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Rooting performance of bahai (*Ormosia calavensis* Azaola ex Blanco) and Bakan (*Litsea philippinensis* Merr.) As affected by different concentrations of indole-3-Butyric acid (IBA)

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Abstract

A macro-somatic propagation study was conducted to assess the effects of rooting hormone and the level of concentration to the rooting performance of Bahai (*Ormosia calavensis* Azaola ex Blanco) and Bakan (*Litsea philippinensis* Merr.). The study was carried out in the clonal nursery of Central Mindanao University, Msuan, Bukidnon, Philippines. The experimental design used was the 2 x 4 factorial experiment arranged in Complete Randomized Design (CRD) replicated five times. The two tree species served as Factor A: Bahai and Bakan; while levels of Indole-3-Butyric Acid (IBA) served as Factor B: control – no auxin , 100 ppm, 300 ppm, and 500 ppm. Findings show that Bahai cuttings exhibited statistically higher rooting performance compared to Bakan in terms of root length and number of roots treated with 300 ppm of IBA. Data show that Bahai had an average root length of 2.042 cm compared to Bakan with only 1.355 cm. For the number of roots, Bahai had an average of 1.855 compared to Bakan with 1.376. Between the two species used, Bahai showed higher survival rate of 84.42% compared to 57.63% for Bakan. Findings suggest that both species can be treated with 300 ppm of IBA to enhance higher rooting percentage where it produces greater number and longer length of roots. It is recommended that Bahai and Bakan tree species can be potential species for propagation using macro-somatic technologies.

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Introduction

The use of conservation programs have been anticipated to promote conservation and cultivation of tree species through the use of proper vegetative propagation technique asexual propagation is one of the best methods to maintain existence of some species and improve or sustain its quality. Cloning is a process by which individual organisms are multiplied asexually - a process of vegetative regeneration or reproduction (Longman, 1993). Cloning process can be both done naturally and artificially. The process of vegetative regeneration can be produced in two ways. One of which involves the relatively new processes of micro-propagation which have arisen from the development of modern biotechnology, and the other one is involving the more traditional techniques of macro-propagation (Leakey, 2014).

In the Philippines, the use of macro-propagation techniques has not been practiced in most of the native tree species. Yet, native tree species become increasingly important in land rehabilitation activities (Butterfield, 1995). In this study, two of the less known native tree species in the country were studied for macro-somatic propagation. These are Bahai (*Ormosia calavensis* Azaola ex Blanco) and Bakan (*Litsea philippinensis* Merr.) of which at present, clonal propagation protocol for such species are yet to be established. Clonal techniques, therefore, would promote conservation of these species by increasing its population and at the same time maintain or improve its quality. Results of the study would also be useful to future researchers and farmers who would work and venture into tree farming using the said species.

Materials and methods

Location of the Study Site

The experiment was carried out under a close misting propagation chamber of Central Mindanao University, Clonal Nursery of the College of Forestry and Environmental Science. The propagation bed was filled with screened sand as the rooting media. The sand was thoroughly washed and treated with fungicide to protect the experimental materials from diseases.

Experimental design

The experiment was set up in a 2 × 4 factorial experiment arranged in Complete Randomized Design with five (5) replications. The stem cuttings of the two species were distributed randomly in each block with 10 samples per experimental unit. There were a total of 8 treatment combination (2 Factor A × 4 Factor B) which were replicated five (5) times. Since there were 10 cuttings in an experimental unit, a total of 400 cuttings were observed throughout the research study.

Preparation of the Rooting Hormone

Indole-3-Butyric Acid (IBA) was used as the hormone for Bahai (*O. calavensis* Azaola ex Blanco) and Bakan (*L. philippinensis* Merr.). The concentration levels of IBA were: 100, 300 and 500 ppm. This was done by adding 1 ml, 3 ml, and 5 ml of IBA per liter of distilled water. To dissolve the hormone, a drop of 70% alcohol was added to the solution. Each hormone solution was dispensed in properly-labelled containers ready for use.

