were arcsine transformed and the number of roots was square root transformed; nevertheless, the results are shown in original units. All statistical analyses were carried out using R 4.0.2 [21].

3. Results and Discussions

This study describes a practical and efficient methodology for vegetative propagation of *T. cacao* by rooting cuttings, with and without the application of IBA and using plastic-covered mini-tunnels with nebulization during the rooting period. This system provides better control capacity against restrictive weather conditions, maintaining a favorable environment for the rooting and growth of plants [13], successfully used in different plant species [14–17]. We believe that this system will improve the production of cacao clones on a large scale and at an affordable cost, to contribute to the technological development of farmers and strengthen their capacities for proper crop management.

The technique of rooted cuttings has been widely used for the propagation of cacao and involves stimulating the growth of adventitious roots at the base of cuttings taken from the branches of selected trees when placed in high humidity environments [6].

According to Amoah, [29] the propagation environment influences the rooting capacity of cuttings since an adequate environment stimulates meristematic activity and minimizes the physiological shock produced after severance, allowing successful rooting. In this regard, substrates have proven to be a key factor for the process of rooting cuttings in various plant species, including cacao [30–32]. An appropriate substrate must provide certain physical conditions that allow proper root development, such as pore space for adequate aeration and good drainage while allowing a proper water retention capacity [33]. In our investigation, a substrate formulated from Canadian Sphagnum moss, commercially known as Jiffy ©, was used. This substrate was characterized by having good moisture retention and high organic content (94–98%), both important characteristics for root development in cuttings obtained from young and leafy branches. Such cuttings, due to their physiological characteristics, are also known to benefit from the nutritional compounds existing in the propagation medium [34].

Other important factors to keep in mind are the temperature and humidity of the rooting environment. Regarding temperature, Hartman et al. [35] suggest that the optimum temperature for rooting of any vegetal species is the one in which it develops adequately in its natural environment. Suchithra [9] points out that the optimum temperature for cacao growth is close to 25°C, slightly higher than those recorded during the rooting phase of the present study (26–34°C). It should be noted, however, that high temperatures during the rooting process can be compensated by high percentages of relative humidity [15] which also allow for compensating fast water loss by evaporation in relation to the slow absorption of water from the incipient roots developed initially [36]. Previous studies have indicated that the optimum relative humidity percentage for rooting cacao cuttings varies between 80% and 100% [20, 37] including the value (above 80%) registered in the present study.

Controlling the conditions mentioned above depends on the propagation system used. The use of mini-tunnels in our experiment allowed control of the relative humidity, and maintenance of temperature and irrigation at optimal levels, which were key factors for the success of the rooting.

3.1. Rooting Percentage. The rooting percentage was evaluated 62 days after planting; this variable showed the highest values with doses of 1000 ppm (90.0%) and 0 ppm (87.0%), without significant differences between them, followed by 2000 ppm (56.7%) and 3000 ppm (50.0%). Rooting percentage displayed a clear tendency to decrease with increasing doses of IBA (Figure 2(a)). According to Leakey [38], a propagation system is considered appropriate when the rooting percentage exceeds 70%, a situation that can be observed in the treatments with 0 ppm and 1000 ppm of IBA, with 87 and 90% of rooted cuttings, respectively. The results obtained in this study are higher than those shown by Tee and Lamin [39] with cacao cuttings, who reported a maximum rooting value of 56% with the dose of 8000 ppm of IBA, using a nonmist wooden propagator which acts as a propagation chamber. Also using cacao cuttings, Mejía-Chávez et al. [19] mentioned that the highest rooting percentage was obtained by the treatment with 200 ppm of IBA and the control treatment (without hormone), with 43% and 37%, respectively. On the other hand, Edward et al. [21] reported up to a 70.3% of rooting using a liquid composition consisting of auxin growth regulators α-napthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) in a nonmist propagator, whereas Kamga et al. [22] using 1/2 tablet of Rhizopon hormone per liter of water in a "Clementine" propagator reported 73.6% of rooted cuttings, evaluating different cacao genotypes. Additionally, it is important to mention that in the Department of San Martín, Peru, using

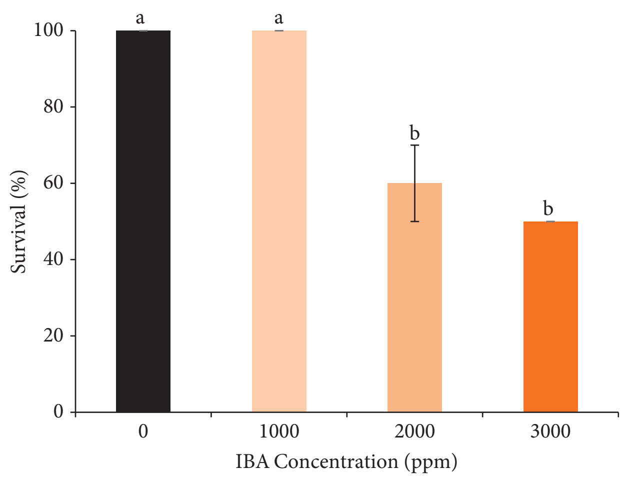

FIGURE 2: Root traits of *T. cacao* cuttings, according to IBA dose, 62 days after rooting induction. Columns with the same letter indicate nonsignificant differences between the treatments (Tukey's HSD, $p < 0.05$). (a) Rooting percentage. (b) Number of roots. (c) Root length. Means and standard error ($n = 30$).

the same propagation system (microtunnels) and the application of IBA in another important economic crop (coffee), similar results (88%) to our study were obtained [40].

