JOURNAL OF DEGRADED AND MINING LANDS MANAGEMENT
Volume 11, Number 4 (July 2024):6081-6088, doi:10.15243/jdmlm.2024.114.6081
ISSN: 2339-076X (p); 2502-2458 (e), www.jdmlm.ub.ac.id

Research Article

Vegetative propagation of Bagoadlau (*Xanthosthemon philippinensis* Merr.) using indolebutyric acid

Romel S. Romano*, Shierel F. Vallesteros, Romnick L. Pascua

College of Forestry, Environment and Resources Management. Nueva Vizcaya State University, Bayombong Bayombong, Nueva Vizcaya Philippines

*corresponding author: romelsromano@gmail.com

Article history:
Received 13 February 2024
Revised 24 March 2024
Accepted 17 April 2024

Keywords:
indolebutyric acid
stem cuttings
Xanthosthemon philippinensis

Abstract

The research investigated the vegetative propagation of Bagoadlau (*Xanthosthemon philippinensis* Merr.) stem cuttings using different concentrations of indolebutyric acid (IBA). Pretreated cuttings were planted in polyethylene bags and observed in a fully covered chamber for 45 days. The study employed a single trial of completely randomized design (CRD) with five treatments (500 ppm, 1,000 ppm, 1,500 ppm, and 2,000 ppm), each replicated four times. Results indicated significant variations among treatment means in terms of percent shooting, shoot length, percent rooting, number and length of adventitious roots, and percent callusing. Notably, 500 ppm and 1,500 ppm demonstrated the highest outcomes in percent shooting, shoot length, percent survival, measurement of adventitious roots, count of roots, and percentage of rooting. In contrast, the survival percentage of T2 (500 ppm) displayed the highest rate and consistent performance compared to other treatments. Hence, the study recommends using 500 ppm and 1,500 ppm for effective rooting of Bagoadlau (*Xanthosthemon philippinensis* Merr.) stem cuttings. The successful propagation of native species like Bagoadlau is particularly relevant to ecosystem restoration efforts in regions marked by land degradation and mining activities. The study addresses several crucial aspects by successfully establishing Bagoadlau on such lands. This includes the restoration of native biodiversity, improved soil, and water quality through stabilizing soil, preventing erosion, and acting as a natural filtration system.

To cite this article: Romano, R.S., Vallesteros, S.F. and Pascua, R.L. 2024. Vegetative propagation of Bagoadlau (*Xanthosthemon philippinensis* Merr.) using indolebutyric acid. Journal of Degraded and Mining Lands Management 11(4):6081-6088, doi:10.15243/jdmlm.2023.114.6081.

Introduction

Forests are the last frontier of our nature reserve because they serve as the shield and cushions against the global effects of climate change as well as the buffer of untapped mineral resources. As highlighted by FAO, forests can act as carbon sinks or sources of carbon at different times; they also hold vast natural reserves of untouched mineral resources.

Implementing and safeguarding forest and tree plantations can enhance carbon sequestration in both aboveground and belowground biomass, thus contributing to the preservation of the natural mineral resource landscape. Trees play a vital role in the ecological system by serving as essential carbon storage and sequestration elements. They accomplish this by capturing and utilizing carbon in physiological processes like photosynthesis. Additionally, trees contribute to maintaining regular biogeochemical cycles, which regulate the equilibrium of beneficial and detrimental mineral components within a natural landscape.

In the Philippines, ecologically important tree species, such as threatened and endemic greatly contribute to the landscape stability, ecosystem sustainability, and biodiversity. Protection and conservation of endemic and threatened species are highly regarded in a sustainable and responsible mining operation. Therefore, the species long-term effect on the co-existence of mining and sustaining forest ecosystem and diversity is significant.

Bagoadlau (*Xanthostemon philippinensis*) is one of the threatened and endangered species in the Myrtaceae family and exists only in a few fragmented populations from the southwestern part of Luzon Island, Philippines.

It is similar to the Mangkono or Philippine ironwood; it is also native to the Visayan, Palawan, and northeastern Mindanao islands. Renowned for its robust and exceptionally durable wood, this plant species faces the threat of habitat loss. *Xanthostemon philippinensis* can attain a height of up to 33 feet and a maximum diameter of approximately 3.8 feet (1.2 meters). According to Fernando et al. (2008), species that thrive in ultramafic forests often have morphological modifications that enable them to endure harsh environmental conditions by reducing their water needs and acquiring nickel and magnesium.

Utilizing vegetative (clonal) propagation by rooting stem cuttings presents an alternative method for propagating endangered tree species, thereby facilitating their rapid multiplication. Cloning holds the possibility of launching a revolution in tree improvement that will significantly impact silviculture, forest policy, harvesting of the forest, and timber consumption. Conventional techniques include grafting and rooting. Newer techniques for cloning include cell, tissue, and organ culture. The aim is to generate and encapsulate embryoids from cell culture to generate high clonal fidelity artificial seeds. Wherever forests are maintained as renewable resources, it appears likely that the switch to clonal forestry will happen swiftly.

