Response of (*Kopsia arborea* Blume) Seed Germination to Rhizosphere Microbial Treatment

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Abstract: *Kopsia arborea* Blume is a plant species with high bioactive potential, yet its cultivation remains underdeveloped. One of the primary challenges in generative propagation is the low viability and slow seed germination rate. This study aims to analyze the effect of generative materials and rhizosphere microbes on the germination of Kopsia seeds. The generative materials used include whole fruits and fruit embryos of Kopsia. The rhizosphere microbes applied consist of a mixture of Trichoderma sp., Bacillus sp., Pseudomonas sp., and Azospirillum sp. The generative materials were cultivated in sand media over a two-month observation period. The observed parameters included the germination process, germination capacity, maximum growth potential, mean germination time, germination rate, vigor index, plant height, seedling length, total fresh weight, total dry weight, and shoot-root ratio. The treatment of generative materials significantly influenced germination capacity, mean germination time, germination rate, total fresh weight, total dry weight, and shoot-root ratio. Microbial applications affected germination capacity, mean germination time, maximum growth potential, vigor index, and seedling length. Overall, both whole fruits and embryos of kopsia are suitable for generative propagation. However, microbial applications are only necessary when propagation is conducted using whole fruits.

Keywords: Generative materials; Germination process; Germination rate; Germination time; Vigor index

Introduction

Kopsia arborea Blume is a flowering plant species from the family *Apocynaceae,* naturally distributed across the tropical regions of Southeast Asia, including Indonesia, Malaysia, and Thailand. This tree is renowned for its elegant white blossoms, making it a popular choice for ornamental use and tropical landscape design. Kopsia is tolerant of suboptimal environmental conditions and demonstrates strong adaptive capacity. It is also commonly planted as a shade tree due to its sturdy trunk, evergreen foliage, slow-growing root system, and resistance to pests and diseases.

Kopsia (*Kopsia* sp.) is widely used as an ornamental and shade plant and has growing potential as a medicinal plant due to its secondary metabolite content. Secondary metabolites play an essential role in plant defense mechanisms (Nabillah & Chatri, 2024). Kopsia contains alkaloids, flavonoids, and tannins, which are known for their antioxidant properties and potential benefits in preventing chronic diseases such as cancer and coronary heart disease (Purwanto et al., 2017). Despite these advantages, scientific information on kopsia cultivation and propagation techniques remains limited.

Previous studies on kopsia propagation are scarce. Rasyad et al. (2019), reported generative propagation using seeds inoculated with endophytic fungi. Kopsia

seeds are also known to be polyembryonic, meaning that a single seed can produce multiple embryos derived from both sexual and asexual processes (Rahmadini et al., 2020). This characteristic may lead to variability in seed viability, vigor, and germination uniformity, posing challenges for nursery production.

Optimizing seed germination is critical for successful plant cultivation, particularly at the nursery stage. However, early plant growth is often constrained by unfavorable environmental conditions and limited nutrient availability. One environmentally friendly strategy to overcome these limitations is the application of rhizosphere microbes. Rhizosphere microorganisms such as *Trichoderma* sp., *Bacillus* sp., *Pseudomonas* sp., and *Azospirillum* sp. have been shown to enhance seed germination and early plant growth through nutrient solubilization, phytohormone production, and pathogen suppression (Tiwari et al., 2023).

Biopriming is an effective method for applying beneficial microbes through seed hydration combined with microbial inoculation (Hadinata et al., 2023). This technique enables microbial colonization of seeds, improving seed viability, accelerating germination, and increasing tolerance to biotic and abiotic stresses (Due et al., 2024). Although positive effects of biopriming have been reported in various crops, its application to kopsia seeds has not yet been investigated.

The novelty of this study lies in evaluating the combined effects of generative seed materials and rhizosphere microbial application on the germination of polyembryonic kopsia seeds. This research addresses a critical knowledge gap in kopsia propagation and provides scientific support for the use of rhizosphere microbes as sustainable biofertilizers in nursery systems.

Method

Sowing Media Preparation

The sowing medium used for germinating kopsia seeds was sand. Before use, the sand was sun-dried for three consecutive days to reduce moisture and minimize potential microbial contamination. Once prepared, the sand was placed into germination trays to serve as the growth substrate.

