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Influence of Rhizomal Buds and Soaking Time in ANAA on the Growth Characteristics of Thai Ginger (*Alpinia galanga* (L.) Willd.)

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Abstract The study investigated the growth characteristics of *Alpinia galanga* (L.) Willd. and their response to varying numbers of rhizomal buds and soaking time in Alpha-naphthalene acetic acid (ANAA). The experiment was designed as a two-factor study following a Randomized Complete Block Design (RCBD) with three replications. Factor A involved different numbers of rhizomal buds, categorized as follows: A_1 - one bud; A_2 - two buds; and A_3 - three buds. Factor B examined the soaking time in ANAA, with the following treatments: B_1 - direct planting (control); B_2 - 30 minutes; B_3 - 60 minutes; and B_4 - 90 minutes. The findings indicate that the number of rhizomal buds and soaking time did not significantly affect the growth performance of Thai ginger at 30, 60, 90, and 120 days after planting, except for specific instances. Notably, the height of the plants at 30 and 120 days, rhizome bud formation, and survival percentage at 120 days were significantly influenced by soaking time in ANAA. The study suggests that Thai ginger plants with three rhizome buds and those directly planted exhibited the best growth performance. Importantly, there was no significant interaction effect observed between the different numbers of rhizomal buds and soaking time in ANAA on the growth characteristics of *Alpinia galanga.* The investigation revealed that the influence of rhizomal buds and soaking time in ANAA on the growth and development of *A. galanga* was independent for each factor, offering insights into optimizing its cultivation.

Keywords Alpha-Naphthalene Acetic Acid (ANAA), *Alpinia galanga*, Rhizomal Buds, Soaking Time, Thai Ginger

1. Introduction

Alpinia galanga (L.) Willd. is a perennial aromatic herb known for its showy flowers, belonging to the *Zingiberaceae* family. This plant is primarily found in India and Southeast Asia, and it holds significant importance in both the pharmaceutical and food industries [1]. *A. galanga* is employed to treat a range of ailments, including throat infections, ulcers, bad breath, fever, whooping cough, bronchial catarrh, and rheumatism [1]. Its rhizomes have demonstrated antibacterial [2], antifungal [3], anti-inflammatory [4], anti-diabetic [5], and antioxidant properties [6]. Moreover, the phytochemical constituents of *A. galanga* are harnessed for their antiulcer [7] and antitumor activities [8].

This plant is known by various names such as Thai ginger, greater Java, and Siamese ginger. Two closely related species were properly propagated like A. officinarum Hance and A. calcarata Rosc., both of which are known as lesser galangal [9]. *A. galanga* relies on rhizomes for vegetative propagation, yet this method proves insufficient to satisfy its commercial demand.

Conventional breeding techniques encounter challenges in rhizomatic plants, primarily due to the absence of seed production [10]. Most of the propagation techniques employed in the production are rhizomal cutting. Macropropagation under *Zingiberaceae* family is through the conventional method. Rhizomes develop as dense, succulent underground root structures that spread horizontally just below the soil's surface. In contrast, tubers consist of thick portions of stems or roots. As new rhizomes and tubers form, the plants connected to the rhizomes extend outward, while smaller roots develop to secure them in the soil. Dividing these structures presents a cost-effective method for expanding production [11]. Increasing the number of explants, the application of rooting hormones using auxins is used to promote the development of roots. Commercially, Alpha-naphthalene acetic acid (ANAA), a kind of synthetic auxin is commonly used to increase the percentage of rooting on cuttings. Conversely, while many plants don't require the use of rooting hormones, the application of auxins tends to accelerate and standardize root formation in these plants. Mass propagation of these plants is needed to secure their availability to industry.

Alpinia galanga is a very important herbaceous plant used in culinary as aromatics and has medical potential. This plant is still understudied. There was limited information on how to propagate these plants given their importance and scientific knowledge in the medical world. Hence, the study aims to determine the influence of rhizomal buds and soaking time in ANAA on the growth characteristics of Thai ginger (*Alpinia galanga* (L.) Willd.). Specifically, it aimed to determine the growth performance of *Alpinia galanga* (L.) Willd. in terms of the number of rhizomal bud formation, height (cm) of plants, and survival rate (%) of explants at 30, 60, 90 and 120 DAP. Determine the growth characteristics of *A. galanga* as influenced by soaking time in ANAA and find out the interaction effect between the number of rhizomal buds and soaking time in ANAA on the growth of Thai ginger. There is severe shortage in the production in other countries such as Thailand, China, and India. Results of the study will provide relevant information on the growth performance as influenced by ANAA that may contribute to the mass production that could meet the demand of the *Alpinia galanga* (L.) Willd. locally and internationally.

