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Short Communication
Effects of Commercial Plant Hormones on the Survival, Rooting and Growth of Stem Cuttings of an Herbal Tea Plant, *Aidia racemosa*

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Abstract

Objective: The effects of three commercial plant hormones (Seradix containing 0.1% indolebutyric acid or IBA, Clonex containing 0.3% IBA and 'A fruit plus' containing cytokinins, gibberellins and auxins) on growth of *Aidia racemosa* (Cav.) Tirveng stem cuttings were investigated. **Methodology:** The cuttings treated with hormones and distilled water (control) were planted in soil and perlite media. The survival and rooting percentages, number and length of new roots per cutting, number and surface area of new leaves per cutting and the relative growth rates were assessed every 4 weeks for 16 weeks. **Results:** Clonex-treated stem cuttings showed slightly higher survival (75% at week 4 and 35% at week 16) and rooting (35% at week 16) percentages than other treatments. There were no significant effects of the hormone treatments on the various growth parameters investigated, however, it seems that stem cuttings treated with seradix and 'A fruit plus' produced higher number of new leaves (0.35 and 0.30, respectively) and clonex-treated stem cuttings produced larger leaves (0.72 ± 0.66 cm^2) than the other hormone treatments. **Conclusion:** This study suggests that clonex is the most suitable commercial hormones for the vegetative propagation of *A. racemosa* but more studies need to be conducted to provide an alternative but efficient way to produce planting materials of *A. racemosa* for the mass production of herbal tea.

Key words: *Aidia racemosa*, plant hormone, stem cuttings, relative growth rate, vegetative propagation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aidia racemosa (Cav.) Tirveng (Rubiaceae), which is an evergreen shrub native to Asian regions, possesses leaves that contain medicinal properties and are beneficial to human health[1]. The decoction of boiled leaves is consumed as a remedy for gastric pain and to strengthen the back muscle especially after heavy physical work. Other species in the family Rubiaceae, such as *Hedyotis auricularia*, *Mussaenda philippinensis* and *Oldenladia corymbosa*, were found to reduce hypertension, cure ulcer, fever and diarrhea and maintain stamina[2]. However, no studies have been made on *A. racemosa* yet. Closely related species, such as *A. densiflora* has been found to contain alkaloids in its leaves and seeds and *A. tomentosa* has been shown to contain saponin in its fruits[1].

In Brunei Darussalam, the local villagers have been harvesting the leaves of *A. racemosa* for the commercial production of herbal tea. In order to expand the market, supply and production issues are the primary constraints as more plants are needed to keep up with the increasing local demand. Since growing *A. racemosa* plants from seeds takes a long time (germination may take 1-2 months)[3], vegetative propagation by stem cuttings is investigated. There have been no previous studies on the vegetative propagation of *A. racemosa*, so this study aims to examine the types of commercial hormones that could be used to effectively mass propagate as well as increase survival and rooting percentages and relative growth rates of *A. racemosa,* which could potentially help to regulate market supply in the long run and conserve the plants in the wild.

MATERIALS AND METHODS

Sampling: Stem cuttings of *Aidia racemosa* were collected in Kampong Kiudang, Tutong, Brunei Darussalam (N 04 42'49.8, E 114 46'35.8, elevation 69 m). The stem cuttings (~14 cm in length) were obtained by cutting the woody shoot tips using sharp secateurs that had been sterilized with 70% ethanol. The stem cuttings were placed in a plastic bag, which was regularly supplied with distilled water to prevent them from getting dehydrated.

Preparation of stem cuttings: Stem cuttings were brought back to the biology laboratory for subsequent treatments with hormones. A total of 20 stem cuttings were used for every hormone treatment including the control treatment, which is using distilled water. Strict hygiene practices were carried out to eliminate the possibility of plant death due to microbial contamination. Any fruits, flowers or flower buds that were found on the stem cuttings were removed and the leaves were cut to about two third of their original size in order to encourage root and shoot growth. Initial measurements of the stem cuttings include the shoot length (cm), diameter of stem (cm) and number of leaves.

The commercial plant hormones used in this study were seradix (0.1% indolebutyric acid or IBA), clonex (0.3% IBA) and 'A fruit plus' (a biostimulant containing unknown concentrations of cytokinins, gibberellins and auxins). The plant hormones were obtained from the local markets, so that they could be used by the villagers for the intended purpose. The hormones were applied to the stem cuttings using the basal quick-dip method following Abidin and Metali[4], whereby the cut end of stem cuttings were dipped 1-2 cm in the hormone powder or solution for 5-10 sec.