Cloning of tree species using vegetative propagation is an interesting aspect to study when considering responsible mining. It is important to assess the performance of trees under cloning techniques to know the possibility of re-planting such threatened species after being affected by deforestation caused by mining operations, which will ameliorate at least the conservation of tree diversity and the ecosystem stability of the affected landscape. There were many scientific publications on vegetative propagation for forest improvement and conservation, but there are few that consider the responsibility of mining operations in preserving the affected tree species. Therefore, the present study took the initiative to pioneer the issue.

Materials and Methods

The experiment took place in Bayombong, Nueva Vizcaya, where the humidity measured 69%, and the temperature hovered around 27°C. Observations on rooting and early growth performance were conducted over a span of 45 days, spanning from November 2022 to January 2023. Employing a completely randomized design (CRD), the experiment involved five treatments replicated four times. Each experimental unit comprised 8 stem cuttings, totaling 40 stem cuttings per treatment and 160 cuttings across 20 experimental units.

Treatment modalities employed

The Bagoadlau (*X. philippinensis*) cuttings underwent treatment with different concentrations of IBA (T1 = control, T2 = 500 ppm, T3 = 1,000 ppm, T4 = 1,500 ppm, and T5 = 2,000 ppm). The specific description of each treatment is presented in Table 1. The layout of the study is shown in Figure 1.

Table 1. Description of treatments.

Treatments	Description
T1- Control	Submerged in distilled water: The stem cuttings were submerged in distilled water for a duration of 1 hour prior to planting them in polyethylene bags and positioning them within a sealed chamber.
T2	500 ppm IBA concentration: The stem cuttings were immersed in a 500 ppm indolebutyric acid solution for 1 hour prior to being planted in polyethylene bags and positioned within a sealed chamber.
T3	1,000 ppm IBA concentration: The stem cuttings were immersed in a 1,000 ppm indolebutyric acid solution for 1 hour prior to being planted in polyethylene bags and positioned within a sealed chamber.
T4	1,500 ppm IBA concentration: The stem cuttings were immersed in a 1,500 ppm indolebutyric acid solution for 1 hour before being planted in polyethylene bags and positioned within a sealed chamber.
T5	2,000 ppm IBA concentration: The stem cuttings were immersed in a 2,000 ppm indolebutyric acid solution for 1 hour prior to planting them in polyethylene bags and positioning them within a sealed chamber.

1 T5R1	2 T1R1	3 T4R1	4 T4R3	5 T1R2
6 T1R3	7 T3R3	8 T2R2	9 T2R3	10 T5R2
11 T2R4	12 T5R4	13 T4R4	14 T5R3	15 T1R4
16 T4R2	17 T3R2	18 T2R1	19 T3R4	20 T3R1

Figure 1. Layout of the study.

Remarks: T1 (control) = soaked in distilled water, T2 = soaked 500 ppm indolebutyric acid concentrations, T3 = soaked 1,000 ppm indolebutyric acid concentrations, T4 = soaked 1,500 ppm indolebutyric acid concentrations, and T5 = soaked 2,000 ppm indolebutyric acid concentrations.

Soil media preparation

The soil mixture comprised of sandy soil, garden soil, and loam soil in equal proportions (1:1:1/2). To guarantee sterility, the prepared soil was sun-dried for twelve hours.

Preparation of rooting hormone

A solution was prepared by dissolving 2 grams of IBA powder in 5 mL NaOH within a 1,000 mL volumetric flask. As a result, the components mentioned above were meticulously blended, followed by adding one liter of distilled water (Benabise et al., 2021). After thoroughly preparing stem cuttings from Bagoadlau (*X. philippinensis*), the basal region of each bundled cutting, measuring at least 1.0 cm in length, was subjected to immersion in various rooting concentrations for one hour. It is important to note that this process did not include the control group submerged in distilled water.

Stem cuttings preparation

Two-node cuttings were carefully obtained from the apical shoot of the matured secondary stem of Bagoadlau (*X. philippinensis*) early in the morning to minimize moisture loss through transpiration. To prevent desiccation and dust collection, the uniform slices were submerged in a bucket of tap water. The leaves were vertically clipped in half to minimize excessive transpiration and facilitate insertion into the rooting medium. The cuttings underwent a one-hour treatment with a fungicide to prevent the formation of bacteria and fungi.

