

THE EFFECT OF *AQUILARIA MALACCENSIS* SEED PRIMED WITH GIBBERELIC ACID AND INDOLE BUTYRIC ACID

Kharnaioir S^{1,*} & Thomas SC²

¹Department of Biotechnology, Assam Don Bosco University, Tapesia, Garden, Kamarkuchi, Sonapur, Assam-782402, India

²Department of Biochemistry, Assam Don Bosco University, Tapesia, Garden, Kamarkuchi, Sonapur, Assam-782402, India

*suklang2015@gmail.com

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The main goal of this study was to produce seedlings of *Aquilaria malaccensis* with rapid growth, uniformity and high quality. The effect of indole butyric acid (IBA) and gibberellic acid (GA₃) at different concentrations and combinations (100, 150 and 200 ppm) on the germination and development of seedlings was investigated. The primed seed was grown in two types of soil, namely, sterile and unsterile soil. The seeds primed with IBA (100, 150 and 200 ppm) and planted in sterile soil showed germination and organ development of 8 seeds from 3 treatments within 4–7 days. However, the seeds primed with GA₃ took 10 to 15 days for germination, and organ development of 14 seeds from 3 treatments. The germination rate of all the primed seeds of IBA and GA₃ grown in unsterile soil was null. The various combination of IBA and GA₃ played a significant role in the germination and organ development of *A. malaccensis* seed in the sterile soil (17.305 ± 0.88), than in unsterile soil.

Keywords: Stressful environment, IBA, GA₃, germination rate, growth hormone, unsterile soil, sterile soil

INTRODUCTION

Plant growth regulators play an important role in plant life cycles such as seed maturation, seed germination, shoot, root and leaf development, as well as plant protection from environmental variables (Shu et al. 2018). *Aquilaria malaccensis* (Thymelaeaceae) is regenerated naturally by seeds that are available throughout the rainy season. It produces high grades of agarwood oil. Agarwood is produced when the plant's endogenous fungi interact with it in a sustainable manner, beginning at a young age and culminating in the creation of resin when the plant begins to mature (Mochahari et al. 2020). Due to its desiccation sensitivity, the seed is vulnerable to microbial infection and other environmental conditions. The main consequences that affect the life cycle of the plant are delayed germination and loss of dormancy. Seed priming is a cheap biochemical and physiological process that improves the health of the plants and speeds up their growth and development in a stressful environment.

Aquilaria malaccensis is an endangered species listed on CITES list since 1995, as well as the IUCN red data list since 2011, as vulnerable and on the verge of extinction in the ecosystem (IUCN 2009).

Environmental degradation, overexploitation and high demand in both national and international trade has threatened this species in its natural habitat. *Aquilaria malaccensis* cultivars are widely available in India, Bangladesh, Myanmar, Sumatra, Peninsular Malaysia, Singapore and the Philippines (Tnah et al. 2012). Mean annual rainfall range of 1500 to 6500 mm, and maximum and minimum temperatures of 22–28 °C and 14–21 °C respectively are preferred for the development of *A. malaccensis* organs (Beniwal 1989). The tree stands 18–20 m tall and has a 1.5–2.0 m diameter. *Aquilaria* trees are traditionally propagated by grafting, stem cutting and seedlings, but environmental factors such as insect attacks, delayed rooting, low germination and low seed shelf-life have impacted the agarwood output. In a seed weight prediction test, heavier seeds have a higher likelihood of excellent germination and seedling growth than lighter seeds (Shankar 2012). To improve on the traditional method, Yasnita et al (2017) developed shoot cutting by treating injured stems with indole butyric acid (IBA) and naphthaleneacetic acid (NAA) at

various concentrations while growing in three different media, namely soil, soil and rice husk, and soil + compost. The authors discovered that seed primed at 100 ppm (IBA and NAA) while growing in soil + rice produced the most roots. Borpuzari and Kachari (2018) discovered that after treatment using various concentrations of IBA, basal (hardwood) cutting responded better in root development than the middle and top positions of the branch. As a result, only shoot cutting was primed or treated with growth hormones. There has been no research on priming the *A. malaccensis* seed, nor has there been any comparative study on growing the primed seeds in sterile and unsterile soil. Research on the effect of plant growth regulators on seed germination of *A. malaccensis* is limited although it is reasonable to increase seed germination of such an economically important plant. Thus, the study was aimed at improving germination and organ development of *A. malaccensis* in a short period of time by priming the seed with plant growth regulators, namely IBA and gibberellic acid (GA₃) separately and in different concentration and combinations and growing it in both sterile and non-sterile soil.