2. Materials and Methods

2.1. Materials

The materials, tools and equipment used in the study were the following: rhizomal buds of *Alpinia galanga* (L.) Willd., commercial Alpha-naphthalene acetic acid (ANAA), distilled water, beaker, carbonized rice hull, garden soil, vermicompost, polyethylene bags, hand trowel, knife, water sprinkler, meter stick and record book.

2.2. Methods

Experimental treatments. The study consisted of two factors such as: number of rhizomal buds (Factor A) and soaking time (Factor B). Factor A has three treatments such as: one bud (A_1), two buds (A_2), and three buds (A_3). The different soaking times using ANAA solution were composed of four treatments like: direct planting (B_1), 30 min (B_2), 60 min (B_3), and 90 min (B_4), respectively.

Experimental design and layout. The factorial experiment was laid out in a Randomized Complete Block Design (RCBD) with twelve treatment combination and three replications. Each treatment combination has 30 sample plants subdivided into three replications. The experimental layout is shown in Figure 1.

BLOCK

I	II	III
A_2B_4	A_3B_3	A_2B_3
A_1B_4	A_3B_2	A_1B_3
A_1B_3	A_2B_1	A_2B_4
A_2B_3	A_1B_1	A_1B_2
A_2B_2	A_3B_4	A_2B_1
A_3B_1	A_1B_3	A_3B_1
A_3B_3	A_2B_4	A_2B_2
A_1B_1	A_3B_1	A_3B_2
A_3B_4	A_1B_4	A_3B_3
A_2B_1	A_2B_3	A_1B_4
A_1B_2	A_2B_2	A_1B_1
A_3B_2	A_1B_2	A_3B_4

Figure 1. Field layout of the study with treatments arranged in a Factorial Randomized Complete Block Design (RCBD)

2.3. Weather Condition of the Study Site

Throughout the study, the weather conditions remained consistent, with daytime temperatures averaging around 30 ℃, while nighttime temperatures felt cooler at approximately 25 ℃. Over the years, Banga typically receives an average of 111.5 mm of rainfall, and the humidity levels stay close to 81%, according to data from [12].

Legend:

Factor A: Number of Rhizomal Bud

A_1 – One
A_2 – Two
A_3 – Three

Factor B: Soaking Time (Minutes)

B_1 – Control (Direct planting)
B_2 – 30
B_3 – 60
B_4 - 90

2.4. Nursery Management Practices

2.4.1. Land Preparation

The land was prepared through flattening the soil to achieve a fine tilth that ensured proper placement of pots.

2.4.2. Collection of Planting Materials

Rhizomes were consistently gathered from a mature *Alpinia galanga* plant that was 12 months old in Altavas, Aklan. Altavas, Aklan is situated at sea level, with a Tropical monsoon climate. The average annual temperature in the municipality is 30.07 ℃ (86.13 ℉), which is 2.85% higher than the Philippines' overall average. Altavas usually experiences approximately 88.81 millimeters (3.5 inches) of rainfall and has 175.23 rainy days annually, accounting for 48.01% of the year [13]. These rhizomes were uniformly sized, cut, and then sorted based on the count of protruding rhizomes as part of the treatment.

2.4.3. Media Preparation

The growing medium utilized in the study was composed of carbonized rice hull, garden soil and vermicompost in a 1:1:1 ratio. The rice hulls were collected from the Aklan State University (ASU) Ranch in Banga, Aklan. Collected rice hulls were carbonized with the use of a carbonizer. The garden soils were taken from the Diversified Field Crop area of ASU, while vermicompost was bought from an accredited source outside the University. When all medium components were completely prepared, it was mixed equally following the 1:1:1 ratio. The mixed soil media were placed in a 4” x 6” polyethylene bags at two inches below the brim ready for rhizomal bud planting.

2.4.4. Soaking

Three plastic basins were prepared and filled with water then mixed with ANAA solution in a diluted ratio of 4 tablespoons per 16 liter of water as recommended in the product label. Rhizomal buds were soaked for 30 (B_2), 60 (B_3) and 90 (B_4) minutes time period considering each treatment prior to planting.

2.4.5. Planting

Each rhizomal bud corresponding to the treatments was planted in the potting bags two inches below the brim. Each bag was composed of one, two and three *A. galanga* rhizomal buds planted according to the treatment.