Preparation of Cuttings

Cuttings of Bahai (*O. calavensis* Azaola ex Blanco) were collected from a mother tree located at the Central Mindanao University-Swine Production Project. The Bakan (*L. philippinensis* Merr.) cuttings were collected from the hardened seedlings as serial cuttings at the Central Mindanao University Clonal Nursery of the College of Forestry and Environmental Science. The cuttings were collected using a sharp pruning shear to an average length of three inches with at least three fully-developed leaves.

The main stem of the planting stocks were cut across the third node down the apex using a pair of pruning scissor. Leaves were trimmed to reduce the leaf size to approximately ⅓ of the original size following the methods used by Leakey *et al.* (1982). To avoid contamination during preparation, disinfection was done to all the materials by soaking the cuttings in a basin with fungicide solution.

Basal long soaked method

The basal parts of Bahai and Bakan cuttings were soaked into the rooting hormone for 1 hour, except for the control where cuttings were only soaked in tap water. The cuttings were then planted following the experimental lay-out.

Data collection and statistical analysis

The research study was conducted for a period of two months. Upon the termination of the study, data on root length, number of roots (callused and rooted), survival rate and root biomass were collected. The collected data were subjected to Analysis of Variance (ANOVA) using MStatC to determine the significant differences of the parameters of the study. The difference of means was done using Tukey's Test.

Results and discussion

Bahai (*O. calavensis* Azaola ex Blanco) cuttings significantly produced longer roots with mean length of 2.042 cm as compared to Bakan (*L. philippinensis* Merr.) with only 1.355 cm (Figure 1). This result maybe attributed to the fact that Bahai is a sun-demanding species, as stated by Milan (undated) and since the study was conducted during summer season when it is more favorable for the Bahai cuttings to root naturally.

Table 1. Mean root length of Bahai and Bakan species as affected by different concentrations of IBA.

Treatment	Mean root length
A. Factor A	
Species	
Bahai	2.042[a]
Bakan	1.355[b]
Level of Significance	**
Coefficient of Variation	41.01
B. Factor B	
Concentration level	
No auxin (control)	1.263[b]
100 ppm	1.668[ab]
300 ppm	2.253[a]
500 ppm	1.610[ab]
Level of Significance	*
Coefficient of Variation	41.01
C. Interaction	
Treatment	
S_1C_1	1.684
S_1C_2	2.404
S_1C_3	2.250
S_1C_4	1.830
S_2C_1	0.842
S_2C_2	0.932
S_2C_3	2.256
S_2C_4	1.390
Level of Significance	ns
Coefficient of Variation	41.01%

Mean length of roots with the same letter are not significantly different from each other using Tukeys at 1% level. Legend; ** = highly significant, * = significant, ns = not significant S_1 = Bahai, S_2 = Bakan, C_1 = 0 ppm, C_2 = 100 ppm, C_3 = 300 ppm, C_4 = 500 ppm.

On the IBA concentration level, 300 ppm obtained the highest root length of 2.253 cm, followed by 100 ppm, 500 ppm and 0 ppm (Control) with 1.668 cm, 1.610 cm and 1.263 cm, respectively (Table 1). Results show significant differences on the species and the levels of concentration for the mean length of roots. The use of rooting hormone in stimulating longer root production was useful.

Table 2. Mean root biomass of Bahai and Bakan species as affected by different concentrations of IBA.

Treatment	Mean root biomass
A. Factor A	
Species	
Bahai	0.734
Bakan	0.734
Level of Significance	ns
Coefficient of Variation	13.16
B. Factor B	
Concentration Levels	
No auxin (control)	0.721
100 ppm	0.735
300 ppm	1.768
500 ppm	1.712
Level of Significance	ns
Coefficient of Variation	13.16
C. Interaction	
Treatment Combination	
S_1C_1	
S_1C_2	
S_1C_3	0.722
S_1C_4	0.752
S_2C_1	0.772
S_2C_2	0.690
S_2C_3	0.720
S_2C_4	0.718
	0.764
Level of Significance	0.734
Coefficient of Variation	ns
	13.16 %

Mean length of roots with the same letter are not significantly different from each other using Tukey at 1% level. Legend; ** = highly significant, * = significant, ns = not significant S_1 = Bahai, S_2 = Bakan, C_1 = 0 ppm, C_2 = 100 ppm, C_3 = 300 ppm, C_4 = 500 ppm.

