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Optimization of Indole Butyric Acid (IBA) in the Propagation of Miracle Fruit (*Synsepalum dulcificum*) by Stem Cutting

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Abstract

This study looked into the best concentration of indole-3-butyric acid (IBA) to use while propagating *Synsepalum dulcificum*, or miracle fruit, from stem cuttings. IBA concentrations ranging from 0 to 500 mg/L were applied to cuttings of semi-hardwood and hardwood in a split-plot Randomized Complete Block Design experiment. The findings indicated that at four weeks after planting, both cutting types started to produce calluses, and at eight weeks, roots started to sprout. For both kinds of cuttings, 400 mg/L was the ideal IBA concentration, resulting in the greatest rooting percentages of 70% for hardwood cuttings and 83.3% for semi-hardwood cuttings. In all treatments, semi-hardwood cuttings showed consistently better rootability than hardwood cuttings. At 400 mg/L IBA, the semi-hardwood cuttings exhibited the largest mean number of roots (8.33). For root length, dry weight, and number of roots, there were no statistically significant variations between treatments. Hardwood cuttings, on the other hand, demonstrated a significant difference in each of these parameters between the control and all IBA treatments; at 400 mg/L IBA, the highest mean number of roots (7.00) was also seen. For both cutting types, the rooting percentage increased from 0 mg/L to 400 mg/L IBA, then decreased at 500 mg/L, indicating an upper limit to the positive effects of IBA. The rooting percentage of semi-hardwood cuttings showed a notable trend, rising from 3.3% (control) to 83.3% (400 mg/L) and then falling to 63.3% at 500 mg/L. These results offer information about the vegetative propagation of *Synsepalum dulcificum*, showing that the best way to root is using semi-hardwood cuttings treated with 400 mg/L IBA. Through the development of effective multiplication techniques, miraculous fruit's potential for large-scale cultivation and a range of uses in the food and pharmaceutical industries is supported.

Keywords

Hardwood, Miracle Fruit, Indole Butyric Acid, Semi Hardwood, Rooting

1. Introduction

The "miracle fruit" plant (*Synsepalum dulcificum*), also known as the miracle berry plant, belongs to the family Sapotaceae. It is a tropical evergreen shrub that grows to about 2 - 5 m tall [1]. The tree is native to tropical West Africa and has distinctive flavors to otherwise bitter or acidic foods [2]. It grows well in wild virgin forests, coastal areas and mountainous forests [3].

The most unfamiliar thing about the fruit is the effect of the fleshy pulp on the taste buds of the tongue that makes every sour or acidic food eaten or drunk to taste very sweet. The flesh of the berries contains miraculin, a glycoprotein substance that causes a change in flavor [4]. Miracle fruit could possibly help diabetic people eat sweet food without taking in sugar, and it has been investigated and considered as a possible source of a natural food sweetener [5].

The miracle fruit plant produces whitish creamy flowers which measure around 0.5cm on the auxiliary buds throughout the year. It has a large seed that is engulfed by a thin layer of flesh [6].

Large-scale propagation and/or production of miracle fruit plants pose some restrictions as the seeds are recalcitrant and propagation by cuttings is difficult to root. Most of the miracle fruit plants available in the market are grown by using seed, but the growth of the seedlings is slow at the nursery stage [7]. A one-year-old plant often is only 5 - 7.5 cm tall and it takes 3 to 4 years for the plant to reach 35 - 50 cm tall and then growth is more rapid [8]. When grown from seed, *Synsepalum dulcificum* took 3 to 4 years to reach maturity and begin to bear fruit whereas plants grown from stem cuttings started to bear fruit earlier [9].

A propagation technique by cuttings needs to be devised to produce early fruiting planting materials [7]. Auxins are well-known phytohormones that promote adventitious roots from stem cuttings of tree species [8]. Indole-3-Butyric Acid [IBA] is believed to be the best synthetic auxin for general use because it is non-toxic to plants over a wide range of concentrations [9]. Although IBA was proven effective in promoting rooting in woody plant species [8], there has been a scanty comprehensive study on the rooting performance of *Synsepalum dulcificum* stem cuttings using IBA. It is for this reason that a study on the determination of the optimum use of Indole-3-Butyric Acid (IBA) concentrations in the rooting performance of the Miracle fruit plant (*Synsepalum dulcificum*) through stem cutting is justifiable to conduct.