Preparation of Generative Materials

Generative materials were collected directly from kopsia trees located around the Faculty of Forestry, IPB University. The fruits were harvested using a pole by selecting fresh, physiologically mature kopsia fruits, characterized by a dark purplish hue and free from pests and diseases. The harvested fruits were placed into bags filled with cocopeat to maintain moisture and prevent physical damage. The exocarp and mesocarp of the fruits were removed, leaving only the embryo. Kopsia seeds

are classified as polyembryonic, meaning that a single seed may contain more than one embryo, with one derived from sexual reproduction and the others from asexual processes.

Preparation of Rhizosphere Microbial Inoculum

The rhizosphere microbes used in this study consisted of a mixture of the fungus *Trichoderma* sp. and the bacteria *Bacillus* sp., *Pseudomonas* sp., and *Azospirillum* sp., all of which were sourced from the Forest Pathology Laboratory, Department of Silviculture, IPB University. A total of 5 grams of bacterial inoculum was weighed and dissolved in 100 mL of sterile water, then incubated for 24 hours at room temperature. After incubation, the microbial solution was transferred into a container containing 1 liter of sterile water and stirred thoroughly to ensure homogeneity

Germination of Generative Materials

The prepared generative material was soaked in a rhizosphere microbial solution for two hours. After soaking, the kopsia materials were sown onto the prepared sand-based germination medium. Each germination tray contained 20 individual kopsia generative units

Maintenance

Maintenance activities included watering, weed removal, and pest-disease monitoring. Watering was carried out routinely once or twice daily, depending on the moisture condition of the sowing medium. Weed removal was performed manually by uprooting weeds that emerged on the surface of the germination media. Pest and disease monitoring was conducted to minimize potential damage or disturbances to the seedlings. In the event of pest or disease attacks, control measures were applied, and affected seedlings were separated from the healthy ones to prevent further spread.

Observation and Data Collection

Observation and data collection were conducted over a period of approximately two months, concurrently with maintenance activities. The observed variables included germination process, germination capacity, maximum growth potential, mean germination time, germination rate, height, seedling length, vigor index, total fresh weight, total dry weight, and shoot-root ratio.

The germination process was monitored from the initial emergence of the plumule on the surface of the sowing medium until full seed germination. Germination capacity and mean germination time were assessed following the method described by Fata et al. (2020); maximum growth potential and vigor index

referred to the procedures outlined by Tefa (2017); germination rate was evaluated based on Fatikhasari et al. (2022); while height, total fresh weight, total dry weight, and shoot-root ratio were measured according to Djara et al. (2022). Seedling length was measured at the end of the observation period, from the tip of the root to the apex of the seedling, using a ruler.

Research Design

The experiment design used in this study was a Completely Randomized Design (CRD) with a factorial arrangement involving two factors. The first factor was the type of generative material, consisting of two levels: (1) whole fruits, and (2) embryos. The second factor was the application of rhizosphere microbes, also consisting of two levels: (1) without microbial application and (2) with microbial application. In total, there were four treatment combinations. Each treatment was replicated three times, with each replicate consisting of 20 kopsia seeds, resulting in a total of 240 seeds across all treatments.

Data Analysis

The data collected were processed using Microsoft Excel and SPSS software. Analysis of variance (ANOVA) was performed using the F-test to determine the interaction effects among the treatments applied in this study. Decision-making based on the ANOVA results followed these criteria: reject H_0 and accept H_1 if the calculated F-value exceeds the critical F-table value; accept H_0 and reject H_1 if the calculated F-value is less than the F-table value, at a 5% significance level. If the P-value > α (0.05), the treatment is considered to have no significant effect on the observed variables. Conversely, if the P-value < α (0.05), the treatment is considered to have a significant effect. In cases where significant effects were observed, further analysis was conducted using a Post Hoc Test (Tukey's test) (Gomez & Gomez, 1984).