MATERIALS AND METHODS

Collection of samples

Fresh *A. malaccensis* seeds were collected from the agarwood plantations of Assam Don Bosco University. The seeds were disinfected with 0.1% Bavistin for 10 min and washed with sterile distilled water. The sample was placed inside the laminar airflow chamber and treated with 70% ethanol for 40–60 seconds which was followed by rinsing with sterile distilled water for 3–5 times. Lastly, the sample was treated with 0.1% mercuric

chloride (HgCl₂) for 40–60 seconds and rinsed with sterile distilled water for 3–5 times.

Medium preparation

Soil collected from the same agarwood plantations were used as the growth medium for the seeds. Sterile soil was prepared by keeping it in an oven at 100 °C for 1 hour and then allowing it to cool to room temperature. The soil was then made sterile by keeping it in an autoclave for 1 hour at 121 °C and then transferred into sterile disposable plastic cups.

Priming of seed

Sterile *A. malaccensis* seeds were primed or treated with IBA and GA₃ separately (Table 1) and in combinations of different concentrations (Table 2). The seed primed with the combination of IBA and GA₃ was classified into three groups as shown in Table 2. The concentrations of IBA and GA₃ were standardised before the experiment. The seeds were immersed in a priming medium and kept in the dark chamber for 24 hours at normal room temperature. Before transferring into the growth medium (sterile and unsterile soil), the primed seeds were washed using sterile distilled water and allowed to dry on sterile filter paper. Five seeds were taken for each treatment and each treatment was replicated three times. Control group (unprimed) of seeds were also kept with treatment groups. Primed and control seeds were planted in sterile and unsterile soil (growth medium) as mentioned above. Daily records were kept of soil moisture, seed germination, organ development and germination time. After the seeds sprouted, they were put into polythene bags to make sure that their organs would grow properly.

Table 1 Priming of *Aquilaria malaccensis* seed independently with indole butyric acid (IBA) and gibberellic acid (GA₃) and planted in sterile and unsterile soil

Plant growth regulators concentrations (ppm)		Growth medium	
IBA	GA ₃		
100	100	Sterile soil	Unsterile soil
150	150		
200	200		

Table 2 Priming of *Aquilaria malaccensis* seed with the combination of indole butyric acid (IBA) and gibberellic acid (GA₃) and planted in sterile and unsterile soil

	Combinations of IBA and GA ₃ (ppm)		Growth medium	
	IBA (ppm)	GA ₃ (ppm)		
Group I	100	100	Sterile soil	Unsterile soil
	100	150		
	100	200		
	150	100		
Group II	150	150		
	150	200		
	200	100		
Group III	200	150		
	200	200		

Measurement of germination rate

The percentage of germination was calculated using the following formula:

$$\text{Total germinated seed (\%)} = \frac{\text{Germinated seed} \times 100}{\text{Total number of primed seed}}$$

To assess the effects of IBA and GA₃ on germination of primed seed and the effect of growth medium, the number of germinated seed, shoot, root and leaves development were measured and recorded.

Statistical analysis

The experiment was carried out in triplicate and results were expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) and completely randomised design (CRD) were carried out on the data using the Web-Based Agricultural Statistical Package (version 1.0).

RESULTS

Effect of sterile Soil on seed germination of primed seeds with indole butyric acid (IBA) and gibberellic acid (GA₃) independently

The seeds primed with IBA (100, 150 and 200 ppm) and planted in sterile soil showed germination and organ development within 4–7 days (Table 3). Out of 15 seeds (3 replicates of 5 each) primed with IBA at 100 ppm, only 2

seeds germinated with root and shoot formation. Similarly, the seeds primed with IBA at 150 ppm and 200 ppm, showed germination and organ development of 3 seeds respectively, as shown in Figure 3 and Table 3.