Collection of data

The following metrics were assessed: number of shootings, shoot length, number and length of adventitious roots, number of calluses, number of leaves, and survival rate. Each parameter was measured for individual cuttings. Shoots, roots, leaves, and calluses were counted separately. The shoot length measurement was conducted using a ruler, with measurements taken from the base to the tip of the shoot. Similarly, root length was determined by measuring the distance from the origin site to the roots' tip. The survival rate calculation was limited to stem cuttings exhibiting roots and shoots as survivors.
The following formulas were employed:

Percent Shooting

$$\%\text{Shooting} = \frac{\text{No. of Stem Cuttings with Shoots}}{\text{Total number of planted stem cuttings}} \times 100$$

Percent Rooting

$$\%\text{Rooting} = \frac{\text{No. of Stem Cuttings with Roots}}{\text{Total number of planted stem cuttings}} \times 100$$

Percent Survival

$$\%\text{Survival} = \frac{\text{No. of Cuttings of Shoots and Roots}}{\text{Total number of planted stem cuttings}} \times 100$$

Percent Callusing

$$\%\text{Callusing} = \frac{\text{No. of Stem Cuttings with Callus}}{\text{Total number of planted stem cuttings}} \times 100$$

Statistical analysis

The STAR software was employed to assess significant variations in the rooting capability and initial growth performance of Bagoadlau (*X. philippinensis*) across different IBA concentrations. Additionally, the Duncan Multiple Range Test (DMRT) was applied to further scrutinize the mean values of statistically significant parameters.

Results and Discussion

Percentage of survival

The study findings indicated that T2 (500 ppm) exhibited the highest survival rate at 43.75%, with T4 (1,000 ppm) following closely (Figure 2). This result is consistent with a study by An et al. (2018) on guava, where cuttings treated with 500ppm IBA concentration showed a survival rate of 40.58%. This could be attributed to the vegetative propagation process, where the plant generated more shoots, roots, and leaves per cutting by allocating the majority of its resources to the leaf buds. Leaf buds serve as significant sites for natural auxin production, essential for both photosynthesis and respiration (Wahab et al., 2001).

Percentage of shooting

Regarding shooting percentage, T2 (500 ppm) exhibited the highest response at 81.3%, followed by T1 (control) at 62.5%, T4 (1,500 ppm) at 37.5%, T5 (2,000 ppm) at 37.5%, and T3 (1,000 ppm) registering the lowest shooting percentage (Figure 3). Notably, compared to the control treatment, IBA concentrations notably increased shooting percentage. This underscores the role of auxins in stimulating root and shoot growth across various plant species (Wu et al., 2007). The Duncan Multiple Range Test (DMRT) indicates a statistically significant difference in means, with T2 (500 ppm) exhibiting the highest significant difference.

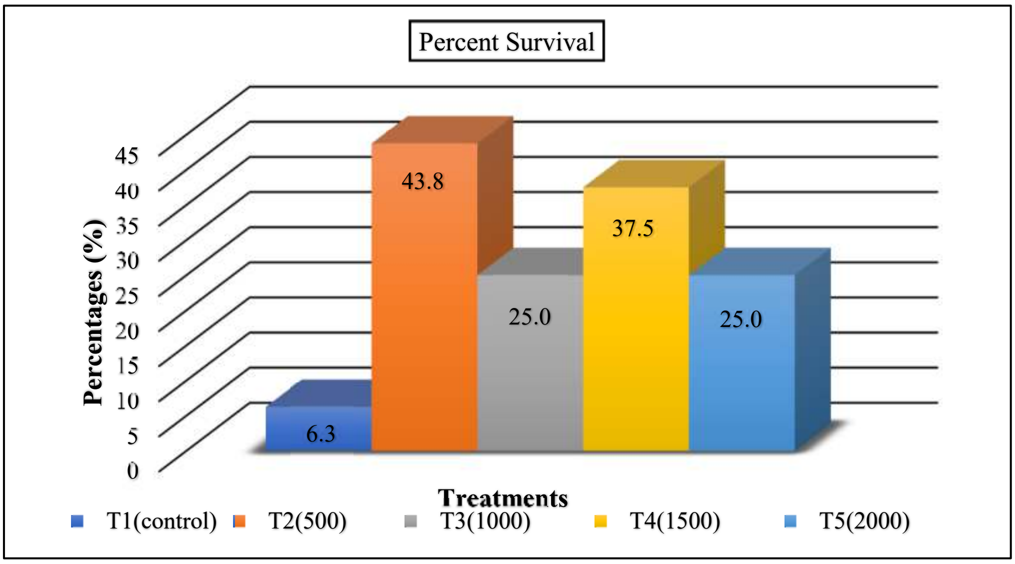

Figure 2. Percentage of survival of *Bagoadlau* (*X. philippinensis*).