Preparation of growth media and growth conditions: The stem cuttings treated with the commercial hormones or distilled water (control) were immediately planted onto a growth medium containing a 1:1 mixture of perlite and soil, which was pre-autoclaved to kill any contaminating microbes. A total of 80 g of the autoclaved soil mixture was poured into each of the well-drained, square-based plastic pot (16 $cm^2 \times 7.5$ cm). A volume of 50 mL of distilled water was subsequently poured onto each pot to avoid the cut surfaces of stem cuttings to come into contact with any dry soil pockets. All the plants were kept in the greenhouse that was installed with an automatic misting system that operated every 5 min to spray water onto the plants. The stem cuttings were grown at 27-31°C, relative humidity of 80-90% and light at 300 μmoL m^{-2} sec^{-1} (measured with an Apogee QuantumMQ-200 PAR Meter, Apogee Instruments, UK). The pots were randomly arranged on the benches and were moved to new random positions every week. A plastic transparent sheet was used to cover the pots of plants on the bench in order to retain high atmospheric humidity, promote rooting and reduce transpirational loss.

Assessment of cuttings: The study was conducted for a total of 16 weeks and the measurements of the stem cuttings conducted every 4 weeks following Aminah *et al.*[5], included survival and rooting percentages (%), number and area of new leaves (cm^2) per cutting, number and length of roots (cm) per cutting and Relative Growth Rate (RGR) based stem length and stem diameter (cm cm^{-1} $week^{-1}$). The Relative Growth Rate (RGR) based on either stem length or stem diameter per stem cutting was calculated according to Hunt[6] as follows:

$$RGR = \frac{\log_e W_2 - \log_e W_1}{(t_2 - t_1)}$$

where, W_1 and W_2 represent the initial and final values, respectively, for either the stem length or stem diameter and t_2 was considered either week 4, 8, 12 and 16, while t_1 as the initial week. Any plants with their leaves and/or stems that turned brown were considered dead and their measurements were stopped.

Statistical analysis: Prior to statistical analysis, assumptions of normality and equal variances of data were checked and found to be violated. Therefore, Kruskal-Wallis non-parametric tests were conducted to compare each of the parameters measured between the different hormone treatments and control. Statistical analysis were conducted using R version 3.2.3[7].

RESULTS

Survival and rooting percentages: The stem cuttings treated with clonex, containing 0.3% IBA as its active ingredient, had the highest survival percentage at week 4 (75%) and week 16 (35%) (Fig. 1a). The stem cuttings treated with seradix, 'A fruit plus' or distilled water (control) had low survival percentages, ranging from 10-20% at week 16. The seradix-treated stem cuttings had the lowest survival percentages at week 12 (15%) and 16 (10%).

None of the stem cuttings rooted at week 4 (Fig. 1b). The stem cuttings treated with clonex had the highest rooting percentages (30-35%) compared to other treatments including distilled water at the remaining weeks (week 8 -16). The seradix-treated stem cuttings had the lowest rooting percentages (5-10%) compared to other treatments including distilled water (control). Rooting percentages for stem cuttings treated with 'A fruit plus' and seradix were found to be lower than the ones treated with distilled water.

Effects of commercial hormones on root and leaf development: There were no significant differences between the different hormones used and distilled water for each of the evaluated parameters at week 4, 8, 12 and 16. Only data at week 16 were shown in Fig. 2.

At week 16, stem cuttings treated with distilled water (control) as well as the commercial plant hormones showed a consistently low mean number of roots produced per cutting ranging from 1.7-1.8 (Fig. 2). The control treatment resulted in higher mean length of roots (29.1±12.5 mm) produced per cutting than the rest of the treatments. Stem cuttings treated with seradix and 'A fruit plus' appeared to produce high number of new leaves produced (0.35 and 0.30, respectively) per cutting but no new leaves were produced for stem cuttings treated with distilled water. However, despite the low number of new leaves produced, clonex-treated stem cuttings appeared to produce larger leaves (0.72±0.66 cm^2) per cutting when compared to the rest of the hormone treatments. The RGR values based on stem length and stem diameter were relatively low for all of the treatments but the greatest mean $RGR_{stem\ diameter}$ was found in stem cuttings treated with 'A fruit plus' (0.003±0.002 cm cm^{-1} $week^{-1}$).