However, the seeds primed with GA₃ took 10 to 15 days for germination and organ development (Table 3). Out of 15 seeds treated with GA₃ at a concentration of 100 ppm, four seeds germinated with the emergence of shoot and root. At 150 ppm five seeds germinated with an appearance of shoot and root development. Likewise, at 200 ppm five seeds germinated with slow development of shoot and root, as shown in Figure 1 and Table 3. Out of 15 unprimed seeds (control), grown in sterile soil, only one seed germinated after 15 days with shoot, root and leaf formation, as seen in Figure 1 and Table 3.

Statistical analysis of the effect of sterile soil on seed germination of primed seed with indole butyric acid (IBA) and gibberellic acid (GA₃) independently

Statistically, it was observed that the seed primed with 100 ppm of IBA had a significant difference from the seed primed with 150 ppm of IBA and 200 ppm of IBA. Whereas, the seed primed with 150 ppm and 200 ppm of IBA showed no statistically significant differences from each other. However, they were significantly different from the control, as shown in Table 3.

The seeds primed with GA₃ at 100 ppm and 150 ppm were not significantly different from each other, but they were significantly different from seeds treated with GA₃ at 200 ppm. Overall, it was discovered that the effect of IBA and GA₃

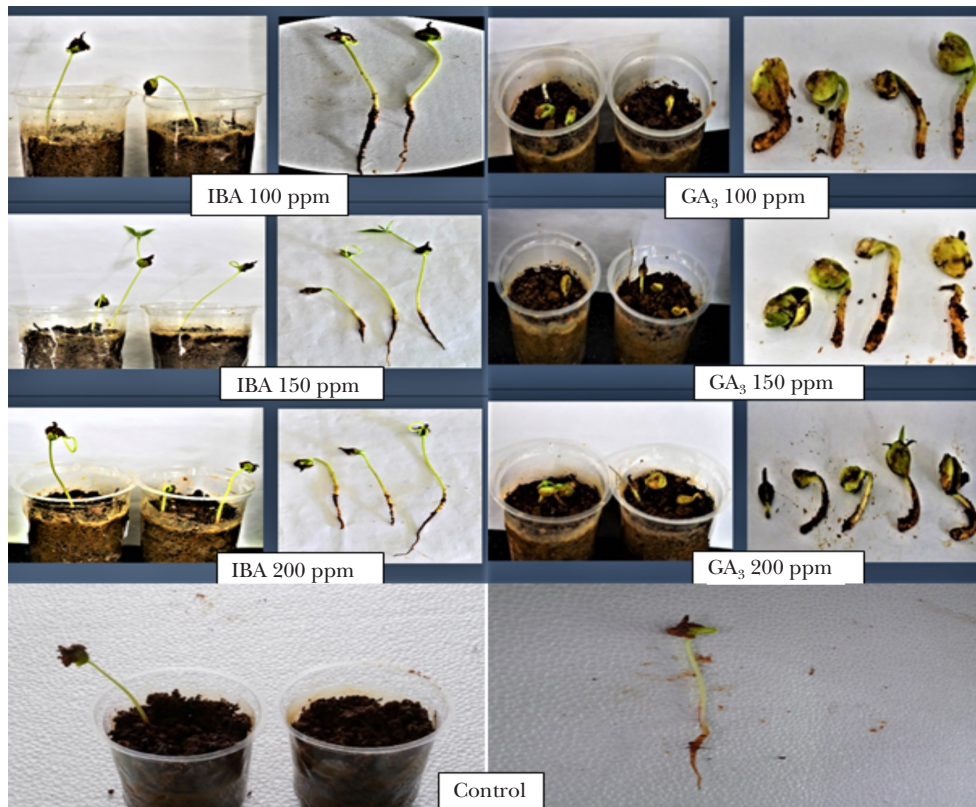


Figure 1 Effect of indole butyric acid (IBA) and gibberellic acid (GA₃) and sterile soil on germination of *Aquilaria malaccensis* seeds

Table 3 Effect of indole butyric acid (IBA) and gibberellic acid (GA₃) and sterile soil on germination of *Aquilaria malaccensis* seeds

