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Levels of concentration of alpha naphthalene acetic acid (ANAA) root hormone on the rooting performance of palisan (*Aquilaria cumingiana* Decne Ridl.) serial cutting

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Key words: Alpha naphthalene acetic acid, Levels of concentration, Palisan, Rooting performance, Serial cutting

Abstract

This original study addresses the persistent issue of indiscriminate cutting of Aquilaria trees by examining the effects of different concentrations of ANAA root hormone on Palisan rooting performance at the DENR Clonal Facility in Upper Pulacan, Labangan, Zamboanga Del Sur. The research employed a Randomized Complete Block Design (RCBD) over a two-month period, with four concentration levels (0ppm, 1000ppm, 2000ppm, and 3000ppm). Each block consisted of ten cuttings, replicated three times, totalling to 120 cuttings for the entire setup. The study's results indicate that, concerning the number of roots, T2 (1000ppm) produced the highest mean (4.4), while the lowest mean (1.8) was observed in the same concentration. Regarding the length of roots, T3 (2000 ppm) exhibited the highest mean (2.21), with T1 (0ppm) showing the lowest mean (0.73). In terms of the number of leaves, T3 (2000ppm) yielded the highest mean (1), while T4 (3000ppm) produced the lowest mean (0.2). However, the study found that the different levels of ANAA root hormone did not significantly impact the rooting performance of Palisan serial cutting. Statistical analysis revealed p-values greater than the 5% significance level for number of roots (0.9336), length of roots (0.8575), and number of leaves (0.2561). Therefore, further studies concentrating on vegetative propagation, specifically serial cutting under both misting and non-misting propagation systems, using ANAA rooting hormones at various levels, is recommended to enhance rooting.

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Introduction

Aquilaria cumingiana, a member of the Thymelaeaceae family referred to as "Palisan" in Tagalog, "Bago" in Manobo, and "Binukat" in Aklan Bisaya, has been extensively covered in literature, with a focus primarily on its conservation and biodiversity status (Lee and Mohamed, 2016). Underscoring the economic importance of Agarwood-Producing Species (APS) for its role in agarwood formation, a process induced by various stressors, (Suharti, 2011; Tan *et al.*, 2019) highlights the depletion of wild populations due to heightened demand and overharvesting, alongside conservation efforts driven by legislation (Persoon and van Beek, 2008; Esyanti *et al.*, 2019). Furthermore, it delves into the challenges associated with seedling production, stressing the necessity for further research, especially concerning *A. cumingiana* in the Philippines (Sitepu, 2011). The Department of Environment and Natural Resources (DENR) has pinpointed eight Aquilaria species, including *A. brachyantha, A. decemcostata, A. parvifolia, A. apiculata, A. citrinicarpa, A. urdanetensis, A. malaccencis, and A. cumingiana*, as vulnerable in the Philippines, which is notable given the country's status as a significant agarwood supplier (DENR, 2017; The Plant List, 2013; Yin *et al.*, 2016).

Aquilaria trees have the potential to produce significant seed quantities annually (Soehartono and Newton, 2001). However, excessive seed harvesting in their natural environment hinders seedling production (Lian *et al.*, 2016; Lee and Mohamed, 2016), compounded by the challenge of large seeds with low compatibility and germination rates (Kundu and Kachari, 2000; Sitepu, 2011; Tabin and Shrivastava, 2014). To address this, researchers have explored micropropagation techniques (Chiu, 2016; Esyanti *et al.*, 2019) and macro-propagation using both young (Loc and Luu, 2002; Yung, 2013; Yusnita *et al.*, 2017) and mature stem cuttings (Borpuzari and Kachari, 2018). However, these methods primarily focus on specific Aquilaria species, particularly A. *malaccensis*, neglecting others like *A. cumingiana*, particularly in the Philippines. Therefore, this study aimed to examine the effects of various levels of concentration of ANAA root hormone on the rooting performance of Palisan.

This study's findings significantly advance our understanding of Palisan's rooting performance, providing valuable insights for conservation and reforestation efforts. By identifying effective vegetative propagation technique and hormone concentration levels, it offers a blueprint for enhancing Palisan cultivation techniques, ultimately contributing to biodiversity conservation and ecosystem restoration. Moreover, the investigator aimed to examine the effects of various levels of concentration of ANAA root hormone on the rooting performance of Palisan.