Result and Discussion

Morphology and Germination Process of Kopsia Seeds

Kopsia fruits exhibit a distinctive shape, ranging from oval to nearly spherical, with a length of approximately 1–3 cm and a diameter of about 0.8–2.2 cm. Immature fruits are green, while physiologically mature fruits turn dark purplish black in color (Figure 1a). The fruit surface is smooth, hairless, and appears clean and glossy. Kopsia fruits consist of several layers: the exocarp (outer skin), mesocarp, and endocarp. The mesocarp is characterized by a firm, fibrous texture. The endocarp contains the testa, embryo, and endosperm (Figure 1b).
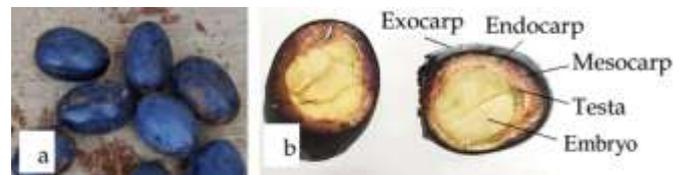

Figure 1. Morphology of kopsia fruit: (a) Whole kopsia fruit, (b) Internal structure of kopsia fruit

The larger the size of kopsia fruit, the greater the number of embryos contained within. Similar findings were reported by Raja (2017) in kaffir lime, where larger seeds tended to contain more embryos. Kopsia fruit is classified as a recalcitrant seed. According to Rasyad et al. (2019), the moisture content of Kopsia fruit exceeds 70%. Recalcitrant seeds typically have a moisture content above 30% and cannot be stored for extended periods (Sudrajat et al., 2017). Seeds with high moisture content have a relatively short shelf life, and their viability declines rapidly when the moisture level is reduced to 15–20% (Indraeni et al., 2019). A moisture content of 70% indicates that the seed remains fresh, physiologically active, and metabolically functional.
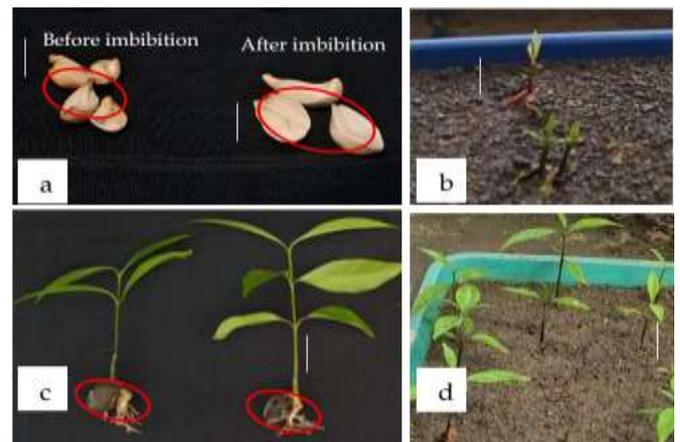

Figure 2. Seed germination process of kopsia: (a) Changes in kopsia seed before and after imbibition; (b) Emergence of plumule kemunculan plumula, (c) Hypogeal seed type tipe benih hipogeal, (d) Emergence of the first true leaf. - = 2 cm

The germination process begins with seed imbibition, marked by an increase in seed size and a softening of the seed coat. In whole Kopsia fruits, the imbibition process is not clearly visible, whereas in isolated embryos, the changes are more apparent (Figure 2a). The next stage is characterized by the emergence of the radicle, followed by the development of the plumule or embryonic shoot, which grows upward toward the soil surface (Figure 2b). Whole Kopsia seeds began to show radicle emergence followed by plumule development on day 15, while Kopsia embryos exhibited these growth stages earlier, on day 9. Kopsia seeds undergo hypogeal germination, as the seed remains below the surface of the growing medium (Figure 2c).

Once the plumule successfully penetrates the soil surface, the first true leaves begin to form around day 14 (Figure 2d).

The germination process of kopsia seeds begins with imbibition. In whole fruits, the imbibition process is not clearly visible, whereas in isolated embryos, the changes are more apparent. Germination is initiated by water absorption, which activates nutrient mobilization through enzymatic reactions involving amylase, protease, and lipase. These enzymes facilitate the transfer of stored nutrients from the endosperm to the embryo, supporting the germination process (Mangardi et al., 2021).

During imbibition, physiological changes occur in the seed, resulting in increased size and softening of the seed coat. Water uptake by the embryo and endosperm causes swelling of both structures, which in turn exerts pressure on the softened seed coat, leading to its rupture and allowing the radicle to emerge. Imbibition directly influences the speed of germination. Pre-treatment methods such as scarification or seed extraction from the fruit can enhance germination rates. The thicker the seed coat, the longer it takes for water to penetrate the internal layers, thereby slowing down the germination process (Wulandari & Nurhayani, 2019).