Plant growth regulators (PGR)	Concentrations (ppm)	Total primed seed	Germinated seed	Percentage of germination = TG * 100/TPs	Germination time (days)	Treatment average
IBA	100	15	2	13.32		11.830 ± 0.33 _{bc}
	150	15	3	20	4–7	14.332 ± 0.57 _{ab}
	200	15	3	20		14.759 ± 0.57 _{ab}
GA ₃	100	15	4	26.66		17.275 ± 0.88 _a
	150	15	4	26.66	10–15	17.275 ± 0.88 _a
	200	15	5	33.33		19.428 ± 0.66 _a
Control (T7)	-	15	1	6.66	10–15	8.447 ± 0.33 _c

on *A. malaccensis* seed independently showed a significant difference from control or unprimed seed using one-way ANOVA and CRD (Table 3).

Effect of unsterile soil on seed germination of primed seeds with indole butyric acid (IBA) and gibberellic acid (GA₃) independently

Figure 2 and Table 5 show the results of *A. malaccensis* seed growing in unsterile soil,

which was treated separately with plant growth hormones (IBA and GA₃ at 100, 150, 200 ppm). The germination rate of all the primed seeds of IBA and GA₃ grown in unsterile soil was null, except the seeds primed with 200 ppm of IBA which displayed the germination of a single seed after 27 days of planting in unsterile soil. Two seeds from the unprimed (control) group showed slight delayed germination after 40 days of planting in unsterile soil (Figure 2).

Statistical analysis of the effect of unsterile soil on seed germination of primed seed

Statistically it was observed that the seeds primed with IBA and GA₃ independently grew in unsterile soil (Table 4). The results showed that 100 ppm and 150 ppm of IBA showed no significant difference from 100 ppm, 150 ppm and 200 ppm of GA₃. Whereas seeds primed with 200 ppm of IBA showed a significantly different response from all the treatments (100 ppm and

150 ppm of IBA and 100 ppm, 150 ppm and 200 ppm of GA₃) and the control.

Effect of sterile soil on seed germination of primed seeds with indole butyric acid (IBA) and gibberellic acid (GA₃) combination

The result of *A. malaccensis* seed primed with various combinations of IBA and GA₃, and planted in sterile soil is shown in Figure 3 and Table 5. The germinated seeds from group I

