Promotion of Adventitious Root Formation of Miracle Fruit (*Synsepalum dulcificum* Daniell) Through Stem Cuttings and Air Layering Technique

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ABSTRACT: *Synsepalum dulcificum* or Miracle fruit plant is a medicinal shrub that belongs to the family Sapotaceace are known to exert an extraordinary effect of changing sour taste to sweet. The increase in industrial demand may cause depletion on the raw materials source as it is a slow-growing plant. Propagations of miracle fruits are restricted due to difficulties in rooting through cuttings and the seeds are recalcitrant. This study was to overcome the needs to supply large number of seedlings for large-scale planting by inducing adventitious root formation through vegetative propagation methods such as stem cutting and air layering (marcotting). A total of 140 softwood stem cuttings were treated with four IBA concentrations (0, 500, 1000, and 2000 mg/L) and planted in sand bed under non-mist system. Another experiment involved air layering was initiated on vertical young shoots treated with commercial rooting hormone Seradix No.2 (3000 ppm). Percentages of rooting (%), mean number of root (%), and mean root length (cm) per rooted cutting were collected 2 months after planting. Stem cuttings soaked in control treatment produced the highest percentage of rooting (54.28%) per cutting. However, mean root numbers per rooted cutting increases with high concentrations of IBA treatment. Adventitious root started to emerge four weeks after propagation period in air layering method. Results showed that 73.33% air layers successfully induced roots with mean root length 5.98cm. Thus, vegetative propagation is a promising technique that can be applied to promote adventitious rooting in hard-to-root species as in *Synsepalum dulcificum*.

KEYWORDS: Adventitous root, Air Layering, IBA, Stem cutting, *Synsepalum dulcificum*

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INTRODUCTION

Miracle fruit or known as *Synsepalum dulcificum* Daniell is an African native shrub that belongs to the family Sapotaceace. Small ellipsoid berries of large seed coated with thin layer of edible pulp are produced throughout the year. The fresh pulps of the fruits were known to exert an extraordinary effect by causing acidic food consumed to taste sweet. This sweetening property is caused by the action of a miraculin; a type of glycoprotein compound found in the tasteless pulp of the miracle fruit. The mechanism works on the taste buds of the tongue by distorting the sweet receptor under acidic condition which makes the food taste sweet (Metcalfe & Chalk, 1972).

Nowadays, nutritionist are more interested into natural food sweetener as compared to some artificial sweetener that contain carcinogenic compound such as cyclamates and saccharine which may cause health problems to the consumer (International Agency for Research on Cancer, 1980). *S. dulcificum* are found to be one of the possible sources of natural food sweetener which the fruit can be used to overcome diabetic patients from consuming sweet food without taking in sugar (Kant, 2005). For centuries, people in West African often use miracle fruit to sweeten their sour foods and drinks such as acidulated maize bread and sour palm wine (Inglett *et al*., 1965).

Demand of miracle fruits in industry has increased drastically due to the need for large scale productions and for further research to enhance the potential uses of the plant in medicinal areas.

Without proper management to maintain the propagation, raw source of *S. dulcificum* will be depleted. The problems will continue to arise as the *S. dulcificum* is a slow growing type plant that takes about 3 to 4 years until it reaches maturity to bear fruits. Propagation of *S. dulcificum* faces some difficulties in which the seeds can lose its viability when dried and rooting through cutting is not easily obtained (Okhapkina, 2006). According to Joyner (2006), fruiting of miracle plant began earlier when grown through stem cutting as compared to seedlings that takes 3 to 4 years to mature and produce fruits. Research has been conducted on the effect of IBA hormone to enhance rooting in stem cuttings however there has been no report on rooting performance of miracle fruit plant through air layering method in this species. The aim of this study is to determine the effect of higher concentrations of IBA hormone on stem cuttings and application of air layering method in promoting adventitious root in *S. dulcificum.*

METHODOLOGY

Source of Explant

Sample of stem cutting explants were collected in the morning from four healthy stock plants planted in Plant and Tissue Culture Laboratory of Faculty Resource Science and Technology in Universiti Malaysia Sarawak (UNiMAS). Explants for stem cuttings measured 5 to 8 cm composed of two-nodes with a single leaf on the upper part were obtained. The cuttings were cut below the second node from the stock plant and placed in a beaker filled with water to avoid the stem from drying due to transpiration. Air layering technique requires young upright branches composed of actively growing buds and leaves that were still attached to the stock plant.