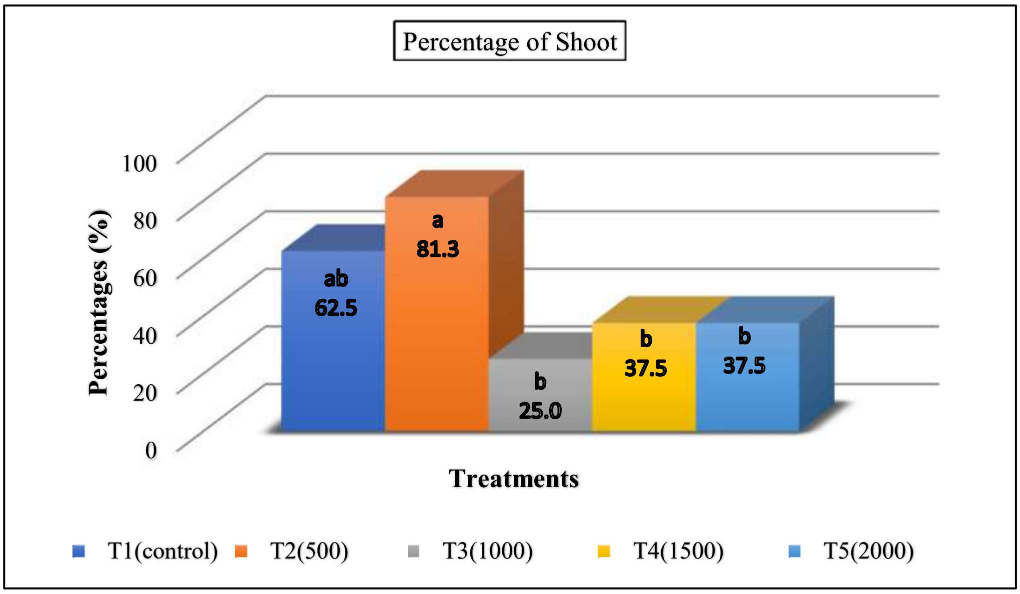

Figure 3. Percentage of shooting of *Bagoadlau* (*X. philippinensis*).

Percentage of rooting

The highest proportion of rooted cuttings was observed at 1,500 ppm (T4) and 1,000 ppm (T3) IBA concentrations, indicating an increase in rooting ability with higher IBA concentrations (Figure 4). Thus, IBA concentration has the potential to enhance root development. Prior research conducted by Nandi et al. (2002) and Sati et al. (2019) has demonstrated the efficacy of IBA in promoting root development in stem cuttings of *Cedrus deodara* and *Ginkgo biloba*, respectively. According to Rahman et al. (2002), the impact of auxin on root development can be ascribed to its function in facilitating cellular division inside the vascular cambium, resulting in the generation of root primordial cells. The significant increase in rooting percentage observed in T4 (1,500 ppm) appears contradictory to the findings of An et al. (2018), where the application of IBA was noted to inhibit root development in hardwood cuttings, with the highest efficacy achieved at 1,500 ppm. Nevertheless, overall, applying IBA to stem cuttings tended to result in a higher root production compared to untreated controls. The discovery above aligns with the findings of Aziz et al. (2020), whose study investigated the impact of different concentrations of IBA on black mulberry hardwood cuttings. The study demonstrated that IBA concentrations were more effective in promoting root development in black mulberry hardwood cuttings compared to untreated cuttings.

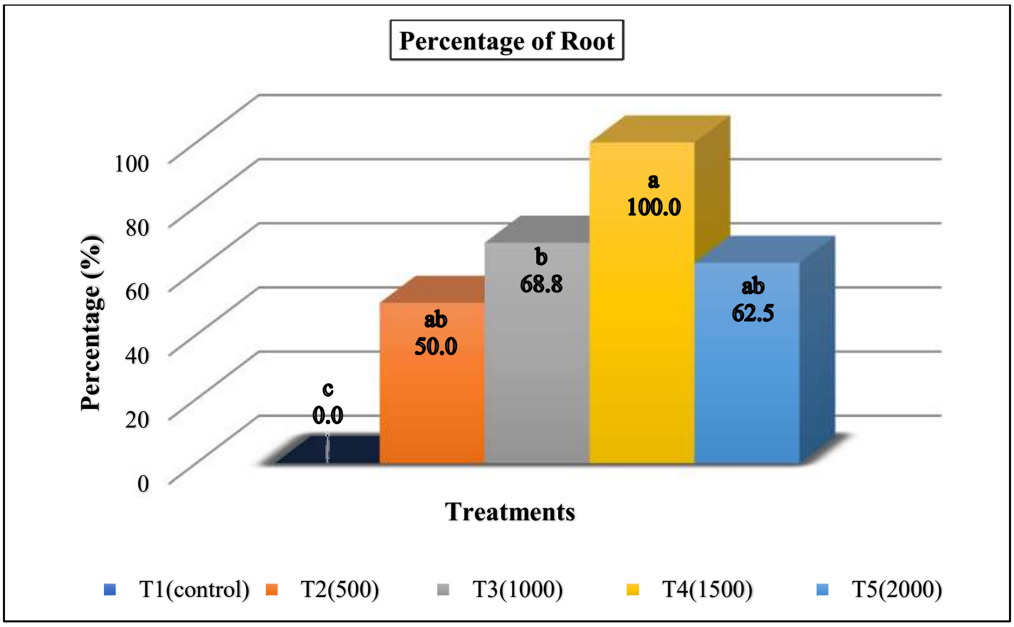

Figure 4. Percentage of rooting of *Bagoadlau* (*X. philippinensis*).