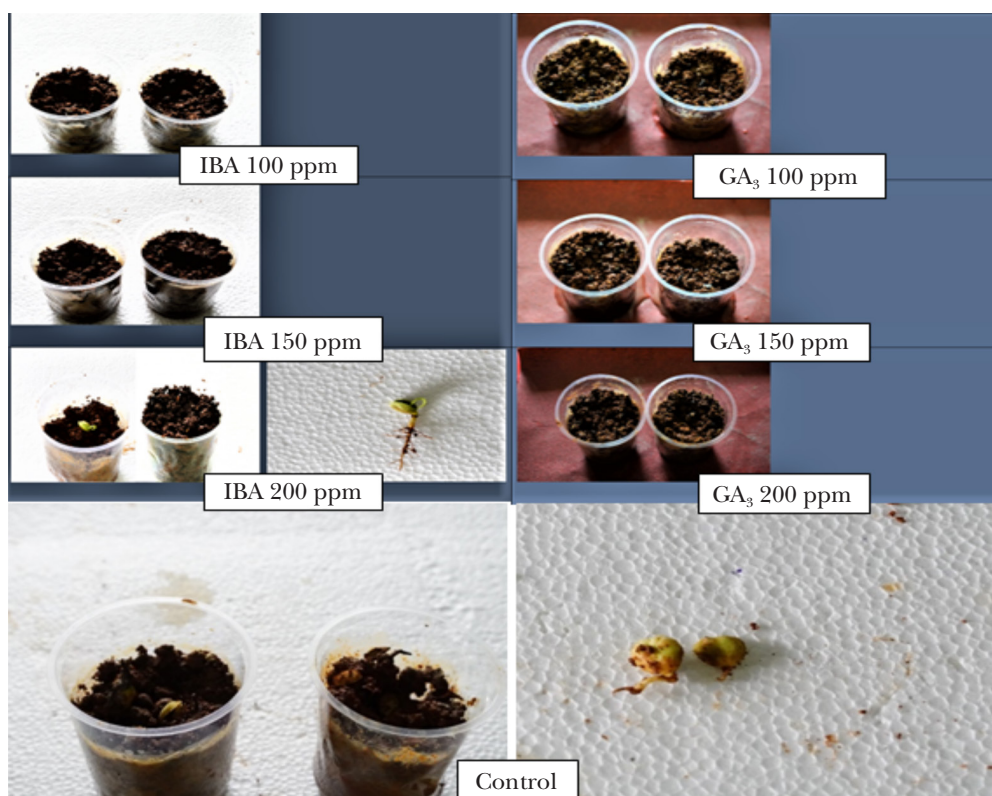


Figure 2 Effect of indole butyric acid (IBA) and gibberellic acid (GA₃) and unsterile soil on germination of *Aquilaria malaccensis* seeds

Table 4 Effect of indole butyric acid (IBA) and gibberellic acid (GA₃) and unsterile soil on germination of *Aquilaria malaccensis* seeds

Plant growth regulators (PGR)	Concentrations (ppm)	Total primed seed	Germinated seed	Total percentage of germination = TG * 100/TPs	Germination time (days)	Treatment average
IBA	100	15	-	-		1.281 ± 0.0 _b
	150	15	-	-		1.281 ± 0.0 _b
	200	15	1	6.66	20–27	8.436 ± 0.33 _a
GA ₃	100	15	-	-		1.281 ± 0.0 _b
	150	15	-	-		1.281 ± 0.0 _b
	200	15	-	-		1.281 ± 0.0 _b
Control (T7)	-	15	2	13.32	30–40	8.569 ± 0.38 _a

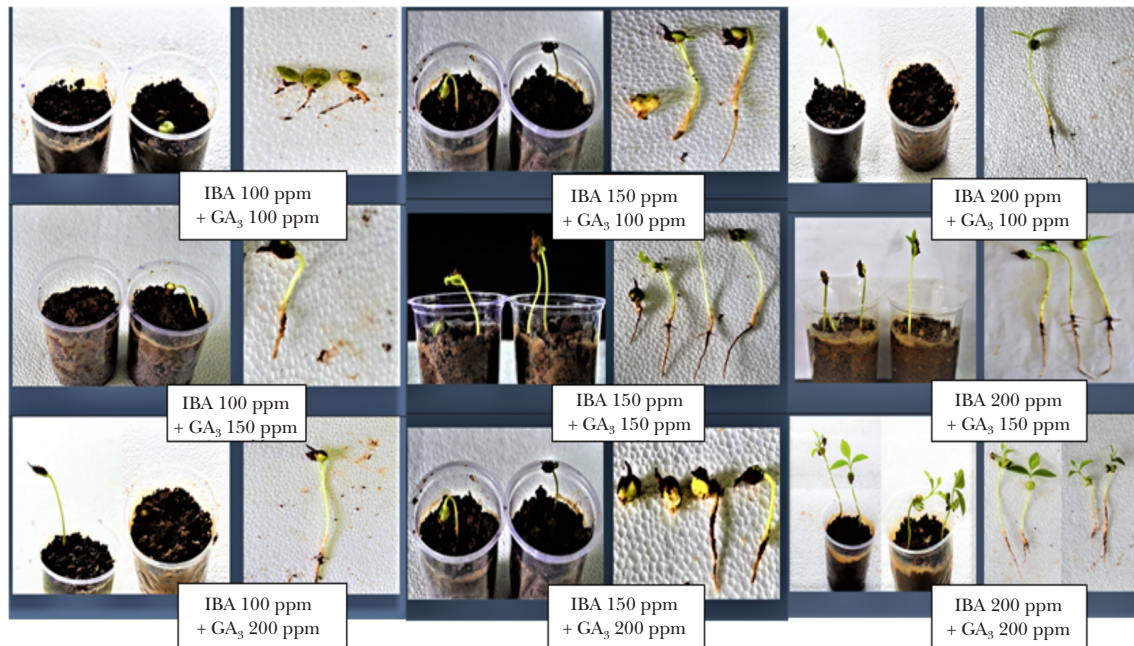


Figure 3 Effect of indole butyric acid (IBA) and gibberellic acid (GA₃) and sterile soil on germination of *Aquilaria malaccensis* seeds