The next stage of germination is marked by the emergence of the radicle and plumule within the germination medium. While the radicle is not visibly observable due to obstruction by the sowing medium, the development of the plumule can be clearly monitored. Morphologically, this stage begins with the appearance of the radicle as the initial root structure, followed by the elongation of the plumule as the shoot primordium (Mangardi et al., 2021). Germinating seeds are characterized by the emergence of the radicle, followed by the growth of the hypocotyl, which appears pale yellowish white (Wulandari & Farzana, 2020). The hypocotyl continues to elongate until it reaches the surface of the growing medium, followed by the emergence of the cotyledons, which remain enclosed within the seed coat and do not yet open. Once the cotyledons fully unfold and orient upward, the plumule begins to develop into the first true leaves. The emergence of the primary root is also associated with the development of the first leaf. Experimental results showed that radicle and plumule emergence occurred earlier in the embryo treatment compared to the whole fruit treatment. Similar findings were reported by Fatikhasari et al. (2022), indicating that larger seeds with thicker seed coats generally require a longer germination period than smaller seeds. It is assumed that seed surface area may influence the rate of imbibition and the activation of enzymes and hormones involved in the germination process. Kopsia seeds possess a thick seed coat structure, which prolongs the time required for germination when used in whole form. The thicker the seed coat, the longer it takes for water to penetrate the seed layers (Wulandari & Nurhayani, 2019), resulting in delayed germination (Krisnawati & Adie, 2016).

Kopsia seeds exhibit hypogeal germination, in which the cotyledons remain below the surface of the growing medium during germination. These seeds are also classified as polyembryonic, as a single fruit contains more than one embryo. This indicates that each Kopsia fruit has the potential to produce multiple seedlings, each capable of developing into an independent plant. Research findings revealed that whole fruits resulted in slower seedling growth compared to isolated embryos. Similar results were reported by Walingkas et al. (2022), in Avocado (*Persea americana* Mill.) and by Piri et al. (2023), in Siam orange (*Citrus nobilis* L.). This phenomenon is likely because separated embryos possess greater nutrient reserves for germination and are not subject to intra-embryo competition, unlike seeds germinating from whole fruits.

Kopsia seeds are classified as polyembryonic, as they contain more than one embryo within a single fruit, allowing multiple seedlings to emerge from a single seed. The number of embryos observed in this study varied considerably: whole fruits produced up to three seedlings from a single seed, while isolated embryos yielded as many as seven seedlings from one seed (Figure 3a). In the whole fruit treatment, the resulting seedlings were generally separable from one another. In contrast, seedlings from the embryo treatment were more difficult to separate due to the fusion of multiple embryos within a single endosperm tissue (Figure 3b).
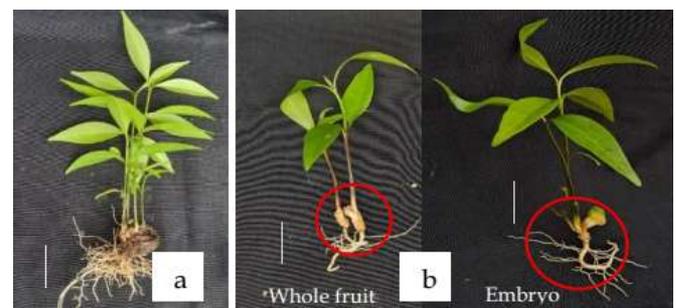

Figure 3. Germination of kopsia seeds: (a) Polyembryony in kopsia seeds, showing the emergence of more than one seedling from a Single seed, (b) Occurrence of polyembryony in kopsia seeds. - = 2 cm

A comparative overview of the germination process between whole kopsia seeds and isolated embryos is presented below. The observed germination stages include imbibition, plumule emergence, full leaf development, occurrence of polyembryony, and polyembryonic seedling formation (Table 1).

Table 1. Recapitulation of Germination Outcomes in Kopsia Seeds

Germination process	Whole fruit	Embryo
Imbibition process	Fruit size remains constant	Embryo size is relatively larger
Plumule emergence (days)	15.00	9.00
Full leaf development (days)	20.00	15.00
Polyembryony occurrence (%)	47.50	5.00
Number of polyembryonic seedlings (seedlings per fruit/embryo)	7.00	3.00

Seed Vigor of Kopsia

Research findings indicate that both microbial application and generative material significantly influenced germination capacity and mean germination time. In contrast, maximum growth potential was affected solely by microbial application. Based on the results of the analysis of variance, there was a significant interaction between microbial application and generative material on germination capacity, mean germination time, and maximum growth potential (Table 2).