Our findings indicate that cacao cuttings without hormone (0 ppm IBA), or treated with the lower dose (1000 ppm IBA) showed a higher rooting percentage. Similar results were reported by Kamga et al. [22] and Charvet-Candela [41], who mention that an excessive concentration of auxin had a negative impact on the percentage of rooted cuttings, slowing the activity of stimulation of rhizogenesis (regeneration of adventitious roots). The good results obtained without the application of hormones during the rooting process in cacao could be economically important and a key factor for farmers.

3.2. Number of Roots per Cutting. The number of roots also showed significant differences between the different doses used, with a similar pattern to that of rooting percentage. On average, more roots per cutting were obtained with doses of 1000 ppm (4.5 roots) and 0 ppm (4.3 roots), forming a group significantly different from 2000 ppm (2.7 roots) and 3000 ppm (1.7 roots) 62 days after planting (Figure 2(b)).

In their study with cacao cuttings, using different concentrations of hormone, Kamga et al. [22] reported between 5.1 and 6.5 roots/cutting, the latter with the lowest dose (1/2 tablet of Rhizopon per liter of water). Also with cacao, Essola et al. [20] reported a number of roots rates of 7.7–11.1, the highest results being obtained with the lowest dose of IBA (25 ppm). Overall, these results show that cacao cuttings need minimal amounts of exogenous hormone for root formation, suggesting that they have a good supply of endogenous auxins. In this regard, Jacob and Opeke [42] also reported that cacao cuttings can root without the need for exogenous hormones, as we found in our experiment. This behavior has also been reported in other plants species [43, 44].

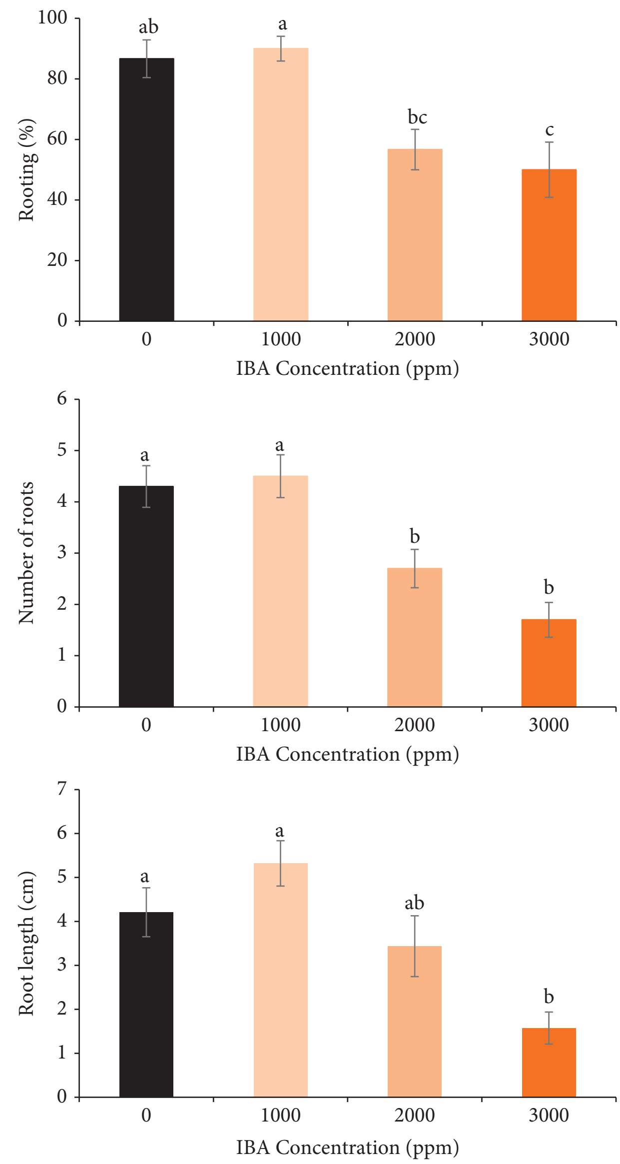

FIGURE 3: Survival percentage of *T. cacao* cuttings according to IBA dose, 62 days after rooting induction. Columns with the same letter indicate nonsignificant differences between the treatments (Tukey's HSD, $p < 0.05$). Means and standard error ($n = 30$).

Likewise, it has been reported that cacao genotypes differ in their rooting abilities, which can explain the differences in results presented by some authors concerning this variable [18, 20, 22, 45–47].