Materials and methods

Description of the study area

The research was carried out at the former Clonal Facility of the Forest and Timber Resources and Research Center (FTRRC) under the Ecosystems Research Development Bureau (ERDB). However, in 2018, it was transferred to the Department of Environment and Natural Resources (DENR) situated in Upper Pulacan, Labangan, Zamboanga Del Sur. The facility spans one (1) hectare and is dedicated to cloning native and indigenous species. The Clonal Facility includes various sections such as the rooting chamber, recovery chamber, potting shed, sterilization area, hardening area, and hedge garden. With a tropical climate characterized by a minimum temperature of 18 °C (64 °F) and significant annual rainfall, the site is positioned at an elevation of 203 to 207 meters above sea level.

Research design and sampling

The study aimed to assess the rooting performance of Palisan serial cuttings treated with ANAA root hormones. A Randomized Complete Block Design (RCBD) was employed in the experiment, along with the different concentrations of hormones. The rooting hormone concentrations included 0ppm, 1000ppm, 2000ppm, and 3000ppm. Each concentration had ten (10) cuttings, and the experiment was replicated

three (3) times, resulting in a total of one hundred twenty (120) propagated cuttings. In this study, the researcher utilized simple random sampling, selecting 120 samples from a pool of 200 cuttings. Each treatment received 10 samples, replicated three times, resulting in a balanced and streamlined dataset. Through this approach, the researcher investigated the effect of various levels of Alpha Naphthalene Acetic Acid (ANAA) root hormone on Palisans' rooting performance.

Preparation of rooting bed

The rooting medium consisted of a blend of river sand and coconut coir dust in a 1:1 ratio. River sand was chosen for its ability to provide adequate aeration to the plant, while coconut choir dust was included to retain sufficient moisture for nutrient availability. Water is sprayed in vapor form every 3-4 hours, estimating misting the plant 3-4 times a day in the well-equipped rooting chamber at the DENR clonal facility in Upper Pulacan, Labangan, Zamboanga Del Sur. Subsequently, this carefully prepared mixture was placed into a fully structured and functional rooting chamber.

Collection of serial cuttings

The collection of serial cuttings commenced once the shoots had matured, as indicated by their brownish color with 3-4 nodal cutting. Segments of juvenile shoots from Palisan seedlings were snipped at right angles with leaves attached using sharp cutting scissors. The freshly cut stems were promptly placed in an ice chest box with a small amount of water to prevent dehydration during transportation to the clonal facility for treatments and planting.

Preparation of serial cuttings

Newly collected shoots were transferred into a basin containing a small amount of water for rehydration purposes while leaves were being trimmed halved in a vertical orientation. In the preparation for sterilization, a fungicide solution with the brand name Dithane M-45 Neotec was done by mixing one tablespoon of solution with 8 liters of water.

After preparing the solution, the 3-4 nodal cuttings of Palisan were sterilized by soaking them for about an hour. In the preparation of the Alpha Naphthalene Acetic Acid (ANAA) root hormone, a syringe served as a measuring tool to indicate varying concentrations: 1000ppm (1ml), 2000ppm (2ml), and 3000ppm (3ml), diluted in a constant 1-liter of tap water. Conversely, in the control treatment, the cuttings were dipped into 1-liter tap water. The root hormone was applied to the basal ends of the cuttings, which were scraped, bundled in the same dimensions at the bottom, and immersed about 3/4-1 inch into a container. The bundled cuttings were soaked for approximately one hour and immediately stuck into the prepared rooting chamber after the treatment.