Table 2. Recapitulation of Germination Outcomes in Kopsia Seeds

Variable	Generative material (B)	Microbial application (A)	B x A
Germination capacity (%)	*	*	*
Maximum growth potential (%)	tn	*	*
Mean germination time (%)	*	*	*

Note: tn: not significant, *: significantly different at the 5% significance level

The highest germination rates were observed in treatments involving embryos without microbial application, embryos with microbial application, and whole fruits with microbial application. In contrast, the lowest germination rate was recorded in the treatment using whole fruits without microbial application. The highest maximum growth potential was achieved in the treatment using whole fruits with microbial application, outperforming all other treatments. Meanwhile, both embryo treatments with and without microbial application resulted in similar values for maximum growth potential. Meanwhile, the average germination time indicates that treatments using embryo-derived generative material resulted in shorter mean germination time compared to treatments using whole generative material (Figure 4).
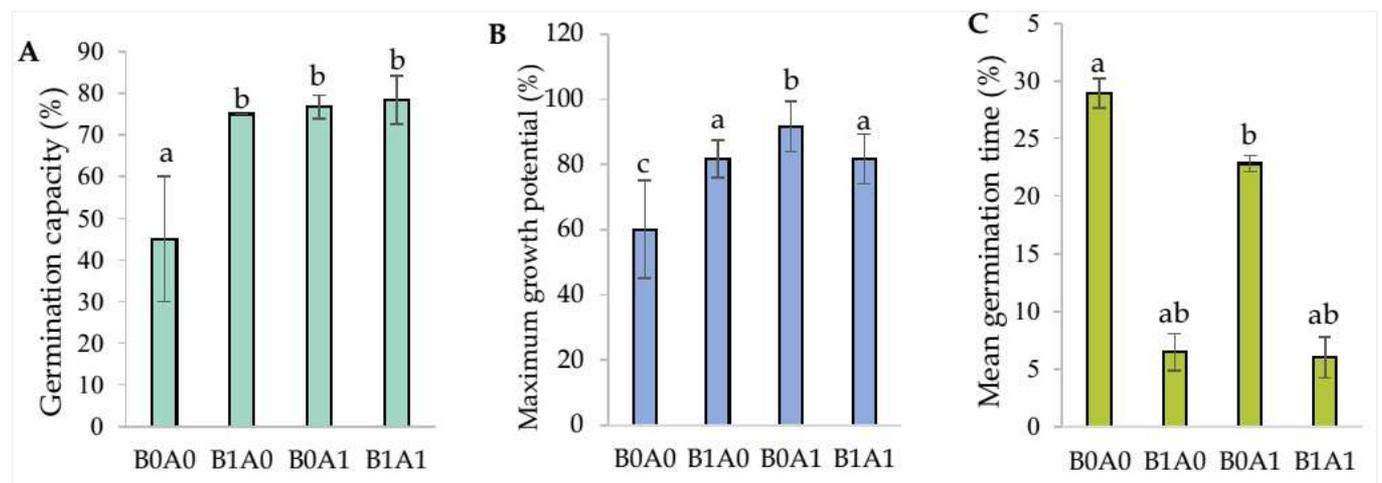

Figure 4. Interaction effect between generative material and microbial application on kopsia seed germination. (B0A0) Whole fruit without microbial application, (B1A0) Embryo without microbial application, (B0A1) Whole fruit with microbial application, and (B1A1) Embryo with microbial application

Research findings indicate that germination capacity, maximum growth potential, and mean germination time of kopsia seeds are influenced by both generative material and microbial application. The best germination performance was observed in treatments involving whole fruits with microbial application, as well as embryos with or without microbial application. Similar results were reported by Rasyad et al. (2019), where the germination success rate of kopsia seeds using whole fruits and endophytic fungi application reached 76%. This suggests that kopsia seeds generally possess good physiological quality and viability, enabling