Table 5 Effect of indole butyric acid (IBA) and gibberellic acid (GA₃) and sterile soil on germination of *Aquilaria malaccensis* seeds

Combination of IBA and GA ₃ (ppm)		Total primed seed	Germinated seed	Percentage of germination = TG * 100/TPs	Germination time (days)	Treatment average
IBA (ppm)	GA ₃ (ppm)					
100	100	15	3	20	7–12	14.717 ± 0.57 _{ab}
100	150	15	1	6.66		8.508 ± 0.33 _b
100	200	15	1	6.66		8.569 ± 0.33 _b
150	100	15	3	20	4–10	14.759 ± 0.57 _{ab}
150	150	15	4	26.66		15.865 ± 0.67 _a
150	200	15	4	26.66		17.305 ± 0.8 _a
200	100	15	1	6.66	4–10	8.524 ± 0.33 _b
200	150	15	3	20		13.974 ± 0.57 _{ab}
200	200	15	4	26.66		17.275 ± 0.66 _a
Control	-	15	1	6.66	10–15	8.447 ± 0.33 _c

were three at 1:1 combination of IBA and GA₃, one at 1:1.5 combination of IBA and GA₃ and one at 1:2 combination of IBA and GA₃. All the five germinated seeds from group I have shown the emergence of shoot and root formation within 9 days of planting in sterile soil.

The germinated seeds from group II were three at 1.5:1 combination of IBA and GA₃, four at 1.5:1.5 combination of IBA and GA₃ and four at 1.5:2 combination of IBA and GA₃ respectively. All the eleven germinated seeds from group

II showed the emergence of shoot and root formation within 9 days of planting in sterile soil. Four seedlings out of eleven germinated and displayed leaf formation, as in Figure 3.

The results of primed seeds from group III planted in sterile soil showed germination of one seed at 2:1 combination of IBA and GA₃, germination of three seeds at 2:1.5 combination of IBA and GA₃, and germination of four seeds at 2:2 combination of IBA and GA₃, correspondingly. All germinated seedlings

showed emergence of healthy shoot, root with root hairs and leaf development.

Statistical analysis of the effect of sterile soil on seed germination of primed seed with indole butyric acid (IBA) and gibberellic acid (GA₃) combination

One-way ANOVA and complete randomised design (CRD) showed a significant difference in the priming of seeds in the combination of IBA and GA₃ at various concentrations. Statistical analysis of seed primed with the combination of IBA and GA₃ growing in sterile soil found in group I, as shown in Table 5, exhibited that 1:1 ppm of IBA and GA₃ combination showed a significant difference to seed primed with 1:1.5 ppm and 1:2 ppm of IBA and GA₃, and unprimed seeds. No significant difference was obtained on seeds primed with 1:1.5 ppm and 1.2 ppm.

Statistically, in group II, it was found that the seeds primed with IBA and GA₃, growing in sterile soil were significantly different from each other and the control, as shown in Table 5. It was also observed that the seed primed in group III growing in sterile soil were statistically significant from each other and the control (Table 5).

Effect of unsterile soil on seed germination of primed seeds of indole butyric acid (IBA) and gibberellic acid (GA₃) combination

Table 6 and Figure 4 shows the results of *A. malaccensis* seed grown in unsterile soil after being treated with various combination of IBA and GA₃. The germinated seeds from group I were one at a 1:1 combination of IBA and GA₃, with poor development of shoot, root and leaf. The germinated seeds were two at a 1:1.5 combination of IBA and GA₃, and one at a 1:2 combination of IBA and GA₃. Three germinated seeds from group I showed the emergence of shoot, root and leaf formation within 17 days of planting in unsterile soil.

The germinated seeds from group II were one at 1.5:1 combination of IBA and GA₃, one at 1.5:1.5 combination of IBA and GA₃ and three at 1.5:2 combination of IBA and GA₃ respectively. All the five germinated seeds from group II showed the emergence of shoot, root and leaf formation within 17 days of planting in unsterile soil. Four seedlings out of 5 germinated and displayed leaf formation, as in Figure 4.

The results of primed seeds from group III planted in unsterile soil showed no germination of seed at 2:1 combination of IBA and GA₃, germination of one seed at 2:1.5 combination of IBA and GA₃, and germination of two seeds at 2:2 combination of IBA and GA₃, correspondingly. All germinated seedlings showed an emergence of healthy shoot, root with root hairs and leaf development.