Data collection and analysis

Two months later, the rooted cutting plants were uprooted for the purpose of data collection. The rooted plants were carefully taken out of the rooting chamber and cleansed with water for measurement. The total number of roots was determined by counting the roots arising from each young Palisan cutting; the overall root length was assessed using a smaller calibrated ruler, measuring the length of the longest developing roots in centimeters (cm); and finally, the total number of leaves was tallied by counting the leaves sprouting from the shoots of the Palisan cutting. The data were analyzed using the Statistical Tool for Agricultural Research (STAR). The ANOVA (Analysis of Variance) was used to determine if there were any statistical difference between the main plot and subplot across different treatments

Results and discussion

Number of roots

The column graph in Fig. 1 showed the mean on the effects of the ANAA treatment on the number of roots in the serial cuttings of *A. cumingiana*. The data revealed that the highest number of roots produced was in T_2 (1000ppm) with a mean of (4.4) in Block 3. On the other hand, the lowest number of roots produced in Block 1 with the same concentration but with a mean of (1.8).

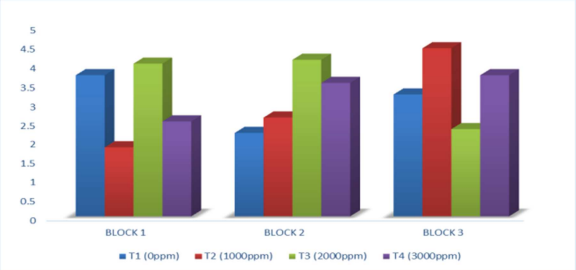

Fig. 1. Mean on the effect of different levels of concentration of ANAA rooting hormone on the number of roots of *A. cumingiana* serial cuttings

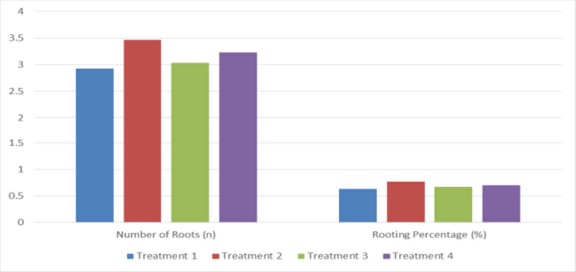

Fig. 2. Means and percentage of the number of roots

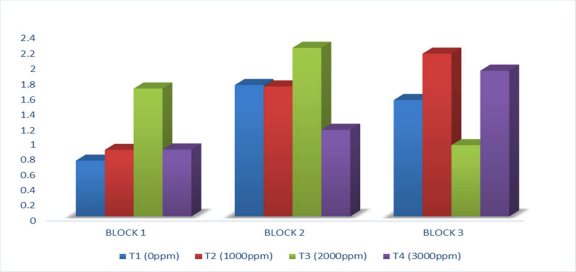

Fig. 3. Mean on the effect of different levels of concentration of ANAA rooting hormone on the length of roots of *A. cumingiana* serial cuttings

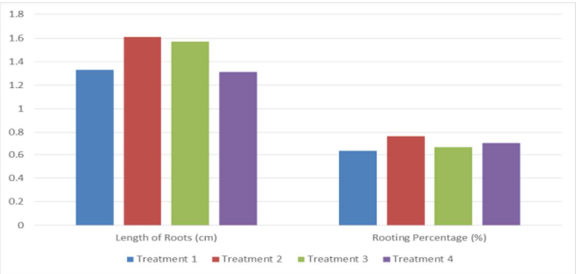

Fig. 4. Means and percentage of the length of roots

Fig. 2 presents the findings indicating that T_2 had the highest mean number of roots (3.47) with a rooting percentage of 76.66%, followed by T_4 (3.23) with 70%, T_3 (3.03) with 66.66%, and T_1 (2.93) with 63.33%, respectively. However, the analysis revealed no statistically significant difference in root number concerning different levels of ANAA rooting hormone concentration (P-value=0.9336). Despite a coefficient variant of 34.69% and a p-value of 0.9336 exceeding the 5% significance level, implying no significant disparity in root number across various ANAA concentrations for Palisan. Notably, the application of 1000ppm ANAA was found to be the most cost-effective for inducing root formation in Palisan cuttings.

The findings of Paul and Aditi (2009) corroborate similar results, demonstrating that 1000 ppm of NAA effectively enhances the rooting process of waterapple air-layers, with the highest root production observed at this concentration. Their experiments underscored survival rates of 63% and 57% at 1000 ppm and 2500 ppm, respectively. Conversely, Tien *et al.* (2020) presented contrasting results, indicating reduced sprouting rates at a 1000 ppm NAA concentration compared to the control group. This suggests potential drawbacks associated with this concentration, such as weakened synergistic effects and lower sprouting rates. While Paul and Aditi (2009) advocate for the efficacy of 1000 ppm NAA in promoting rooting, the disparity in findings underscores the complexity of variables affecting *A.cumingiana* responses to hormone treatments, necessitating further investigation in plant physiology.