2.4.6. Water Management

The plants were uniformly sprinkled with water using a water sprinkler after planting. Watering was dependent on the weather condition. During sunny weather, misting was done. However, in rainy days, misting is not necessary.

2.4.7. Weeding

Manual weeding was done every week for four months to minimize weed occurrence and nutrient competition. Some weeds like false daisy *(Eclipta prostata)*, indian acalypha *(Acalypha indica)*, itchgrass *(Rottboellia cochinchinensis)* and purslane *(Portulaca oleracea)* were observed growing in between beds and pots.

2.4.8. Pest and Disease Management

Keen observation on the attack of different pests and diseases was implemented. Necessary control measures were taken immediately on planted *A. galanga* upon observation of pest presence. There were few pests like garden snail *(Cornu aspersum)*, black with yellow and white spot caterpillar, trail ridge scrub grasshopper *(Melanoplus ordwayae)* in which the researcher took some necessary actions like handpicking and crushing needed to eliminate their presence and prevent its further damage to plants. Beneficial insects like common frog *(Rana temporaria)*, soil centipede *(Geophilus vittatus)*, earthworm *(Lumbricus terrestris)*, black millipede *(Californiulus euphanus)* and ladybug (*Coccinellidae septempunctata)* were also found in the area.

2.5. Data Gathering

Data were obtained from ten representative sample plants selected randomly for the observation of growth characteristics of *A. galanga* rhizomal buds. The following data were gathered:

Rhizomal bud formation. The emerging shoot of rhizomes was counted manually one month after planting. This was done through visual observation on each sample plant.

Height of plants (cm). The height of plant was measured one month after planting for four consecutive months. The height of sample plants was measured from the base of the plant up to the tip of the last leaf with the use of a meter stick.

Survival rate (%) of explant. The survival rate of explant was determined using the formula below:

$$\text{Survival rate (\%)} = \frac{\text{Number of explants survived}}{\text{Number of explants planted}} \times 100$$

2.6. Statistical Tools and Analysis

All data gathered were analyzed using the Sirichai Statistics version 6.07. The analysis of variance (ANOVA) for a two-factor experiment arranged in Randomized Complete Block Design (RCBD) was used. Further tests for significant differences were computed using the Least Significant Difference Test (LSD) of significance.

3. Results and Discussion

3.1. Number of Rhizomal Buds Formed

The mean number of rhizomal buds formed by *A. galanga* at 30, 60, 90, and 120 DAP as influenced by the number of rhizomal buds and soaking time in ANAA is shown in Table 1. Analysis of the study revealed that the *Alpinia galanga* across time points: 30, 60, 90 days and 120 days after planting (DAP) shows no significant influence on the mean number of the rhizomal buds' formation. Specifically, at 30 DAP, the bud numbers ranging from 0.93 to 0.99 were not affected by the number of buds or the soaking time. The same trend was maintained at 60 DAP, where the number of rhizomal buds (1.06 to 1.22 shoots) and soaking time (1.10 to 1.19 shoots) did not show significant differences.

Table 1. Mean number of rhizomal buds formed of *Alpinia galanga* at 30, 60, 90, and 120 DAP as influenced by the number of rhizomal buds and soaking time in ANAA

Number of Rhizomal Buds	DAYS AFTER PLANTING (DAP)			
	30^{ns}	**60^{ns}**	**90^{ns}**	**120^{ns}**
One	0.93	1.06	1.50	2.43
Two	0.97	1.13	1.45	2.57
Three	0.99	1.22	1.61	2.47
Soaking Time (min)	**DAYS AFTER PLANTING (DAP)**			
	30^{ns}	**60^{ns}**	**90^{ns}**	**120****
Direct Planting	1.01	1.19	1.57	2.80^{a}
30	0.90	1.10	1.54	2.20^{b}
60	0.99	1.16	1.59	2.62^{ab}
90	0.94	1.10	1.38	2.33^{ab}
Interaction (NRB x ST)	ns	ns	ns	ns
CV (%)	**9.31**	**14.35**	**17.47**	**14.75**

**Highly significant; Means having a common letter are not significantly different at 1% level by LSD; nsNot significant; DAP – days after planting.

A similar pattern emerged at 90 DAP (bud range: 1.45 to 1.61) and 120 DAP (bud range: 2.43 to 2.57) with no significant impact from the number of rhizomal buds or soaking time. Importantly, there was no significant interaction effect between these factors at any of the observed time points. Essentially, the formation of rhizomal buds in *A. galanga* did not seem to depend much on the number initial number of buds and the soaking time in ANAA over the course of the experiment.