successful germination under suitable environmental conditions. Consistent with Putri et al. (2022), the highest maximum growth potential was achieved through microbial treatments using rhizobacteria such as *Flavobacterium* sp., *Bacillus polymyxa,* and *B. licheniformis.* Comparable findings were also reported by Dewi et al. (2022), in the germination of *Gmelina arborea* Roxb. seeds under various microbial treatments. It is hypothesized that microbes, acting as biological agents, play a role in accelerating seed germination by facilitating seed softening through enzymatic particularly cellulase, protease, and pectinase. This aligns with studies by Widowati et al. (2023) and Putri et al. (2023), which demonstrated that these enzymes contribute to fruit softening. Widowati et al. (2018) further showed that pectinase and cellulase enzymes derived from *Bacillus* microbes were effective in clarifying fruit juice and accelerating mesocarp softening. The mesocarp of kopsia fruit is fibrous and contains bioactive compounds such as alkaloids, flavonoids, and tannins, which function to protect cells and enhance membrane permeability. Cellulase hydrolyzes cellulose, the main component of cell walls, thereby weakening fruit tissue structure. Pectinase breaks down pectin in the cell wall, leading to tissue softening (Widowati et al. 2023), while protease softens the texture and degrades structural proteins (Putri & Saptarini, 2023).

One possible reason why whole kopsia fruits without microbial application exhibited lower germination capacity, maximum growth potential, and longer mean germination time is the presence of thick and hard endocarp and exocarp layers. These rigid structures encase the embryo, hindering its emergence and delaying germination in the sowing medium. In contrast, whole fruits treated with microbial application underwent imbibition more rapidly than untreated fruits, which required more time to initiate germination.

Seed germination begins with physiological activity triggered by water uptake, as water plays a crucial role in activating metabolic processes. The smaller the mean germination time, the faster the seeds germinate (Wulandari & Farzana, 2020). The highest results observed across all microbial treatments further demonstrate that microorganisms can enhance enzymatic activity, stimulate cell enlargement, and improve nutrient absorption efficiency, factors that are essential for successful seed germination. Similar findings were reported by Rasyad et al. (2019), who showed that endophytic fungi significantly improved germination performance. A higher value of maximum growth potential reflects better physiological performance of the seed in completing the initial growth phase optimally.

Kopsia seeds can be germinated generatively using two approaches: whole fruits and isolated embryos. Each generative material has its own advantages and limitations. The advantage of using whole fruits is that no extraction process is required. However, its limitations include: (1) a longer germination period, and (2) the need for microbial applications to accelerate germination and improve the germination rate. On the other hand, the use of embryo-derived generative material offers the following advantages: (1) faster germination time, and (2) no requirement for microbial application to initiate germination. Its main limitation is the need for prior extraction of the embryo.

Seedling Vigor of Kopsia
Germination Rate

The research findings indicate that generative material significantly influenced the germination rate, while microbial application affected the vigor index. In this case, the vigor index of kopsia seeds was influenced by a single factor, namely generative material for germination rate, and microbial application for vigor index. No interaction was observed between microbial application and the type of generative material in affecting either variable (Table 3).

Table 3. Summary of Analysis Variance Results on the Effect of Microbial Application on the Vigor Index of Kopsia Seeds

Variable	Generative material (B)	Microbial application (A)	B x A
Germination rate (%)	*	tn	tn
Vigor index	tn	*	tn

Note: tn: not significant, *: significantly different at the 5% significance level

Embryo-derived generative material demonstrated a faster germination rate compared to whole fruit treatment. Meanwhile, microbial applications had a significant influence on the vigor index (Table 4).

The research findings revealed that the highest germination rate was obtained from treatments using embryo-derived generative material, compared to whole fruit treatments. Jasmi (2018) similarly reported that citrus seeds containing a single embryo exhibited superior growth performance compared to seeds with multiple embryos (whole fruit). This supports the conclusion that the use of embryo-based generative material can accelerate and enhance the germination process of kopsia seedlings. It is further indicated that the distribution of nutrient reserves within the embryo is sufficient and plays a crucial role in determining both

the speed and quality of germination. Embryo-derived seeds face no physical barriers to plumule emergence, unlike whole fruit seeds, where thick fruit layers may hinder the plumule from emerging. A higher germination rate reflects faster physiological activation and synchronized seedling emergence (Wulandari & Farzana, 2020). Rapid germination also contributes to better early seedling growth (Rasyad et al., 2019).