Rooting of Stem Cuttings

A total of 140 selected *S. dulcificum* stem cuttings were treated with IBA using the modified quick-dips method as described by Hartman (2002). Each treatment consists of 35 cuttings and soaked for 5 minutes in different concentrations (0, 500, 1000, and 2000 mg/l) of Indole-3-butyric acid (IBA) hormone. The experiment was repeated three times. The cuttings were then planted on rooting media composed of sieved river sand that were filled with water equivalent to half of the sand depth. The experiment was conducted in a polystyrene container (18″x12″x12″) and was tightly sealed to allow non-mist system to function. Watering was done at every interval of 4 weeks. Stem cuttings treated with 0 mg/l IBA was the control treatment of the experiment.

Air Layering Technique (Marcotting)

Three healthy stocks of *S. dulcificum* plants were used and 15 upright branches with actively growing shoot were selected for marcotting exercise. Each branch about 10-15 cm long was selected. A cutting of 2-3 cm was cut to remove the cambium. Commercial rooting hormone Seradix No. 2 (2000 mg/l IBA) was applied to the marcot area and wrapped tightly with Jiffy-7 medium containing sphagnum peat in a transparent plastic tied at both ends. This is to maintain high humidity level of the marcot to promote rooting. Rooting performance of explants through air layering was compared with stem cuttings method.

Observation and Data Analysis on Rooting Response

Observation and data for both stem cuttings and air layering method were collected after 8 weeks. Rooted cuttings are counted only to those cuttings that clearly form initial root measured ≥ 2 mm (Nandi *et al*., 1996). Data on percentages of rooting (%), mean number of roots per rooted cutting, and mean length of roots per rooted cutting (cm) were recorded and statistically analysed. The analysis of variance (ANOVA) procedures in Statistical Package for Social Sciences (SPSS) version 22 were used to test on the significant effect of the treatment on rooting response. Different between mean are tested by using Duncan Multiple Range Test ($p \leq 0.05$).

RESULT AND DISCUSSION

Rooting of stem cuttings

Propagation through stem cutting has been widely used to overcome rooting problem in most woody plant and shrubs (Evertt *et al*., 1978; Hassanein, 2013). This method was applied to enhance rooting capacity in *S. dulcificum* stem cuttings because this species was known as difficult-to-root woody plant (Duke & DuCellier, 1993). Rooting response of *S.dulcificum* cuttings was examined inside polystyrene box separated based on the treatments given. Observation after 4 weeks of planting showed that majority of cuttings in control treatment initiates primary roots as compared to cuttings treated with IBA hormone. The data obtained for rooting ability of *S. duclificum* stem cuttings are given in Table 1 in the final observations (8 weeks), and it reveals that there is no significant difference in the concentration of IBA on the rooting response based on the three parameters given in Table 1 ($p > 0.05$). Present study showed that higher rooting percentage was achieved in control treatment which 54.28% cuttings were success in forming roots (Figure 1A). However, cuttings treated with IBA (500mg/l) were found to be slightly effective resulted in the lowest success of rooting with only 37.14% cuttings produce roots. Each rooted cutting in the control treatment produce longer mean root length of 1.45 ± 0.32 cm as compared to other treatments (Figure 1B). Cuttings treated with 1000mg/l (Figure 1C) found to be effective for highest average of 1.46 ± 0.34 roots per rooted cuttings (Table 1). Over the three parameters, it can be concluded that there is a tendency to increase the rooting response within *S. dulcificum* cuttings with respect to increase in IBA concentration but higher concentration (2000mg/l) can exert inhibitory effect that will reduce the rooting capacity (Figure 1D).