Shoot length

T2 (500 ppm) demonstrates the longest shoot length among the treatments at 32.5 cm, followed by T1 (control) at 25.8 cm, T5 (1,000 ppm) at 20.3 cm, T3 (1,000 ppm) at 11.6 cm, and T4 (1,500 ppm) at 8.8 cm, respectively (Figure 5). This result can be contrasted with the conclusions of Benabise et al. (2021), who found that different concentrations of IBA had no significant impact on the shoot length generated by cuts. Nevertheless, the cuttings subjected to 2,000 ppm of IBA (14.75mm) and 1,000 ppm IBA (12.52 mm) exhibited the shortest length, while those treated with 500 ppm IBA yielded the longest shoots (8.81mm).

Adventitious root length

The adventitious root length is shown in Figure 6. These findings are consistent with the results reported by Smith et al. (2020) and An et al. (2018). More precisely, the research revealed that the roots exhibited the greatest length in T4 (1,500 ppm) and T5 (2,000 ppm) compared to lower concentrations of IBA. The observation above aligns with the research conducted by An et al. (2018), wherein they observed a substantial increase in the mean number of roots per cutting when IBA was applied. However, no significant effect was observed on the mean root length in hardwood cuttings. The cumulative results presented in this study offer further substantiation for the notion that exogenous IBA has the potential to stimulate the growth of adventitious roots while maintaining their average lengths.

Number of adventitious roots

More adventitious roots were observed in T4 (1,500 ppm) with 2.94 roots and T5 (2,000 ppm) with 2.13 roots, compared to lower IBA concentrations (500 ppm and control treatment) (Figure 7). This could be due to the action of IBA, which facilitates the hydrolysis and transport of carbohydrates and nitrogenous substances near the base of cuttings, leading to accelerated cell elongation and division under favorable climatic conditions. This is similar to the role of plant hormones, including auxins like IBA, in growth, development, and responses to environmental cues, which provides a comprehensive understanding of the cellular mechanisms involved in plant hormone actions, as Davies mentioned in his Plant Hormone book in 2010.

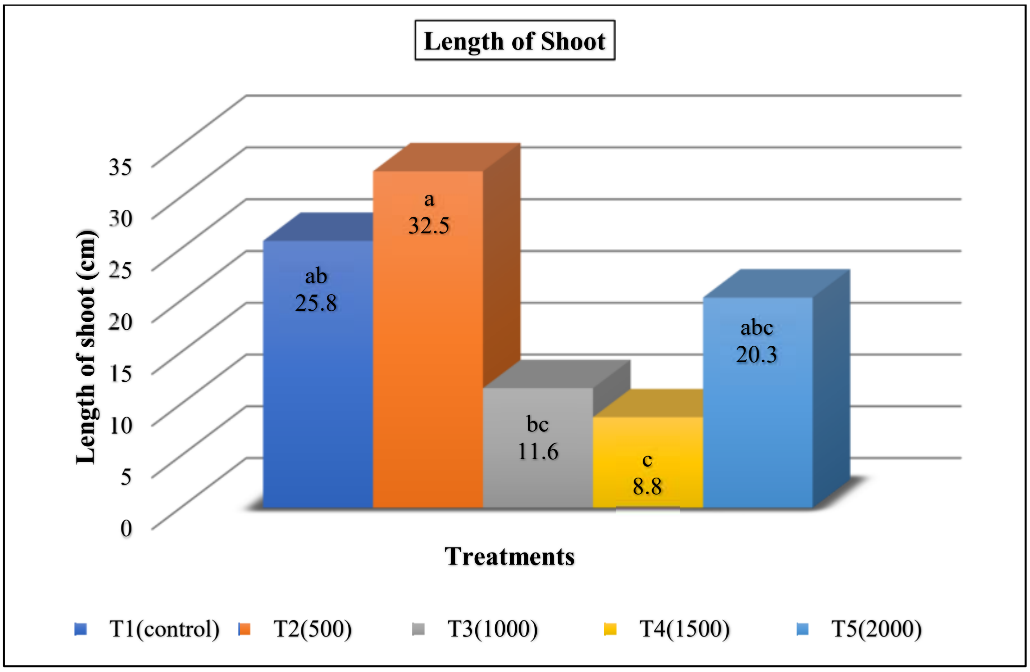

Figure 5. Length of shoots of *Bagoadlau* (*X. philippinensis*).