The findings from this study conform with Elhaak *et al.* (2015) who reported that *Rosmarinos officinalis* presoaked with IBA for 1 hour increased the root length by 17 % in comparison with the control. Nevertheless, research findings tend to agree in two respects that auxins are promotive of adventitious rooting when compared with non-treated controls, however, high concentrations of applied auxin are often damaging (Loach, 1988). High concentration of the hormone may produce growth abnormalities and probably hinders the root formation for some tree species. Successful root formation depends upon many factors like season and time of cutting, portion and diameter of stem, treatment of the rooting hormones, growing media, moisture level, nutrient status and temperature.

Table 3. Percent survival (%) Bahai and Bakan species as affected by different concentrations of IBA.

Treatment	Percent survival
A. Factor A	
Species	
Bahai	84.42[a]
Bakan	57.63[b]
Level of Significance	**
Coefficient of Variation	28.24 %
B. Factor B	
Concentration Levels	
No auxin (control)	59.52
100 ppm	65.63
300 ppm	78.05
500 ppm	80.92
Level of Significance	ns
Coefficient of Variation	28.24 %
C. Interaction	
Treatment Combination	
S_1C_1	34.84
S_1C_2	41.85
S_1C_3	45.99
S_1C_4	46.17
S_2C_1	24.68
S_2C_2	23.78
S_2C_3	32.06
S_2C_4	34.75
Level of Significance	ns
Coefficient of Variation	28.24 %

Mean length of roots with the same letter are not significantly different from each other using Tukey at 1% level. Legend; ** = highly significant, * = significant, ns = not significant S_1 = Bahai, S_2 = Bakan, C_1 = 0 ppm, C_2 = 100 ppm, C_3 = 300 ppm, C_4 = 500 ppm.

Meanwhile, there is no significant interaction effect between the species used and the level of hormone concentration as shown in Table 1. However, it revealed that Bahai and Bakan treated with 300 ppm of IBA produced the longest roots with means of 2.250 cm and 2.256 cm, respectively.

As shown in Figure 1, a significant difference was observed between the two species used. Bahai (*O. calavensis* Azaola ex Blanco) cuttings had higher mean number of roots with 1.855 compared to Bakan (*L. philippinensis* Merr.) with only 1.376. This suggests that Bahai cuttings had a better rooting performance compared to Bakanin terms of mean number of roots. Furthermore, the capacity of Bahai to form adventitious roots is greater than Bakan was due to the reason that Bahai is a sun-demanding species compared to Bakan, hence the better response of Bahai in terms of roots formaton.