2. Methodology

2.1. Experimental Site

The experiment was conducted at the ornamental garden of the Institute of Crop

Science Cluster, College of Agriculture and Food Science, University of the Philippines, Los Banos

2.2. Source of Cuttings and Their Preparation

The stem cuttings of the miracle fruit plant were collected from a farm at Mabacan-Calauan, Laguna. The hardwood cuttings were taken from mature shoots, which were cut to 15 - 20 cm in length with an average of 3 to 4 nodes in each cutting while semi hardwood cuttings were taken from plants six weeks after the tree flushes at a length of 15 - 20 cm with an average of 3 to 4 nodes.

2.3. Experimental Design and Procedure

In the experiment, the rooting response of cuttings of the miracle fruit (*Synsepalum dulcificum*) plant as affected by different treatment levels of IBA concentrations was conducted. Semi-hardwood and hardwood stem cuttings were treated with the following IBA concentration levels; 0 mg/L, 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L and 500 mg/L. The experiment was laid out in a split plot in Completely Randomized Design (CRD) with six (6) treatments, three (3) replications and ten (10) plants per treatment.

The cuttings were each dipped in measured concentration of IBA for one (1) hour. After treatment, they were sown in mist bed with fine river sand as rooting medium.

2.4. Data Collection

The total cuttings planted in each experimental block per treatment were allowed to root for 75 days after planting. Three plants were selected randomly per treatment and were labeled in all the replications. The data was recorded for all the parameters of each treatment in the three experimental replicates.

2.5. Percentage of Rooted Cuttings

The percentage of rooted cuttings was calculated by taking the ratio of rooted cuttings to a total number of cuttings planted in each treatment and expressed in percentage 75 days after planting.

$$\text{Percentage rooted} = \frac{\text{Number of cuttings rooted}}{\text{Total number of cuttings}} \times 100$$

2.6. Number of Roots per Cutting

The total number of adventitious roots per cutting was counted under each treatment and the mean was calculated and expressed in number 75 days after planting.

2.7. Length of the Longest Root per Cutting (cm)

The total length of the longest root per cutting in each treatment was recorded by scale and their mean was calculated and expressed in centimeters 75 days after planting.

2.8. Dry Weight of the Roots (g)

The total roots of each sample of the treatment were collected and they were oven dried at a temperature of 80ºC for three days and then the dry weight was recorded with the help of an electronic balance. The mean dry weight of the root was calculated and expressed in grams, 75 days after planting.

2.9. Data Analysis

Data collected from the experiment was subjected to the analysis of a split plot in CRD using Statistical Analysis System (SAS) version 9.1 and Means were separated using Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

The research conducted on the propagation of miracle fruit (*Synsepalum dulcificum*) utilizing stem cuttings and varying concentrations of Indole-3-Butyric Acid (IBA) produced important results that advance our knowledge of this species' vegetative proliferation.

After planting, callus started to form on both semi-hardwood and hardwood cuttings four weeks later, and adventitious roots appeared eight weeks later. This finding is in line with current research conducted on many woody species. For example, in their study on the vegetative growth of miraculous berry, [7] showed similar durations for callus production and root emergence.

For both semi-hardwood and hardwood cuttings, the results shown in **Table 1** and **Table 2** show that IBA treatments considerably increased rooting percentages when compared to the control. This is consistent with the research conducted by [8], who highlighted the pivotal function of auxins in facilitating adventitious root development in woody plant species.

The best IBA concentration for semi-hardwood cuttings (**Table 1**) was determined to be 400 mg/L, which produced the maximum rooting percentage of 83.3%. Compared to the control (3.3%) and other concentrations, this is a significant improvement. It is interesting to see that the rooting percentage increased from 0 mg/L to 400 mg/L and then started to decline at 500 mg/L. This decline implies that there may be an IBA concentration range that is ideal, above which the effects might become inhibitory. [9] explored the hormonal regulation of root development in their review of sugar sensing and signaling in plants, noting that similar patterns have been identified in other species.

The ideal IBA concentration for hardwood cuttings (**Table 2**) was 400 mg/L, which produced a 70% rooting percentage. Even while this is less than the semi-hardwood cuttings, it is still a considerable improvement over the control group, which had a 26.7% yield. Hardwood cuttings had a lower rooting percentage than semi-hardwood cuttings, which is in line with research by [3], which found that the juvenility of the plant material affects *Synsepalum dulcificum* rooting success.

Hardwood and semi-hardwood cuttings both displayed the largest mean number of roots per cutting, 400 mg/L IBA (8.33 and 7.00, respectively). It is interesting, nonetheless, that for semi-hardwood cuttings, the number of roots, length of roots, and dry weight of roots did not change statistically significantly across treatments (**Table 1**). This implies that miracle fruit semi-hardwood cuttings might be more receptive to endogenous auxins or might need less exogenous auxin in order to start rooting. This observation merits more research since it provides context for our understanding of the auxin requirements in various cutting types.