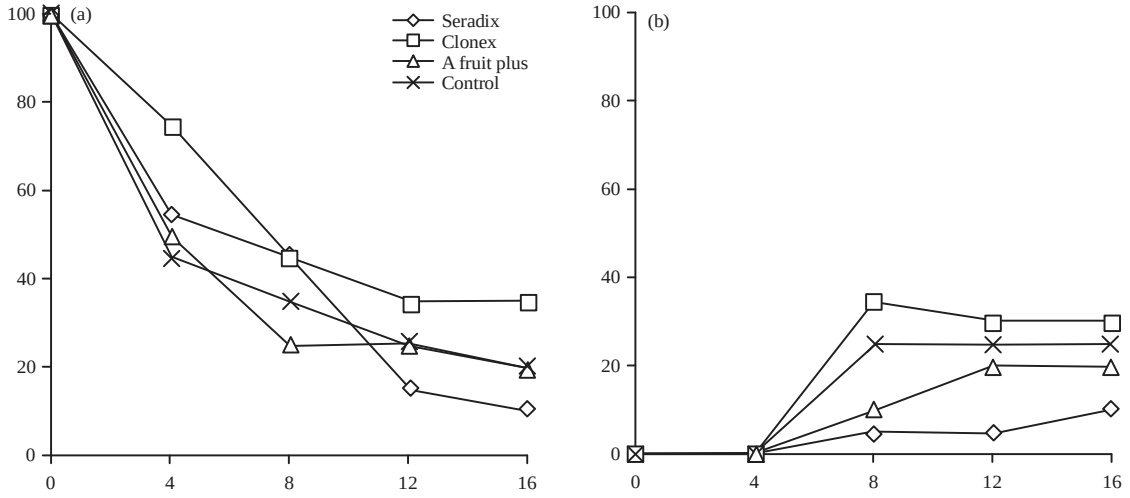

Fig. 1(a-b): (a) Survival and (b) Rooting percentages of *Aidia racemosa* stem cuttings (initial number of stem cuttings = 20) treated with commercial hormones or distilled water (control) over 16 weeks