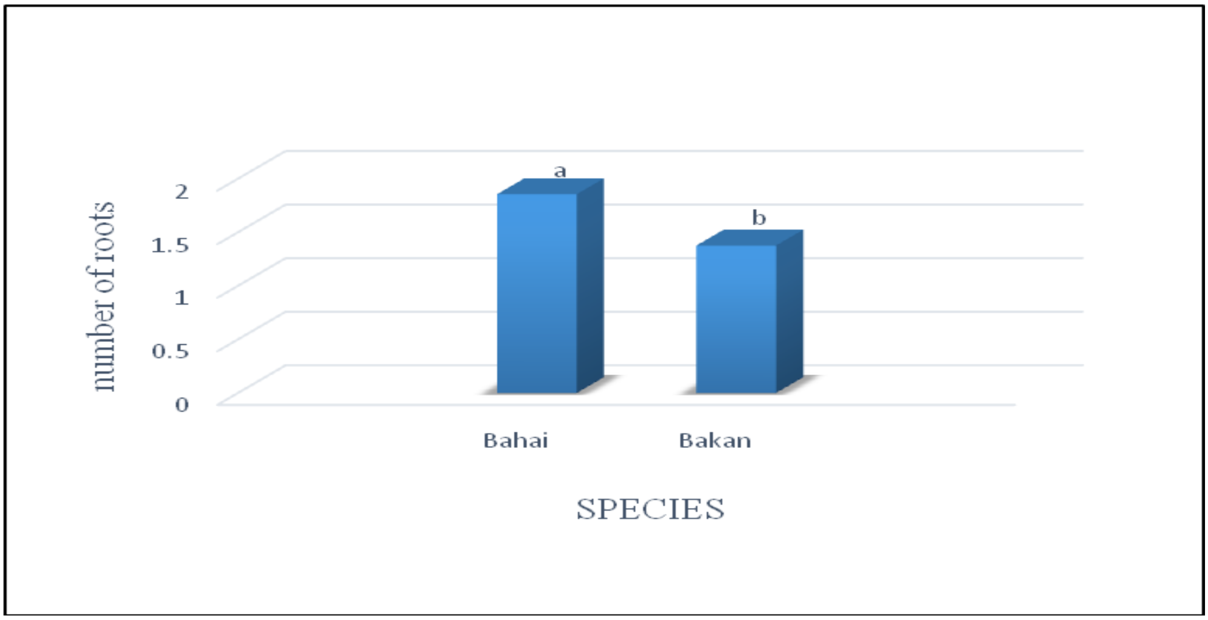

Fig. 1. Graphical presentation on mean number of roots of Bahai (*O. calavensis* Azaola ex Blanco) and Bakan (*L. philippinensis* Merr.).

The success of Bakancuttings to form adventitious roots may have been affected by the temperature inside the rooting chamber. When the temperature got too high, plant tissues deteriorated, resulting in a decrease in respiration. Respiration decreases when the temperature of the plant decreases. More reduction on temperature level affects the rate of photosynthesis, which is the ability of the plant to produce energy. This loss of energy directly affects the formation of roots (Haskell, undated).

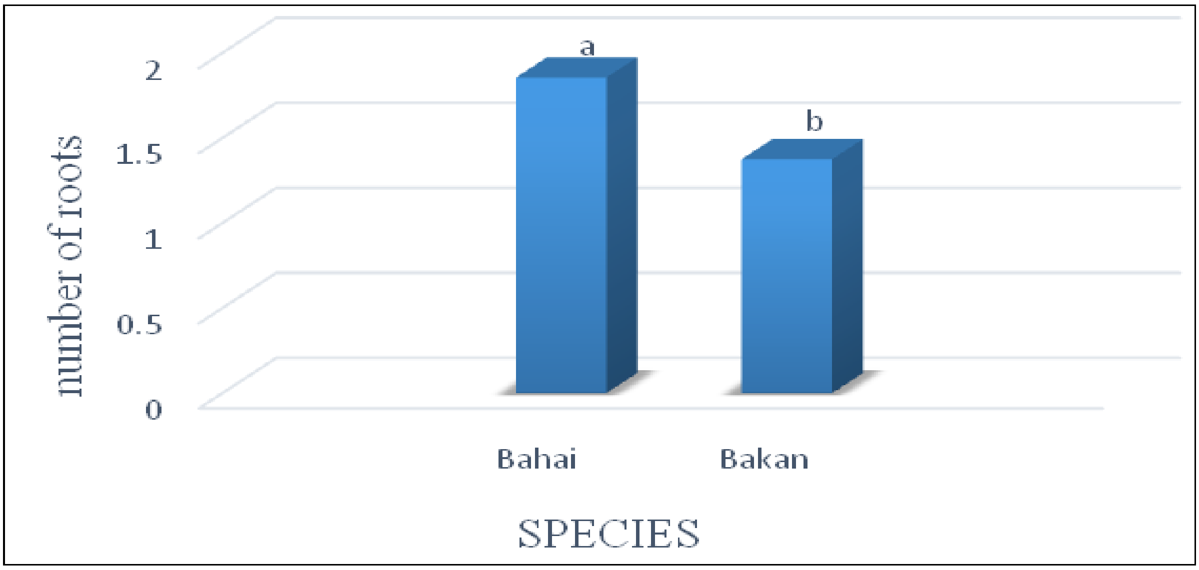

Fig. 2. Graphical presentation on the mean number of roots in response to the level of concentrations.

In terms of the levels of concentration, 300 ppm had the highest mean number of roots with 1.835, followed by 500 ppm, 0 ppm (control) and 100 ppm with 1.620, 1.559 and 1.449, respectively (Figure 2).