Regarding hardwood cuttings (**Table 2**), the number of roots, length of roots, and dry weight of each IBA treatment differed significantly from the control group. The 100 mg/L and 500 mg/L treatments, however, were statistically equivalent. This suggests that even lower IBA concentrations may be useful in encouraging roots in miraculous fruit hardwood cuttings, which may have real-world applications for low-cost propagation techniques.

One of the study's main conclusions is that semi-hardwood cuttings perform better than hardwood cuttings in every way. This is consistent with recent research by [2], who emphasized the significance of cutting type in miraculous fruit proliferation. In their phenotypic and molecular characterization of *Synsepalum dulcificum*, [6] proposed that the higher plasticity and regenerative potential of semi-hardwood cuttings could be the reason for their enhanced rootability.

The results of [4] and [8], who highlighted the potential of miraculous fruit for diverse uses and the necessity for effective propagation methods, are supported by the study's overall positive reaction to IBA treatments. This species is economically valuable, and the excellent rooting that was accomplished here specially with semi-hardwood cuttings—offers encouraging prospects for large-scale multiplication.

The absence of significant differences between IBA concentrations (especially in semi-hardwood cuttings) raises the possibility that there are other factors influencing the rooting process, even though IBA treatments significantly improved rooting compared to the control. The complex interaction of elements affecting rooting success was highlighted in recent work by [7] on the influence of cutting type and hormone concentration in miraculous berry propagation.

An important finding that supports the idea of hormonal balance in plant growth is the decline in rooting percentage for both cutting types that was shown at 500 mg/L IBA. While auxins are essential for root initiation, high quantities can impede root development, as [8] have discussed. This emphasizes how crucial it is to determine the ideal hormone concentrations for particular species and cutting styles.

Table 1. Rooting percentage, mean root numbers per rooted cutting, mean root length per rooted cutting and root dry weight per rooted cutting of semi hardwood miracle fruit cuttings under different IBA concentrations.

Conc. Mg/L	Rooting %	Mean No. of roots	Mean root length (cm)	Mean dry weight (g)
0 mg/L	3.3	0.33^{b}	0.33^{b}	0.00^{a}
100 mg/L	56.7	5.67^{a}	3.18^{a}	0.28^{a}

Continued

200 mg/L	53.3	5.33[a]	4.22[a]	0.59[a]
300 mg/L	50.0	5.00 [a]	3.09[a]	0.17[a]
400 mg/L	83.3	8.33[a]	3.43[a]	0.25[a]
500 mg/L	63.3	6.33[a]	3.14[a]	0.20[a]

Means within column with the same letters are not significantly different at 5% LSD level.

Table 2. Rooting percentage, mean root numbers per rooted cutting, mean root length per rooted cutting and root dry weight per rooted cutting of hard miracle fruit cuttings under different IBA concentrations.

Conc. Mg/L	Rooting %	Mean No. of roots	Mean root length	Mean dry weight (g)
0 mg/L	26.7	2.67[a]	2.06[a]	0.01[a]
100 mg/L	46.7	4.67[a]	3.97[a]	0.18[a]
200 mg/L	56.7	5.67[a]	2.23[a]	0.19[a]
300 mg/L	66.7	6.67[a]	3.21[a]	0.22[a]
400 mg/L	70.0	7.00[a]	2.58[a]	0.15[a]
500 mg/L	56.7	5.67[a]	3.71[a]	0.27[a]

Means within column with the same letters are not.

4. Conclusions

Different amounts of Indole-3-Butyric Acid (IBA) have been effectively used to optimize the stem cutting method for miracle fruit (*Synsepalum dulcificum*). Both hardwood and semi-hardwood cuttings can be successfully rooted, as this study has shown, with semi-hardwood cuttings exhibiting better rootabilty under all conditions.

It's interesting to note that although IBA treatments greatly accelerated rooting in comparison to the control, the lack of substantial variations between different IBA concentrations, especially in semi-hardwood cuttings, raises the possibility that other elements may also be important for rooting. This provides opportunities for more investigation into the intricate interactions among variables influencing *Synsepalum dulcificum* rooting success.

The study also demonstrated the significance of the type of cutting, showing that semi-hardwood cuttings regularly outperform hardwood cuttings. Horticulturists and nursery managers looking to maximize their propagation methods will find this information to be very helpful.

In summary, this study adds a great deal to our knowledge of miraculous fruit vegetative growth and offers useful information for growing the fruit. In order to further improve the propagation techniques for this species, future research could examine how IBA interacts with other elements like ambient conditions and rooting material.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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