Ginger is often propagated by rhizomes, has a low growth rate (about 10-15 shoots per plant per year), and is susceptible to soil pathogens such as bacterial wilt (Pseudomonas solanacearum), soft rot (*Pythium aphanidermatum*). and nematodes. (*Meloidogyne spp*.), causing heavy yield loss [14].

According to the study conducted by Shamsudheen [15] titled "High frequency shoot multiplication of *Alpinia galanga* (L.) Willd. using Rhizome Buds", the researchers have successfully developed a rapid shoot multiplication system utilizing rhizome buds of *Alpinia galanga*. This study has established a simple and efficient method for the regeneration and propagation *of A. galanga*. It specifically investigated the roles of different cytokinins and auxins in inducing shoot and root formation from the rhizome bud explants.

3.2. Height of the Plant

The mean height of *A. galanga* at 30, 60, 90, and 120 DAP as influenced by the number of rhizomal buds and soaking time in ANAA is presented in Table 2. The investigation into the mean height of *A. galanga* at different intervals (30, 60, 90, and 120 days after planting) was meticulously conducted, exploring the influence of the initial number of rhizomal buds and the duration of ANAA soaking. Some significant results emerged out of these results. Initially, during 30 days after planting (DAP), there was no significant difference among the mean heights with two or three rhizome buds, as it varied from 10.54 cm to 12.32 cm respectively. On the other hand, there were significant differences in the duration of soaking in ANAA, the highest height was obtained of the plant soaked for 60 min (13.04 cm), but there were no statistically significant variations. In addition, there were no significant differences in bud number and soaking time in plant height at 60 DAP, as statistics show. However, a noteworthy shift occurred at 90 DAP, indicating a significant effect of the initial number of rhizomal buds on plant height (ranging from 39.81 cm for one bud to 46.45 cm for three buds).

In contrast, soaking time did not yield such an impact, and no interaction effect was identified between the two factors. Intriguingly, at the advanced stage of 120 DAP, the number of rhizomal buds did not yield a significant height variation, while the duration of ANAA soaking significantly affected plant height. Direct planting resulted in the tallest plants (56.51 cm), surpassing those soaked for 60 minutes (54.36 cm) and 90 minutes (49.39 cm), but this result was comparable with plants soaked for 30 minutes.

Notably, the interaction effect between the initial number of rhizomal buds and soaking time on plant height remained non-significant at all observed intervals. In summary, while the number of rhizomal buds exhibited varying effects on height at different growth stages, soaking time significantly influenced height at 120 DAP, and these factors operated independently, presenting opportunities for optimizing *A. galanga* growth based on bud number and ANAA soaking duration.

Table 2. Mean height (cm) of *Alpinia galanga* at 30, 60, 90, and 120 DAP as influenced by the number of rhizomal buds and soaking time in ANAA

Number of Rhizomal Bud	**DAYS AFTER PLANTING (DAP)**			
	30^ns	**60^ns**	**90***	**120^ns**
One	10.98	20.07	39.81^b	49.78
Two	10.54	21.24	43.55^ab	52.10
Three	12.32	23.79	46.45^a	54.83
Soaking Time (min)	**DAYS AFTER PLANTING (DAP)**			
	30**	**60^ns**	**90^ns**	**120***
Direct Planting	11.96^ab	23.80	46.19	56.51^a
30	10.64^ab	20.73	41.73	48.67^c
60	13.04^a	22.60	43.97	54.36^ab
90	9.46^b	19.67	41.18	49.39^bc
Interaction (NRB x ST)	ns	ns	ns	ns
CV (%)	**18.74**	**18.29**	**12.79**	**10.42**

**Highly significant; *Significant; Means having a common letter are not significantly different at 1% and 5% level by LSD; nsNot significant

Alpha naphthyl acetic acid (ANAA) functions as a synthetic auxin, akin to the natural plant hormone indole-3-acetic acid (IAA). It works by stimulating cell division and elongation of plants, which enhances root growth, improves nutrient uptake, and stimulates overall plant growth. However, factors may have influenced the performance of a crop such as optimum light, nutrient, and water requirements of *A. galanga* [16]. The growth of *A. galanga* may also be impacted by insect pests and diseases. To minimize such occurrences, it is essential to engage in proper maintenance and consistent weeding. Weeds can decrease plantation productivity, encroach upon crops, vigorously compete for essential resources like water and nutrients, and potentially attract more insects. Additionally, certain common weed species can act as host plants for numerous insect pests, leading to considerable damage to crops and reduced yield and produce quality. Employing ongoing maintenance and protective measures, such as under brushing and ring weeding, can effectively reduce weed and insect pest issues in agriculture [17].