Figure 1. Formation of adventitious roots in *S.dulcificum* cuttings treated with A) control, B) 500 mg/l IBA, C) 1000 mg/l IBA and D) 2000 mg/l IBA after 8 weeks of planting.

Table 1. Rooting percentage, mean root numbers per rooted cutting and mean root length per rooted cutting of two-node miracle fruit cuttings under different concentrations of IBA hormone.

IBA (mg L^{-1})	Rooting (%)	No. of roots per rooted cutting	Root length per rooted cutting (cm)
0	54.28^{a}	0.80 ± 0.15^{ab}	1.45 ± 0.32^{a}
500	37.14^{a}	0.63 ± 0.16^{a}	1.07 ± 0.27^{a}
1000	45.71^{a}	1.46 ± 0.34^{b}	1.20 ± 0.28^{a}
2000	42.86^{a}	1.11 ± 0.33^{ab}	0.71 ± 0.17^{a}
Sig.	**0.66**	**0.13**	**0.25**

Mean values ± Standard Error of Mean (SEM) values followed by the same letter within a column are not significantly different ($p \leq 0.05$ using Duncan Multiple Range Test)

Application optimum concentration of hormone is the key of success in rooting of cuttings for most difficult-to-root species (Leakey *et al.*, 1982; Purohit *et al.*, 2009). Growth hormone plays an important role in the production of roots through cell division, multiplication and specialization (Davis & Hassig, 1990). Ability to produce roots in hard-to-root species was also highly related to the presence of endogenous hormone produced naturally in the shoot apices and young leaves. This hormone plays an important role in controlling the growth and development of adventitious roots (Krajnc *et al.*, 2013; Went & Thinman, 1937). Auxin is the best known rooting hormone that can exist endogenously or applied exogenously to the plants. Common used auxin was Indole-acetic acid (IAA) or Indole-butyric acid (IBA). Current study showed that not only rooting percentage (54.28%) gave the highest results but also the longest mean root length (1.45±0.32 cm) per rooted cuttings in control treatment. This clearly explains the role of endogenous auxin within *S. dulcificum* cuttings that aids in the formation of roots throughout the process. Same situation occurs in past study during rooting process of pea plants, there was increase in the concentration of endogenous auxin at the base of cuttings when no external hormones applied (Blahova, 1969). Contrast to the results obtained in previous study on *S. dulcificum* which control gave the lowest rooting percentage (0.86±0.86%). The results varied within the concentration of endogenous IAA that was highly dependent to each individual species (Blakesley *et al.*, 1991; Baraldi *et al.*, 1993).

Treating cuttings with external rooting hormones will also help in accelerating the formation of roots and to increase percentage, homogeneity, quality and number of roots per cutting. IBA is a synthetic auxin that was previously tested on the same species with high percentage of success. However, the present study did not agree to the previous findings conducted by Chen *et al.*, (2012) on the same species that high concentration of IBA (800 mg/l) gives highest rooting percentage of *S. dulcificum* stem cuttings. A study showed that application of hormones at doses higher than 8000mg/L were known to cause damage in woody ornamental plants (Loach, 1988). Hence, increase in IBA concentration does not necessarily increase the rooting percentage which cuttings treated with 2000 mg/l IBA shows a slightly decline in all the three parameters. Some other species however, failed to root although when treated with optimum concentration of hormone due to high sensitivity of rooting of cuttings towards the auxin formulation (Beyl & Trigiano, 2016). Previous research recorded that certain plant species also showed decrease in rooting response when cuttings were treated with IBA concentration greater than 0.2%. This indicates that certain level concentration of IBA will give inhibitory effect to the formation of roots in cuttings (Leakey *et al.*, 1990). Furthermore, auxin was said to give effect as promotory at low concentration and inhibitory at supra-optimal concentration on formation of roots (Sugiyama, 1999).