With regards to the mean root biomass, findings showed no significant differences among treatment means as shown in Table 2. However, both of the species had similar weight of 0.734 g.

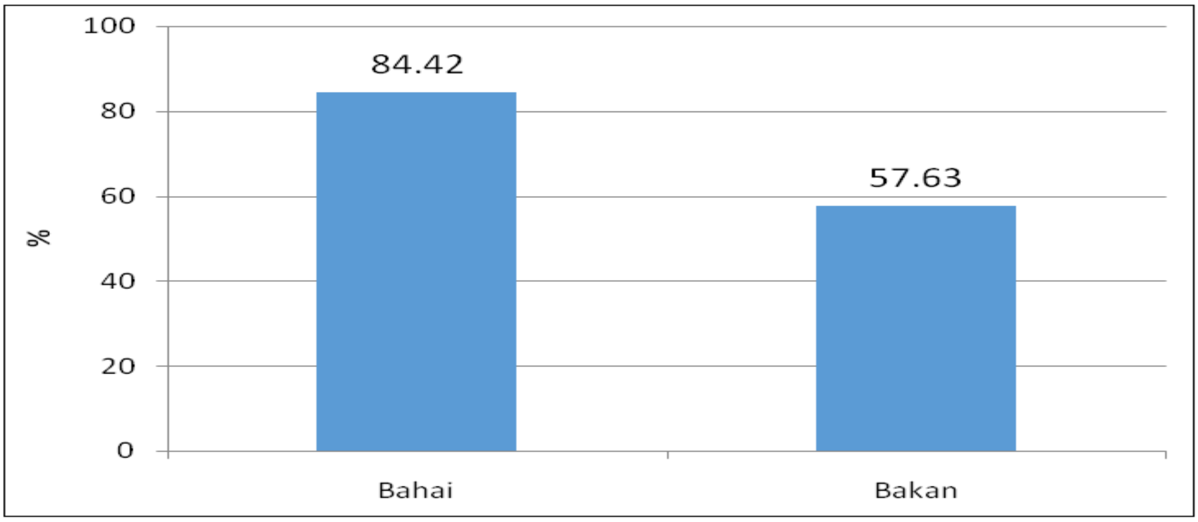

Fig. 3. Graphical presentation on the mean percent survival of Bahai (*O. calavensis* Azaola ex Blanco) and Bakan (*L. philippinensis* Merr.).

This may due to the varying characteristics of the roots and of the two species. Roots of Bahai were thin, long, white in color and hydrophilic in nature while roots of Bakan were large, short, dark in color, turgid, and hydrophobic in nature. Thus, during oven drying, roots of Bahai tend to lose more moisture because of its root characteristics.

In terms of the levels of concentration, 300 ppm had the highest mean root biomass with 1.768 g, followed by 500 ppm, 100 ppm and 0 ppm (control) with 1.712 g, 0.735 g and 0.721 g, respectively. Meanwhile, there was no significant interaction between the species used and the level of IBA concentration (Table 2). However, it reveals that Bahai and Bakan treated with 300 ppm IBA produced the heavier mean root biomass.

Bahai had higher percent survival with 84.42 % compared to Bakan with only 57.63 % (Figure 3). Bahaiwas on its best condition to propagate and respond well to the rooting hormone used in the study. However, it showed no significant differences among the concentration levels. The highest percent survival was observed at 500 ppm with 80.92 %, followed by the 300 ppm with 78.05 % while the 100 ppm and 0 ppm (control) had 65.63 % and 59.52 %, respectively (Table 3). Findings shows that increasing IBA concentration also increases the survivability of the plant.