Statistical analysis of the effect of unsterile soil on seed germination of primed seed with indole butyric acid (IBA) and gibberellic acid (GA₃) combination

The effects of various concentrations and combinations of IBA and GA₃ on *A. malaccensis* were analysed using one-way ANOVA and CRD as shown in Table 6. The primed seed from group I growing in unsterile soil showed that 1:1 ppm (IBA and GA₃) was significantly different from 1:1.5 ppm (IBA and GA₃), but not significantly different from seed primed with 1:2 ppm (IBA and GA₃), and unprimed seed.

The seed primed with IBA and GA₃ in group II, was found that 1.5:1 ppm and 1.5:1.5 ppm (IBA and GA₃) were not significantly different, but they are significantly different from 1.5:2 ppm and control, as shown in Table 6. In group III, it was observed that the seeds primed with IBA and GA₃ growing in unsterile soil were not significantly different from each other and the control (Table 6).

DISCUSSION

Effect of sterile and unsterile soil on seed germination of primed seeds with indole butyric acid (IBA) and gibberellic acid (GA₃) independently

Seed germination is an important stage in the development of various plant organs, particularly the seed's ability to withstand unfavorable environments such as drought, temperature, less rainfall, insect attack and some microbial contamination. Yusnita et al. (2017) propagated the *A. malaccensis* by treating injured shoots with IBA and NAA at different concentrations and growing in different types of medium (soil, soil + rice husk, and soil + compost). It was discovered that 100 ppm of hormone solution growing in soil with rice husk had a significant effect on root



Figure 4 Effect of indole butyric acid (IBA) and gibberellic acid (GA₃) and unsterile soil on germination of *Aquilaria malaccensis* seeds

Table 6: Effect of indole butyric acid (IBA) and gibberellic acid (GA₃) and unsterile soil on germination of *Aquilaria malaccensis* seeds

Combination of IBA and GA ₃ (ppm)		Total primed seed	Germinated seed	Percentage of germination = TG * 100/TPs	Germination time (days)	Treatment average
IBA (ppm)	GA ₃ (ppm)					
100	100	15	1	6.66	15–20	8.508 ± 0.33b
100	150	15	2	13.33		11.830 ± 0.66ab
100	200	15	1	6.66		8.569 ± 0.33b
150	100	15	1	6.66		8.524 ± 0.33b
150	150	15	1	6.66		8.549 ± 0.33b
150	200	15	3	20		14.332 ± 0.57a
200	100	15	-	-		1.281 ± 0.0c
200	150	15	1	6.66		8.436 ± 0.33b
200	200	15	2	13.33		8.508 ± 0.33b
Control	-	15	2	13.33	30–40	8.569 ± 0.33b

formation. It was also reported that the explants (hardwood, middle and top position) treated with IBA hormones and leaf trimming showed a great impact on the vegetative propagation of *A. malaccensis* and *A. cumingiana* (Borpuzari & Kachari 2018, Pinon & Reyes Jr 2021).

The hormones used for priming in this experimental design were IBA and GA₃, and the explant used was the seed. The seed primed

separately with IBA and GA₃ growing in sterile soil was recorded. Physiologically, it was observed that the seed primed with IBA had a thin shoot and good development of organs such as leaves, shoots, roots and hair roots, whereas the seed treated with GA₃ had a slow, uniformly germinated, thick shoot and no hair roots (Figure 1 and Table 3). Statistically, it was observed that seeds primed with IBA and GA₃ separately were significantly

different from the unprimed seeds. Whereas, the seeds primed growing in unsterile soil showed no germination, except at 200 ppm of IBA and control. The two germinated seeds of unprimed seeds growing in unsterile soil were not healthy and died after 40 days of planting. Statistically, it showed that there is no significant difference and experimental error (Figure 2 and Table 4). Hence it was proved that the seed primed with IBA and GA₃ independently growing in sterile soil is more effective than the unsterile soil.