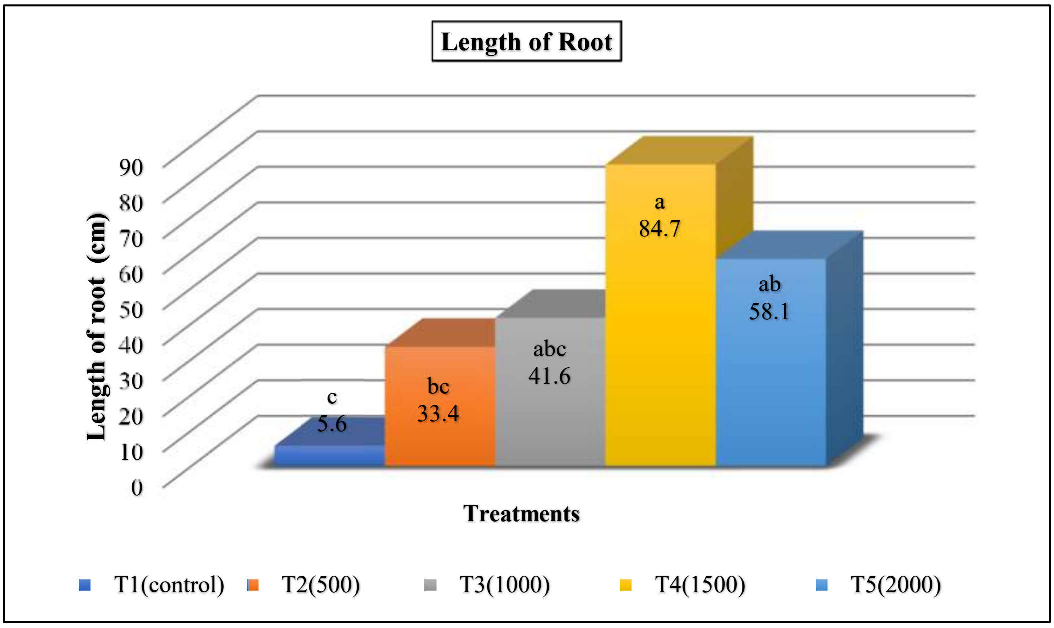

Figure 6. Length of roots of *Bagoadlau* (*X. philippinensis*).

Percentage of callusing

The study revealed that the highest IBA concentration (2,000 ppm) in T5 induced 93.8% callus formation in Bagoadlau (*X. philippinensis*) (Figure 8), consistent with Kordzadeh and Hassan (2021) findings on *Prunus amygdalus* X *Prunus persica*, indicating the positive impact of auxin and H_2O_2 treatments on callus development and rooting of cuttings. This underscores the significance of internal auxin levels in callus formation and cutting root development across plant species, which coincides with the study of Phillips (2010), which delves into the role of auxin in callus formation and rooting of cuttings in various plants.

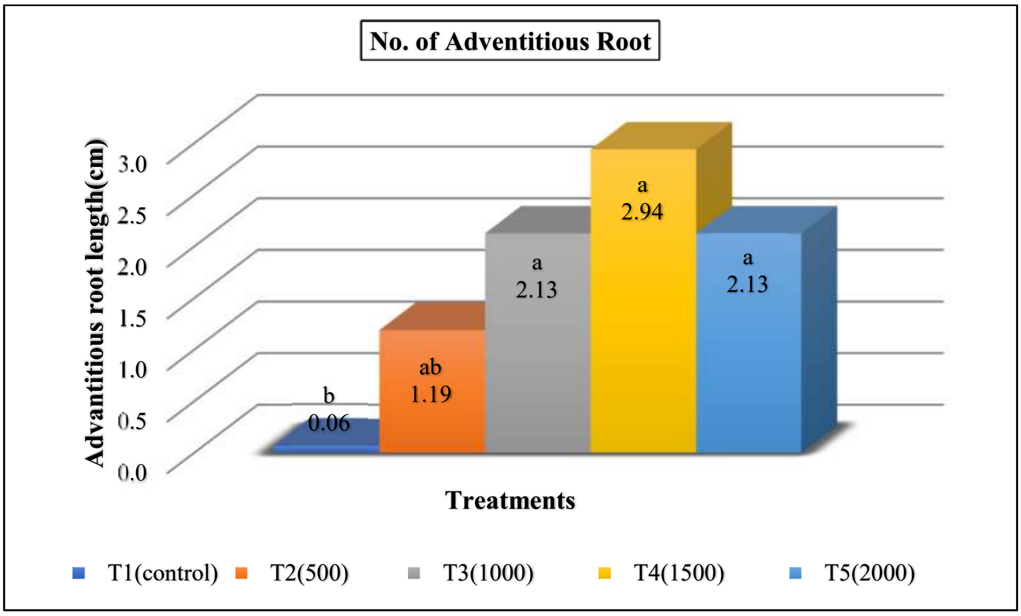

Figure 7. A number of adventitious roots of *Bagoadlau* (*X. philippinensis*).