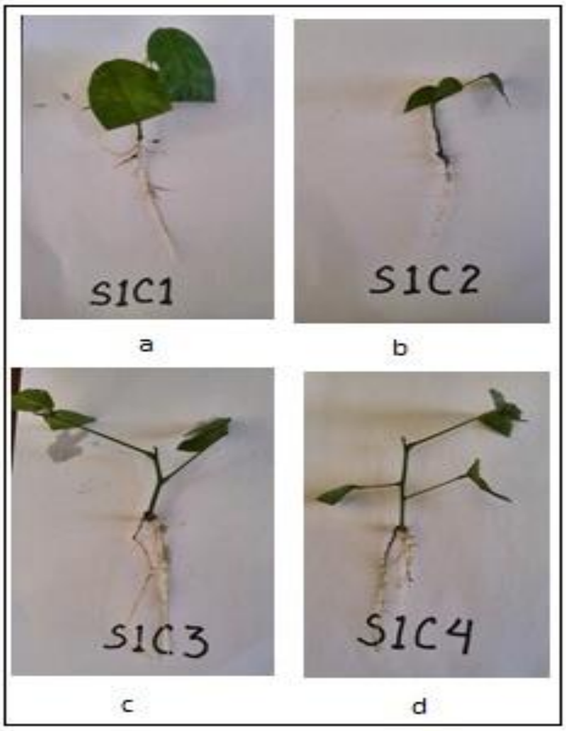

Fig. 4. Bahai (*O. calavensis*) cuttings treated with IBA at four level of concentrations (a) no auxin (control), (b) 100 ppm, (c) 300 ppm and (d) 500 ppm.

The result was supported by Elhaak *et al.* (2015) that lesser percent survival was observed under the control (0 ppm) while it increased upon IBA application. A study conducted by Akwatulira *et al.* (2011) revealed that stem cuttings of *Warburgia ugandensis* propagated in sand produced the least percentage and number of roots per rooted stem cutting.

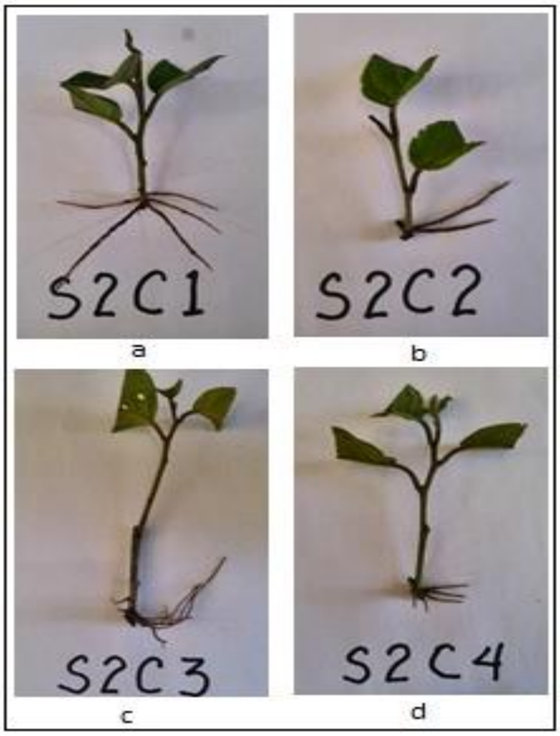

Fig. 5. Bakan (*L. philippinensis* Merr.) cuttings treated with IBA at four levels of concentration (a) no auxin (control), (b) 100 ppm, (c) 300 ppm and(d) 500 ppm.

This result was interesting given that high rooting percentage have been obtained from Bahai propagated in sand media but having a similar findings for Bakan.

Sand was also identified as the best rooting medium for *Gongronema latifilia* stem cuttings by Agbo and Omaliko (2006). However, there was a variation in response to different rooting media as was reported on many tree species.

The differences in the rooting ability of various species propagated in different rooting media could be explained by their xeromorphic or hydromorphic status (Loach, 1992) as cited by Akwatulira *et al.* (2011) and the effects of this status on the water relations of the cuttings (Mensen *et al.*, 1997) as cited by Akwatulira *et al.* (2011).

Further investigation is needed with regards to the relatively low level of survival for Bakanstem cuttings propagated in sand.

Among the two native tree species, Bahai (*O. calavensis* Azaola ex Blanco) cuttings exhibited better rooting performance based on the length of the roots, number of roots and percent survival.

The 500 ppm concentration gave and exhibited greater survivability for the two species. On the other hand, the 300 ppm concentration exhibited better rooting performance in terms of number and length. Both indigenous tree species are potential for macro-somatic propagation technology to produce quality planting materials.

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