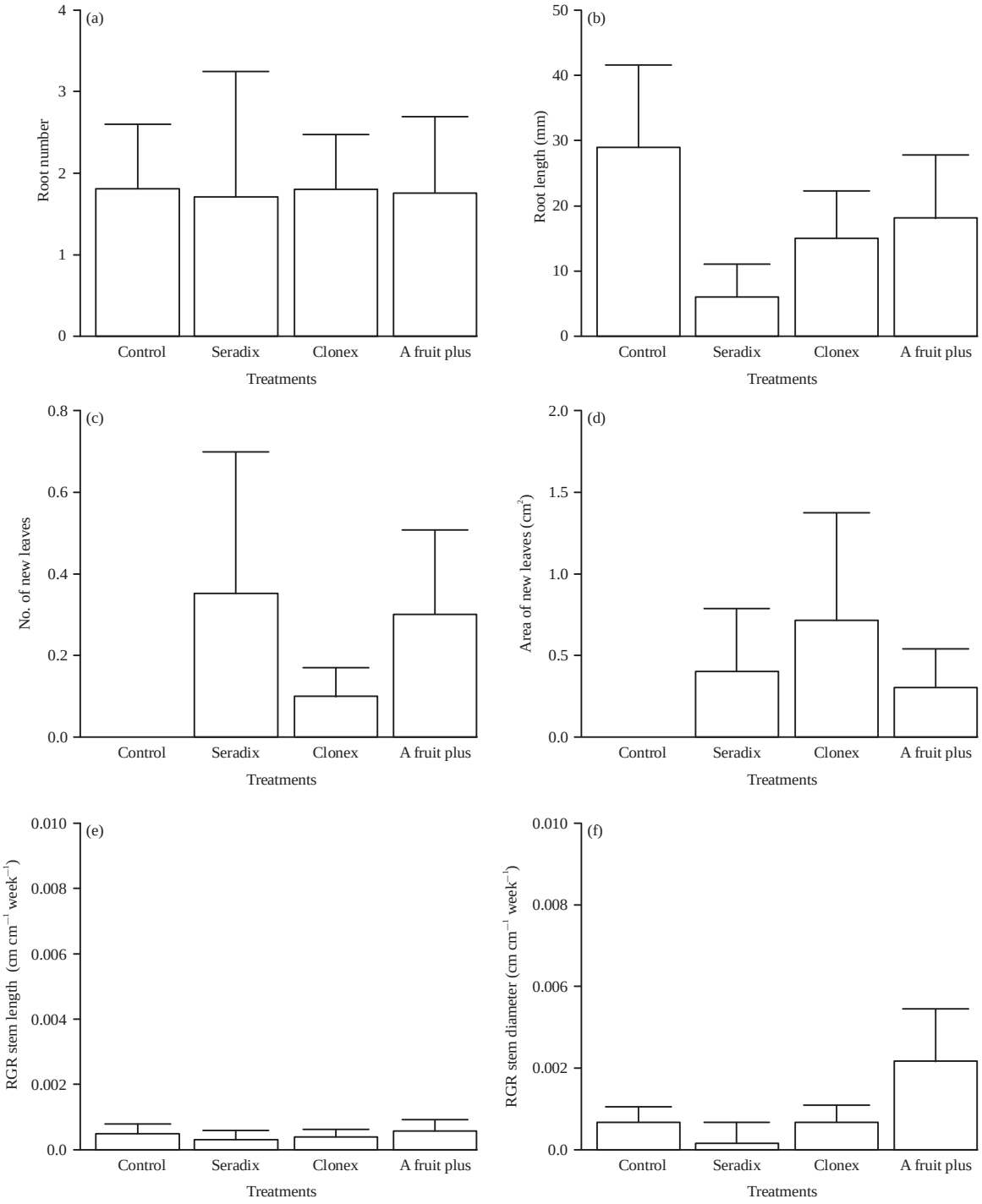

Fig. 2(a-f): (a) Root number per cutting, (b) Root length (cm) per cutting, (c) No. of new leaves per cutting, (d) Area of new leaves (cm^2) per cutting, (e) RGR based on stem length and (f) RGR based on diameter of *Aidia racemosa* stem cuttings subjected to different treatments (distilled water, seradix, clonex and 'A fruit plus') at week 16. Values are Means±Standard Error (SE)

DISCUSSION

Vegetative propagation method provides the facilitation of rapid multiplication of plants to meet the increasing demand for planting materials of *Aidia racemosa* which is the main focus of this study. It is hoped that the use of vegetative propagation would minimize any need to collect the wild plants from the forest and this would help in protecting our

pristine forest. This study revealed that stem cuttings treated with clonex had the highest survival and rooting percentages than the rest of the treatments. Mehrabani *et al.*[8] reported that the immediate formation and the subsequent growth of roots are the most influential factors affecting the survival of cuttings. The successful rooting of stem cuttings could be influenced by many other factors, such as the rooting medium, the environmental conditions as well as the physiological status of the stock plant itself[9]. Although the survival percentage for stem cutting treated with clonex is enough to propagate *A. racemosa*, the overall low survival and rooting percentages for stem cuttings suggest that the commercial hormones used in this study are probably not completely suitable for the propagation of this species using stem cuttings.

Although there was lack of studies on vegetative propagation using clonex, the active ingredient IBA had commonly been used in the propagation of several species of plants from stem cuttings. However, the results varied depending on the concentration of IBA used. A study conducted by Maile and Nieuwenhuis[10] showed that the application of 0.8% IBA did not effectively influence the adventitious root formation on *Eucalyptus nitens* stem cuttings. However, a study by Kipkemoi *et al.*[11] showed that stem cuttings of *Strychnos heningsii* treated with IBA and seradix 2 powder produced more and longer roots and had higher rooting percentage than those treated with NAA and IAA. The study also showed with hormone treatments (IBA, NAA and IAA), the mean number of roots as well as rooting percentage of cuttings increased with increasing concentrations (up to 0.015%). The Seradix hormone used in this study contained 0.1% IBA so this could explain the low survival and rooting percentages. The commercial hormone 'A fruit plus' contains biostimulant and cytokinins, gibberellins and auxins. Cytokinins and gibberellins are not commonly used in studies involving vegetative propagation and generally known to have little or no effects on rooting. This could explain the failure of stem cuttings to produce more roots when 'A fruit plus' was used.

The present study reported no significant effects of the hormone treatments and distilled water for each of the parameter investigated, but seradix and A fruit plus seem to increase leaf production and clonex to produce larger leaves. This current work also suggested that more studies on different growing media need to be conducted as this factor may improve the growth rates, number and length of roots and number and surface area of leaves. For example, Saradha and Samydurai[12] claimed that coconut fiber and vermiculite are preferably used as growing media to improve the aeration and moisture capacity. Other methods of propagation, such as *in vitro* propagation, could be explored for the mass propagation of *A. racemosa*. Khalil *et al.*[13] successfully propagated *Stevia rebaudiama* using micropropagation techniques with 85% success rate when compared to propagation using stem cuttings (60%).

CONCLUSION

From the present findings, treating *A. racemosa* stem cuttings with clonex (0.3% IBA) resulted in high survival and rooting percentages, however, the use of commercial hormones as well as distilled water did not produce a significant difference in terms of the growth parameters under investigation. However, it appeared that stem cuttings treated with seradix and 'A fruit plus' produced high number of new leaves (0.35 and 0.30, respectively) and clonex-treated stem cuttings produced larger leaves (0.72 ± 0.66 cm^2) than the other hormone treatments. More research needs to be done to provide an alternative but effective approach in the mass production of *A. racemosa* plants for the herbal tea market.

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