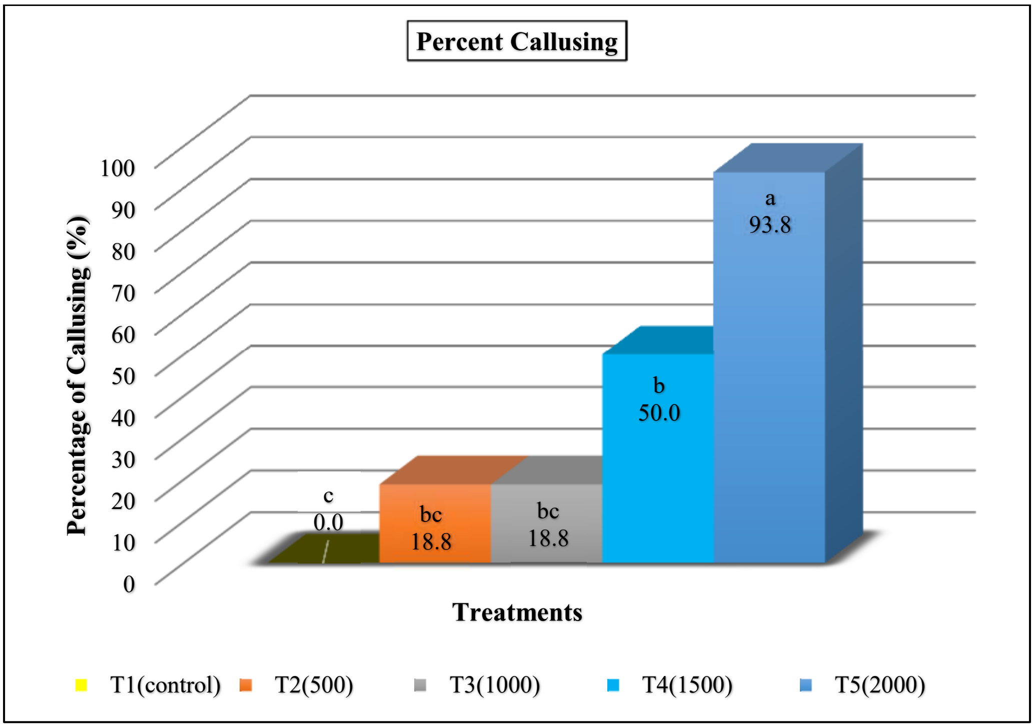

Figure 8. Percentage of callusing of *Bagoadlau* (*X. philippinensis*).

Summary of analysis of variance for Bagoadlau (X. philippinensis)

The analysis of variance revealed significant differences among treatments for percent rooting, number of adventitious roots, length of roots, and percent callusing in Bagoadlau (*X. philippinensis*), as indicated by p-values below the 0.01 level of significance (Table 2). Similarly, significant differences were observed for the length of shoots and percent shooting across treatments, with p-values below the 0.05 level of significance. However, no significant differences were found for percent survival among treatment means, as the p-value exceeded the 5% level of significance. These findings are in accordance with the principles and practices of plant propagation, including factors influencing rooting, callusing, shooting, and survival, as outlined by Hudson et al. (2014).

Table 2. Summary of analysis of variance for Bagoadlau (*X. philippinensis*).

Parameters	Percent Callusing	Percent Survival	Length of shoot	Length of root	No. Adventitious root	Percent Shooting	Percent Root
T1 (control)	0.00^{c}	6.25	25.78^{ab}	5.62^{c}	0.06^{b}	62.5^{ab}	0.00^{c}
T2 (500)	18.75^{bc}	43.75	32.50^{a}	33.44^{bc}	1.19^{ab}	81.25^{a}	50.00^{ab}
T3 (1,000)	18.75^{bc}	25.00	11.56^{bc}	41.56^{abc}	2.12^{a}	25.00^{b}	68.80^{b}
T4 (1,500)	50.00^{b}	37.50	8.75^{c}	84.69^{a}	2.94^{a}	37.50^{b}	100.00^{a}
T5 (2,000)	93.75^{a}	25.00	20.31^{abc}	58.12^{ab}	2.12^{a}	37.50^{b}	62.50^{ab}
F value	20.82**	1.89^{ns}	4.09*	7.43**	7.07**	3.60*	16.45**
P value	0.0000	0.1636	0.0194	0.002	0.0024	0.0297	0.0001
Interpretation	Highly significant	Not Significant	Significant	Highly significant	Highly significant	Significant	Highly significant

**-significant at 0.01, *-significant at 0.05.

Conclusion

Based on the investigation data, the researchers reached the conclusion that the rooting capacity and initial growth of Bagoadlau (X. *philippinensis*) stem cuttings were considerably influenced by different concentrations of IBA. Among the tested concentrations, 500 ppm and 1,500 ppm of IBA yielded optimal results in terms of shooting percentage, shoot length, survival percentage, length and number of adventitious roots, and rooting percentage. Hence, it is strongly recommended to utilize 500 ppm and 1,500 ppm as rooting hormones for the vegetative propagation of Bagoadlau (*X. philippinensis*).