Table 4. Vigor Index and Germination Rate of Kopsia in Relation to Growth

Variable	Generative material		Microbial application	
	Whole fruit	Embryo	Non-microbial	Microbial
Germination rate (%)	0.55[a]	3.62[b]	3.43[a]	3.17[a]
Vigor index	227.33[a]	279.48[a]	196.98[a]	309.83[b]

Values in the same row followed by the same letter are not significantly different at the 5% significance level (Tukey's test)

Vigor Index

The vigor index of kopsia seedlings was higher in treatments involving generative material combined with microbial application, compared to treatments without microbes. Similar findings were reported by Handayani et al. (2024), who demonstrated that microbial utilization could enhance the vigor index of coffee seeds (*Coffea robusta* L.). This effect is presumably due to the active role of rhizosphere microbes in improving the physiological quality of seeds. Microbes capable of producing phytohormones and enzymes such as gibberellins and proteases may contribute to the acceleration of dormancy release and activation of the seed embryos (Mildaziene et al., 2022). Seeds with a high vigor index exhibit rapid and uniform growth (Farida et al., 2017). While high vigor is often associated with high germination rates, it is important to note that seeds with high germination rates do not necessarily possess high vigor.

Seedling Height and Length

The research findings indicate that neither microbial application nor generative material significantly affected the height growth of kopsia seedlings. However, microbial applications did influence the total seedling length. The vigor index of kopsia seedlings, which includes both height and length parameters, was affected by single factors only. No interaction was observed between microbial application and the type of generative material in influencing the germination process of kopsia (Table 5).

Table 5. Summary of Analysis of Variance Results on the Effect of Microbial Application on the Height and Length Growth of Kopsia Seedlings

Variable	Generative material (B)	Microbial application (A)	B x A
Height (cm)	tn	tn	tn
Length (cm)	tn	*	tn

Note: tn: not significant, *: significantly different at the 5% significance level

Based on the data presented, the comparison of treatments on kopsia seedlings showed similar responses in terms of height and length variables. The research findings indicated that seedling length was greater in treatments with microbial application compared to those without microbes (Table 6).

Table 6. Height and Length Values of Kopsia Seedlings Treated with Microbial Application

Variable	Generative material		Microbial application	
	Whole fruit	Embryo	Non-microbial	Microbial
Height (cm)	3.49[a]	3.62[a]	3.14[a]	3.97[a]
Length (cm)	7.77[a]	9.03[a]	7.30[a]	9.50[b]

Values in the same row followed by the same letter are not significantly different at the 5% significance level (Tukey's test)

The research conducted demonstrated that microbial application significantly influenced plant elongation, whereas plant height was not affected by either the type of generative material or microbial treatment. Similar findings were reported by Mangungsong et al. (2019), who observed optimal plant growth with the addition of microbes. This suggests that microbial application plays an active role in promoting phytohormone synthesis and contributes to enhanced stem elongation. Auxins and gibberelins are known to stimulate stem elongation and the rapid development of vegetative tissues (Murrinie et al., 2021). The increase in plant length not only accelerates growth but also improves tissue quality and enhances the absorption of essential macronutrients, such as nitrogen (N) and phosphorus (P), which are crucial for cell elongation. Moreover, microbial applications can support root elongation processes. Microbes can produce plant

growth hormones such as cytokinins, gibberellins, and indole-3-acetic acid (IAA), which stimulate cell division, regulate cell enlargement, and promote root development.

Total Fresh Weight and Total Dry Weight

The research findings indicate that the generative material significantly affected the variables of total fresh weight, total dry weight, and shoot root ratio. The microbial application did not show any significant effect on the observed variables. The biomass of kopsia seedlings was influenced solely by the generative material factor, with no interaction observed between microbial application and the type of generative material (Table 7).

Table 7. Summary of Analysis of Variance Results on the Effect of Microbial Application and Generative Material on Biomass Growth

Variable	Generative material (B)	Microbial application (A)	B x A
Total fresh weight	*	tn	tn
Total dry weight	*	tn	tn
Shoot root ratio	*	tn	tn

Note: tn: not significant, *: significantly different at the 5% significance level

The total fresh weight (TFW) and total dry weight (TDW) values obtained from the conducted research indicated that the highest TFW and TDW were recorded in treatments using whole fruit, while the lowest values were observed in treatments using isolated embryos (Figure 5).
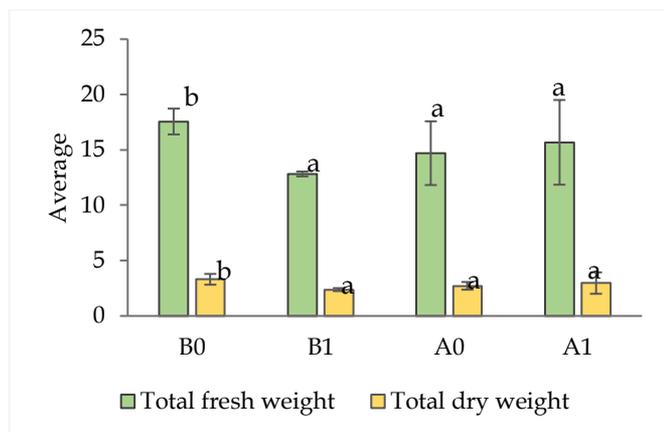