Length of roots

The mean on the effects of the ANAA treatment on the length of roots in the serial cuttings of *A. cumingiana* was shown graphically in Fig. 3. This investigation revealed that in Block 2 produced the highest mean length of roots is with T_3 (2000 ppm) and the lowest is the Block 1 with T_1 (0ppm), with a mean of 2.21cm and 0.73cm, respectively.

Fig. 4 displays a comprehensive analysis wherein T_2 exhibits the highest mean root count of 1.61cm and a rooting percentage of 76.66%, followed by T_3 with a mean of 1.57cm and a rooting percentage of 66.66%. T_1 and T_4 follow suit with rooting percentages of

63.33% and 70% respectively, and mean root lengths of 1.33cm and 1.31cm. However, the effects of ANAA rooting hormone concentrations on root length were statistically insignificant (P-value = 0.8575). Despite a coefficient variant of 37.05% and a p-value exceeding the 5% threshold of significance, suggesting no notable differences in root length among Palisan cuttings exposed to different ANAA concentrations, the most cost-effective method to achieve longer roots was found to be applying ANAA at 1000ppm concentration levels.

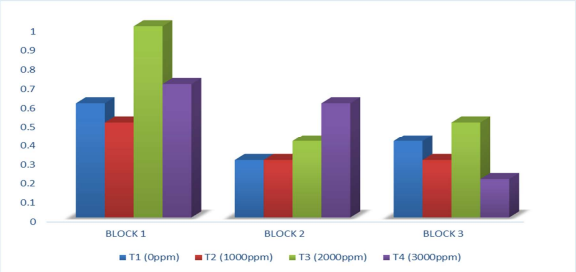

Fig. 5. Mean on the effect of different levels of concentration of ANAA rooting hormone on the number of leaves of *A. cumingiana* serial cuttings

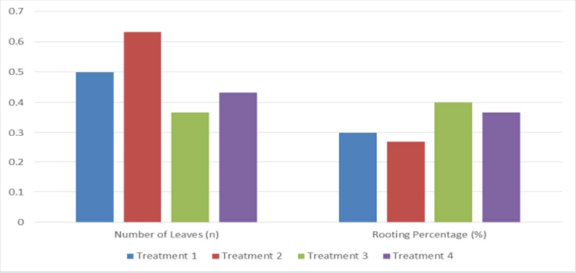

Fig. 6. Means and percentage of the number of leaves

The findings presented are in line with the research of Paul and Aditi (2009), who suggested that employing IBA rooting hormone resulted in notably longer average root lengths, particularly with concentrations of 2500ppm and 500ppm. However, they highlighted the effectiveness of NAA at 1000ppm in promoting root emergence, especially in waterapple air-layers. Additionally, Javier and Mamicpic (n.d.) noted that applying NAA at 100ppm produced the longest average root length. Their field-based study further supported these laboratory findings, showing that NAA enhanced both root number and length during the early stages of plant growth, specifically 80 days post-planting. Hence, it can be deduced that *A. cumingiana* demonstrated favorable root development in response to NAA rooting hormone.

Number of Leaves

The graphical representation in Fig. 5 depicted the mean effect of ANAA treatment on the number of leaves in the serial cuttings of *A. cumingiana*. Exploring further, the data revealed that Block 1, when combined with T_3 (2000ppm), yields the highest number of leaves. On the contrary, in Block 3 also but with T_4 (3000ppm) produced the lowest number of leaves, with a mean of 1 and 0.2, respectively.