Acknowledgments

The authors thank the Nueva Vizcaya State University, Bayombong, Philippines, for supporting this study.

References

An, H., Meng, J., Xu, F., Jiang, S., Wang, X., Shi, C. and Zhang, X. 2018. Rooting ability of hardwood cuttings in highbush blueberry (*Vaccinium corymbosum* L.) using different indole-butyric acid concentrations. HortScience 54(2):194-199, doi:10.21273/ hortsci13691-18.

Aziz, R.R., Mohammed, A.A. and Ahmad, F.K. 2020. Effect of IBA concentration and water soaking on rooting hardwood cuttings of black mulberry (*Morus nigra* L.). *Journal of Zankoy Sulaimani - Part A* 22(1):153-158, doi:10.17656/jzs.10781.

Benabise, E.V., Quinan, J.J. and Carig, J.G. 2021. Response of 'Bignay' [*Antidesma bunius* (Linn.) Spreng] to cutting origins, IBA and BioGroe treatments. *Plant Science Today* 8(3):596-603, doi:10.14719/pst.2021.8.3.1063.

Fernando, E.S., Suh, M.H., Lee, J. and Lee, D.K. 2008. Forest formations of the Philippines. ASEAN-Korea Environmental Cooperation Unit. ISBN: 8992239408, 9788992239400.232 pages.

Hudson, L.N., Newbold, T., Contu, S., Hill, S.L.L., Lysenko, I., De Palma, A., Phillips, H.R.P……. and Purvis, A. 2014. The database of the PREDICTS (Projecting Responses of Ecological Diversity in Changing Terrestrial Systems) project. *Ecology and Evolution Ecology and Evolution* 7(1):145-188, doi:10.1002/ece3.2579.

Kordzadeh, S. and Hassan, S. 2021. Effect of different concentrations of indole butyric acid, putrescine and hydrogen peroxide on stem cuttings of the rootstock GF677 (*Prunus amygdalus* × *Prunus persica*) according to the cutting season. *Revista Facultad Nacional de Agronomía Medellín* 74(2):9571-9582, doi:10.15446/rfnam.v74n2.92414.

Nandi, S.K., Tamta, S. and Palni, L.M.S. 2002. Adventitious root formation in young shoots of *Cedrus deodara*. *Biologia Plantarum* 45(3):473-476, doi:10.1023/A:1016298524177.

Phillips, A.L. 2010. Genetic and transgenic approaches to improving crop performance. In: Davies, P.J. (ed.) *Plant Hormones*. Springer, Dordrecht, doi:10.1007/978-1-4020-2686-7_27.

Rahman, A., Hosokawa, S., Oono, Y., Amakawa, T., Goto, N. and Tsurumi, S. 2002. Auxin and ethylene response interactions during Arabidopsis root hair development dissected by auxin influx modulators. *Plant Physiology* 130(4):1908-1917, doi:10.1104/pp.010546**.**

Sati, P., Dhyani, P., Bhatt, I.D. and Pande, A. 2019. *Ginkgo biloba* flavonoid glycosides in antimicrobial perspective with reference to extraction method. *Journal of Traditional and Complementary Medicine* 9(1):15-23, doi:10.1016/j.jtcme.2017.10.003.

Smith, A.D., George, N.S., Cheung, L., Bhagavathy, G.V., Luthria, D.L., John, K.M. and Bhagwat, A.A. 2020. Pomegranate peel extract reduced colonic damage and bacterial translocation in a mouse model of infectious colitis induced by *Citrobacter rodentium*. *Nutrition Research* 73:27-37, doi:10.1016/j.nutres.2019.11.001.

Wahab, F., Nabi, G., Nawab, A.M. and Shah, M. 2001.Rooting response to semi-hardwood cuttings of guava (*Psidiuim guajava* L.) to various concentrations of different auxins. *Journal of Biological Science* 1:184-187, doi:10.3923/jbs.2001.184.187.

Wu, G., Lewis, D.R. and Spalding, E.P. 2007. Mutations in Arabidopsis multidrug resistance-like ABC transporters separate the roles of acropetal and basipetal auxin transport in lateral root development. *The Plant Cell* 19(6):1826-1837, doi:10.1105/tpc.106.048777.