Figure 5. Total fresh and dry weight of kopsia seeds treated with microbial application for biomass development. (B0) whole fruit, (B1) embryo, (A0) non-microbial, (A1) microbial

The results indicated that the whole fruit treatment yielded the best value in supporting plant growth, as evidenced by increased fresh and dry biomass. Similar findings were reported by Surya et al. (2020), who showed that seed size and weight can enhance plant biomass accumulation. The observed increase in total fresh and dry weight of kopsia plants confirms that whole fruit material has strong potential to accelerate and strengthen biomass formation. This is presumably due to the higher nutrient reserves stored in whole fruit, resulting in more vigorous and robust seedlings that rely on the available food supply. Moreover, both internal and external factors associated with whole fruit material appear to function optimally, contributing to plant development through increased height and leaf number. Whole fruits, being larger than embryo-derived seeds, offer greater storage capacity and can supply sufficient nutrients (Astutik & Ardiarini, 2021). Higher dry weight production reflects better plant growth, indicating efficient photosynthesis and enhanced absorption of essential nutrients and water (Valentine et al., 2017).

Shoot Root Ratio

The research results showed that the highest shoot root ratio (SRR) was obtained from the embryo treatment, which was significantly higher than that of the whole fruit treatment. Kopsia seedlings across all treatments exhibited SRR values ranging from 1.9 – 2.7 (Figure 6).
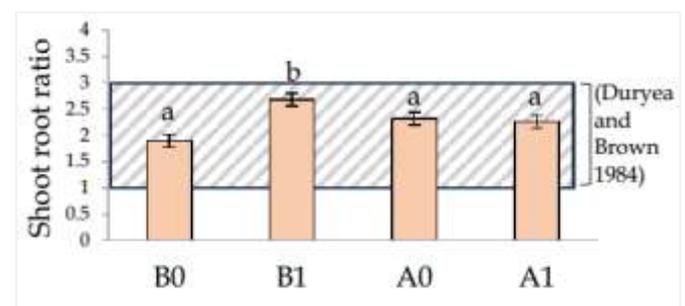

Figure 6. Shoot root ratio of kopsia seeds treated with microbial application for biomass development. (B0) whole fruit, (B1) embryo, (A0) non-microbial, (A1) microbial

The research findings indicated that kopsia seedlings derived from embryo treatment exhibited the highest shoot root ratio (SRR) compared to those from whole fruit treatments. This aligns with the study by Budi et al. (2020), which reported that an SRR greater than 1 in *Toon sinensis* Roem. Seedlings are considered favorable. According to Duryea et al. (1984), an optimal SRR typically ranges between 1–3. In this study, SRR values ranged from 1.9 to 2.7, indicating a healthy

balance in shoot–root development. This is attributed to root development in the soil. Both whole fruit and embryo-derived treatments demonstrated a balanced allocation of growth between shoot and roots. An SRR between 1,5 and 2,0 is generally regarded as ideal during the early growth phase, as it reflects a proportional distribution of energy and photosynthetic products between above and below ground organs. Vigorous shoot growth is supported by a strong root system, which enhances nutrient and water uptake, thereby promoting faster and more robust plant development. Additionally, embryo-derived seeds are presumed to have higher water absorption capacity, which may contribute to accelerated plant growth. A high SRR is indicative of rapid and vigorous seedling development (Hariyadi et al., 2023).

Conclusion

The generative material of kopsia significantly affected germination and early growth, as indicated by germination capacity, mean germination time, germination rate, total fresh weight, total dry weight, and shoot–root ratio. The application of rhizosphere microbes also had a significant effect on germination capacity, mean germination time, maximum growth potential, vigor index, and seedling length. The use of whole-fruit-derived generative material required the support of rhizosphere microbes to accelerate the germination process and improve early seedling growth quality, whereas when the generative material was derived from embryos, microbial application was not a determining factor for successful germination, although it still had the potential to enhance seedling vigor and growth.

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