Fig. 6 demonstrates that T_2 had the highest average number of leaves at 0.6333, followed by T_1 at 0.5000, T_4 at 0.4333, and T_3 at 0.3667. Despite these variances, the statistical analysis, as indicated by a P-value of 0.2561, reveals the lack of significance in the impact of ANAA rooting hormone concentrations on leaf count. Moreover, with a coefficient variation of 30.84% and a p-value of 0.2561 surpassing the 5% significance level, the study fails to detect any substantial differences in leaf count among Palisan cuttings exposed to various ANAA concentration levels. The data also highlights that using 1000ppm of ANAA proves to be the most cost-effective method for inducing leaf emergence in Palisan cuttings.

A thorough analysis of leaf count in response to different NAA treatments can be gleaned from Paul and Aditi (2009), where the highest leaf count resulted from a 2500 ppm NAA spray, closely followed by a 1000 ppm dose, consistent with Setiawati *et al.* (2018) findings indicating inhibitory effects of 100% NAA treatment on shoot growth and leaf expansion. Despite careful experimental protocols and cultural practices, this study primarily focused on ANAA rooting hormone concentrations' impact on leaf quantity, yielding less-than-expected results within a short two-month timeframe. This underscores the complexity of NAA treatments on leaf growth, as elucidated by Paul and Aditi (2009) and Setiawati *et al.* (2018). While the study successfully

enhanced root induction and leaf growth with various treatments, particularly at 1000 ppm concentration, it revealed poor leaf development overall, confirming the hypothesis but indicating the intricate nature of hormonal interactions in Palisan rooting performance.

Conclusion

Based on the findings of this study, the following conclusions were created:

1. The investigation explored the impact of different ANAA concentrations on the serial cutting of Palisan, with particular emphasis on T2 (1000 ppm), which demonstrated notable enhancements in root number (mean count: 3.47), root length (mean count: 1.61), and leaf count (mean count: 0.6333). These results suggest a positive association between ANAA concentration and the vegetative growth of Palisan, indicating the potential efficacy of hormone treatments in enhancing rooting performance.
2. Despite variations in ANAA concentrations, the study found no significant differences in the number of Palisan roots across treatment groups. Mean counts for root number (0.9336), root length (0.8575), and leaf count (0.2561) exceeded the 5% significance level, suggesting that ANAA treatments did not lead to statistically significant changes in root formation. This indicates that factors beyond ANAA concentration may influence Palisan root development.
3. The absence of a significant relationship between hormone concentration and Palisan root performance underscores the intricate nature of plant hormonal interactions and root development processes. While ANAA treatments may have impacted certain aspects of vegetative growth, their effects on root formation were not statistically significant. Further research is warranted to elucidate the underlying mechanisms governing Palisan root development and the potential role of hormonal treatments.
4. Among the tested ANAA concentrations, T2 (1000 ppm) emerged as the most promising treatment, exhibiting the highest mean counts for both root number (3.47) and root length (1.61). In comparison to other concentrations (T1, T3, and T4), T2 consistently demonstrated superior rooting performance. These findings suggest that moderate ANAA concentrations may be optimal for promoting Palisan root growth, highlighting the importance of hormone concentration in vegetative propagation techniques.

Recommendation

Based on the findings and limitations of the study, several recommendations are proposed to further research and enhance the propagation methods for *A. cumingiana* and Palisan planting materials:

Firstly, additional research should be undertaken to verify the effect of Alpha Naphthalene Acetic Acid (ANAA) and the quantity of nodal cuttings on the rooting efficiency and shoot count of *A. cumingiana*. This would involve exploring the optimal concentration of ANAA and determining the ideal number of nodal cuttings to maximize propagation success;

Secondly, investigating different propagation mediums could enhance the juvenile cutting-based regeneration of high-quality Palisan planting materials by identifying substrates that promote better root development and overall plant vigor. Additionally, extending the observing period to four, six, and eight months would allow for a more comprehensive evaluation of the Palisan cuttings' ability to root, providing valuable insights into long-term viability;

Furthermore, examining other commercially available organic rooting hormones may offer alternative options for improving root formation in Palisan cuttings, thereby enhancing propagation efficiency. Lastly, implementing a propagation system that incorporates misting, particularly with ANAA hormone at a concentration of 1000 ppm, can optimize conditions for root development in *A. cumingiana*, leading to increased production of *A. cumingiana* species for various applications.

These recommendations aim to advance propagation techniques and contribute to the sustainable management of these plant species.

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