3.3. Root Length. On average, longer roots were obtained with doses of 1000 ppm (5.32 cm in length) and 0 ppm (4.21 cm in length), forming a group significantly different from 2000 ppm (3.44 cm in length) and 3000 ppm (1.57 cm in length) (Figure 2(c)).

Essola et al. [20] obtained root lengths in cacao cuttings of 5.4 to 8.1 cm, being the highest length of roots with 25 ppm of AIB. Likewise, Kamga et al. [22] reported 7.7–9.1 cm in length, the latter with the lowest dose of

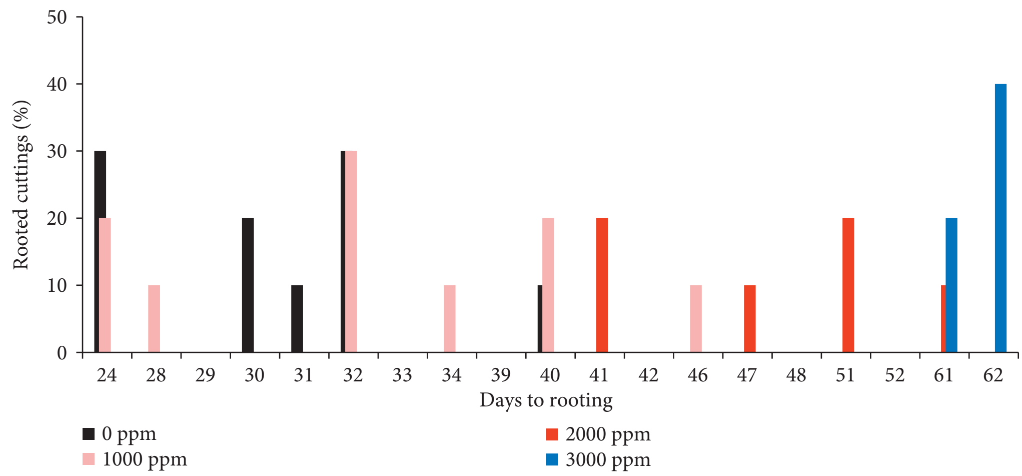

Figure 4: Rooting time of *T. cacao* cuttings treated with different concentrations of IBA.

hormone. These results suggest that as the dose increases, the length of the roots decreases, which could be due to the toxicity caused by an overdose of IBA, as reported by Mesén [48]. As mentioned in previous sections, the difference found in root length between the different studies could be related to the different cacao genotypes evaluated [49], as well as the age of donor plants, time of evaluation, rotting systems, and differences in substrates used, among others.

3.4. Percentage of Survival. After 62 days, the highest survival (100%) was obtained with doses of 0 and 1000 ppm, followed by 2000 and 3000 ppm, with 60 and 50% survival, respectively (Figure 3). Likewise, 100% survival was obtained during the acclimatization process in the greenhouse/nursery of the rooted cuttings with 0 ppm of IBA, after 90 days. The survival rate seems to vary with the propagation system used: Mejía-Chávez et al. [19] obtained a 51% survival rate under a sub-irrigation system without the application of IBA. Essola et al. [20] evaluated cacao rooted cuttings treated with IBA in "Clementine" propagators and obtained a survival rate of approximately 75%, whereas Koko et al. [23], using plastic tunnels and IBA, reported an 80% survival rate. On the other hand, Edward et al. [21] reported 52.7% of survival in nonmist propagators; while Tee and Lamin [18] obtained 25.8% of survival of cacao cuttings rooted with IBA using a nonmist wooden propagator which acted as a propagation chamber. Compared to previous results, our cacao cuttings established in microtunnels had the maximum percentage of survival (100%) and were highly successful.

3.5. Emergence of Roots. Although the use of auxins can be effective for the rooting of cuttings in many plants, in some species the use of high hormonal concentrations generates toxicity and inhibition of root growth, resulting in a delay in rooting [17, 50]. This can explain our findings related to the number of days required for the emergence of roots, which indicated that cuttings treated with 0 ppm rooted after 24–40 days, and this period increased with doses of 1000 ppm (24–46 days), 2000 ppm (41–46 days), and 3000 ppm (62–63 days) (Figure 4). No other investigations were found that evaluated the number of days required for the emergence of roots since they all had only one evaluation day: 160 days [47], 60 days [18], 70 days [51], and 90 days [22]. Despite this, we consider that it is of key importance to consider the minimum and maximum days to obtain high rooting percentages because this could be used as an indication of the viability and speed of the propagation system used.

4. Conclusion

In this study, we tested a promising and cost-effective methodology for vegetative propagation of *T. cacao* plants by rooting of cuttings in plastic-covered mini-tunnels. We showed that cacao cuttings treated with 0 and 1000 ppm IBA can be successfully rooted after 24–46 days, with a high survival percentage of cuttings and with good root traits. All rooted cuttings survived and grew well after being transplanted to pots. The results suggest the success of our protocol for vegetative propagation and acclimatization of cacao plants to obtain high-quality plants quickly and economically (without hormone).

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgments

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