3.3. Survival Rate of Explants

The mean survival rate of *A. galanga* explants at 30, 60, 90, and 120 DAP as influenced by the number of rhizomal buds and soaking time in ANAA is reflected in Table 3. The comprehensive investigation into the survival rate of *A. galanga* explants at various stages (30, 60, 90, and 120 days after planting) revealed several noteworthy insights regarding the influence of the initial number of rhizomal buds and the duration of ANAA soaking. The results consistently indicated that the number of rhizomal buds did not have a significant impact on the survival rate, with percentages ranging from 92.50% (one bud) to 98.25% (three buds) at both 30 and 60 DAP. This trend extended to 90 DAP, where survival rates varied from 95.00% (one bud) to 99.17% (three buds).

Table 3. Mean survival rate (%) of *Alpinia galanga* explant at 30, 60, 90, and 120 DAP as influenced by the number of rhizomal buds and soaking time in ANAA

Number of Rhizomal Buds	**DAYS AFTER PLANTING (DAP)**			
	30^ns	**60^ns**	**90^ns**	**120^ns**
One	92.50	92.50	95.00	95.83
Two	95.75	95.75	97.50	96.67
Three	98.25	98.25	99.17	98.34
Soaking Time (min)	**DAYS AFTER PLANTING (DAP)**			
	30^ns	**60^ns**	**90^ns**	**120***
Direct Planting	100.00	100.00	100.00	100.00^a
30	91.00	91.00	95.56	93.33^b
60	96.67	96.67	97.78	100.00^a
90	94.33	94.33	95.56	94.45^b
Interaction (NRB x ST)	ns	ns	ns	ns
CV (%)	**7.87**	**7.87**	**5.19**	**5.51**

*Significant; Means having a common letter are not significantly different at 5% level by LSD; nsNot significant

However, at 120 DAP, soaking time in ANAA exhibited a crucial role, significantly affecting survival rates. Plants directly planted or soaked for 60 minutes achieved the highest survival rates of 100%, while rates were lower for 30-minute (93.33%) and 90-minute soaking (94.45%). Importantly, no significant interaction effect between the number of rhizomal buds and soaking time was observed at any stage, emphasizing the independent nature of these factors on the survival rate of *A. galanga* explants. These results suggest that while the number of rhizomal buds played a consistent role in survival, particularly in the early stages, strategic adjustment of ANAA soaking time holds significant potential for enhancing survival rates, most notably evident at 120 DAP.

According to the study conducted by Miri [18] auxins used in micropropagation, callus induction and regeneration of ginger had a positive effect on in vitro root formation of ginger shoots, thus, increasing the chance of survival. According to Tiwari and Das [19], the use of hormones or auxins plays an important role in affecting growth/sprouting and survival of stem cuttings. Furthermore, the establishment of a well-developed root system represents a pivotal stage in the vegetative propagation of plants, playing a vital role in ensuring a heightened survival rate for micro propagated plantlets as they transition into the acclimatization phase.

4. Conclusions

The study's findings indicate that both the number of rhizome buds and the duration of soaking of ANAA had no impact on the growth of Thai ginger at 30, 60, 90, and 120 days after planting. However, there were instances where the number of rhizome buds and the soaking time significantly affected the plant's growth, particularly with ANAA. On some occasions, soaking time did influence Thai ginger development. Notably, the duration of soaking with ANA appeared to influence plant height at 30 and 120 days, rhizome bud formation, and plant survival at 120 days. Therefore, it is suggested that this timing can enhance growth and development.

Additionally, the most favorable growth outcomes were observed when directly planting three rhizome buds. This offers valuable insights for farmers, growers, and anyone seeking to enhance the yield and quality of Thai ginger. The influence of soaking time on the growth performance of Alpinia galanga, as determined by the number of rhizomal buds, was not significant for ANAA. Thus, these two factors individually affect plant growth, leading to various cultivation techniques.

Furthermore, this research provides valuable information for cultivating Thai ginger. These results form the foundation for optimizing the use of ANNA and underscore the importance of selecting appropriate buds for improved growth responses. The outcomes of this study can contribute to the advancement of *Alpinia galanga* cultivation methods, which have diverse applications in both medicine and food production.

Conflict of Interest

The author declares no conflict of interest.

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