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Origin of adventitious roots in stem cuttings also affects the rooting performance of a species. Cross-section of the stem in woody plants is different from herbaceous plants in terms of the arrangement of vascular bundles, presence of sclerenchyma and cork cambium. Generally, young secondary phloem is the origin for adventitious roots formation while in some cases rooting may arise from vascular bundle, cambium or pith (Davies and Hartmann, 1988; Hartmann & Kester, 1983). Rooting of *S. dulcificum* originated from secondary phloem which penetration of root primordia through the cortex was visible 5 weeks after planting (Chen *et al*., 2012). The same observation was found in sweet potato (*Ipomoea batatas* (L.) Lam.), the primordial root arises from procambium protrudes through the cortex and epidermis of the stem (Belehu *et al*., 2004). Failure in rooting of *S. dulcificum* cuttings were the cause of lignification and sclerefication processes that occur in the sclerenchyma located in between phloem and cortex as the stem matured and gets older. The process then leads to formation of sclerenchyma ring that blocks the emergence of roots that arise in the phloem (Hartman, 2002).

Air layering technique (Marcotting)

Other technique applied to promote rooting in *S. dulcificum* is through air layering (marcotting). Marcotting is a horticultural technique used to propagate new plants that are difficult to grow from cuttings due to rooting problems. Lychee, jabong, hibiscus and guava are examples of plants that commonly grew by marcotting (Bornhorst, 2005). A total of 15 young branches are randomly chosen from three healthy stock of *S. dulcificum* plant. Each branch is girdled by removing 4-5 cm of the bark and vascular cambium to interrupt the growth and phloem transport downwards towards the roots. High deposition of nutrients, carbohydrates and hormone reserves essential for rooting will remain above the wound area. Commercial rooting powder is then applied at the scratch inch of the bark to enhance rooting process (Sadhu, 1989). Within 4 weeks after the treatment, 46.67% marcots began to produce roots as it is visible through sphagnum peat medium (Figure 2A). Results recorded after 8 weeks gave 73.33% success in promoting roots for *S. dulcificum* through marcotting which the mean number of roots is 2.93 ± 1.04 and 3.99 ± 1.14 cm mean root length per rooted marcot (Figure 2B & 2D).

Several air-layers were found to form only callus at the upper part of the cut (Figure 2C). Failure to form root are due to lacking several factors that may lead to unfavourable condition for rooting to occur. Factors such as time for treatment, size of branch, type and amount of rooting hormone used and the moisture content in peat moss media may be varied that will affect rooting potential of the plant. The air-layers are prepared early in the morning which the rate of transpiration is the lowest. Previous research claims that hormone injury can affect the rooting of the air-layers because of too much rooting powder applied on the cut (Wyman, 1951). Maintaining optimum moisture content in the air-layers is crucial because rooting does not favour in a wet or dry condition. Most plants can be propagated from both "hardwood" and "softwood" branches but in some species only one of the branch type will enhance rooting (Wyman, 1986). Softwood branches of *S. dulcificum* used are found to be effective for inducing roots with more than 50% cuttings successfully forms roots.

Table 2. Rooting percentage, mean root numbers per rooted cutting and mean root length per rooted cutting of miracle fruit stem through marcotting.

Rooting Hormone	Rooting (%)	No. of roots per rooted marcot	Root length per rooted marcot (cm)
Seradix	73.33	2.93 ± 1.04	5.98 ± 1.14

Mean values ± Standard Error of Mean (SEM) values

Figure 2. Rooting of *S. dulcificum* air-layers 4 weeks (A) and 8 weeks (B, C, D) after treatment.

CONCLUSION

There are positive results obtained showing that formation of adventitious roots in *Synsepalum dulcificum* can be propagated through stem cutting and air layering technique (marcotting). Application of commercial rooting hormones (Seradix No.2) was proven to increase the rooting capacity of *S. dulcificum* through air layering technique. However, higher concentrations of IBA hormone does not affect the rooting performance but the study showed that endogenous auxin plays an important role in regulating the growth of adventitious roots in *S. dulcificum* cuttings. Among all the treatments, control achieved the highest percentages of rooting and longest mean length of root per rooted cuttings in *S. dulcificum* cuttings. On the contrary, auxin can also exert inhibitory effects that cause reduction in the rooting capacity of *S. dulcificum* cuttings when applied at high concentration of 2000 mg/l IBA.

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