Effect of sterile soil and unsterile on seed germination of primed seeds with indole butyric acid (IBA) and gibberellic acid (GA₃) combination

The seed primed with the combination of IBA and GA₃ at different concentrations growing in sterile soil was found that, from the three groups, groups II and III showed high germination. Morphologically, it was observed that the seeds primed in group III had improved health and development of plant organs such as shoot, roots and leaves. Statistically, it was found that the seed primed with 1.5:2 ppm of IBA and GA₃ in group II and 2:2 ppm of IBA and GA₃ in group III were significantly different from the other treatments and control (Table 5).

Whereas, the seed primed of IBA and GA₃ growing in unsterile soil was found that, among the groups, seed primed in group II gave the highest germination, as shown in Figure 4 and Table 6. The seed primed in group I did not give complete development of plant organs. Whereas, the seed primed in groups II and III, complete development of organs such as leaves and shoot, and root was obtained. Statistically, it was found that the combination of IBA and GA₃ at 1.5:2 ppm showed a highly significant difference from all the combinations and concentrations of IBA and GA₃, and the control.

A seed primed growing in sterile soil showed effective germination, health and development of organs compared to unsterile soil. Growing primed seed (2:2 ppm of IBA and GA₃) in sterile soil showed a significant improvement in germination, health and development of plant organs such as shoots, roots, hair roots and leaves when compared to all other seed treatments and control.

A combination of these two plant growth regulators (IBA and GA₃) at various concentrations (Table 1 and 2), and an unprimed (control)

sample sown in a sterile soil, showed more potential and rapid germination with a minimum of 4 days and a maximum of 15 days. Therefore, it exhibited a positive effect, such as improved seed germination and organ development. The seed primed with the combination of IBA, with an increase in the concentration of GA₃ and planting in an unsterile soil, improved plant organ development such as shoot, root and leaf at the germination stage or log phase. Seed priming with GA₃ improved tolerance to a variety of stresses, including salinity, cadmium, drought and flooding (Rhaman et al. 2020). The increase in IBA and BAP concentrations demonstrated effective antioxidant and antimicrobial activity (Paric et al. 2017). Thus, the current study found that mixing IBA and GA₃ makes it easier for seeds to grow in places where the soil isn't clean, or where there is a lot of stress.

In comparison to unsterile medium, seeds primed independently and unprimed seeds planted in sterile soil improved significantly in germination, plant health and organ development. The same was observed in the combination of both hormones (IBA and GA₃). The combination of hormones with an increase in concentration showed a great impact on germination and plant organ development (shoot, root, hair root, as well as leaves) compared to the separate primed seed growing in unsterile soil. As shown in Figures 1 and 3, the control grown in sterile soil outperformed the unsterile soil.

ANOVA and CRD test of IBA and GA₃ priming of *A. malaccensis* seed separately or in combination confirmed that the effect of various concentrations and interaction with the medium (soil) is significant in germination. As a result of this study, it was found that the IBA and GA₃ improved the growth rate with an increase in the concentration of the hormones, and at the same ratio of IBA and GA₃ (200 ppm IBA: 200 ppm GA₃).

The GA₃'s mechanism is to accelerate germination, promote uniform growth and lengthen plant stems (Ma et al. 2018). As for GA₃ in priming of *A. malaccensis* seeds, the process of germination was slow, the length of the shoot was thick and short in sterile soil, and there was no growth in nonsterile soil.

CONCLUSION

Seed priming is a physiological technique that improves plant germination by acting as a chemical messenger and allowing plants to

grow or germinate in a stressful environment. It is a physiological method that involves seed hydration to improve metabolic processes before germination, increasing the rate of percentage germination and improving seedling growth under normal and variable environmental conditions. It was observed that the germination rate of *A. malaccensis* seed primed separately with growth hormones (IBA and GA₃), and unprimed culturing in an unsterile soil, is low, unhealthy and slow. This may be due to some environmental factors such as bacterial or fungal infection, which affect the growth, but in combination it improved in all three cases (growth rate, health and organ development). The seed primed with combination hormones improved tolerance to biotic stress environments. This technique can be used to maintain sustainable crop production in contaminated soil. Choosing the soil for germination and developing scientific techniques to germinate and grow plants for crop production or balancing environmental loss are thus the primary goals of the researchers. More research into the effects of IBA and GA₃ on germination and their impact on environmental stress is needed.

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