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Potential propagation seedling of ramie (*Boehmeria nivea*) from various types of stem cuttings

Abstract. Ramie is a fiber-producing plant that can replace cotton as a raw material for the textile industry. In general, ramie plants are reproduced using rhizomes, but it takes a long time, around two years, to be used as a source of planting material. Therefore, other sources of explants besides rhizomes are required, such as stem cuttings. Explants from stem cuttings are quickly available and only need 2-3 months to be used as planting material. This study aims to determine the potential of effective stem cuttings that can be used as planting material for the propagation of ramie seedlings. The research was conducted from July to December 2021 at the experimental garden in the Indonesian Instruments Standardization Testing Center for Sweetener and Fiber Crops, Malang, East Java. The research used a Completely Randomized Design (CRD) consisting of four treatments of ramie seedling material of the Ramindo 1 variety, namely rhizome, shoot cuttings, middle stem cuttings, and basal stem cuttings. Each treatment was repeated 5 times. The result showed that all parameters had a significant effect. Ramie seedling source from basal stem cuttings showed the best growth percentage (92%), stem diameter (4.8 mm), and plant wet weight (44.6 g). Shoot cuttings showed the best number of roots (31.6), plant height (60.8 cm), and number of leaves (41.4). Cuttings from shoot and basal stems can be used as planting material for producing ramie seeds other than rhizomes.

Keywords: Basal stem · Natural fiber · Ramindo 1 · Rhizome

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Introduction

Natural fibers are widely developed in many countries, including Indonesia, which can produce biomass such as jute, flax, cotton, abaca, sisal, kenaf, and ramie, which may be biodegradable. (Elfaleh et al., 2023). Although ramie plants have long been developed in China since 5000-3000 BC (Sen & Reddy, 2011), they have also been widely developed in Japan, the Malay Peninsula (Bayan et al., 2018), India, and Indonesia (Roy & Lutfar, 2020). Ramie fibers are extensively used in the textile industry. (Ullah et al., 2016; Bakshi et al., 2024). Ramie stems can be turned into paper pulp, biocomposites, catalysis, sound absorption, biomedical materials, and fuel. (Sharma et al., 2014; Zhong et al., 2020; Cho et al., 2022), leaves can be used as compost and high-nutrient animal feed (Tang et al., 2021), and rhizomes (roots) are used as planting materials (Nuraini et al., 2022).

The development of ramie to support the textile industry and other textile products has not been optimal due to the lack of use of the superior Ramindo 1 variety. This variety has the advantage of a high fiber yield (Maideliza et al., 2017) and high fiber productivity, reaching 2-2.7 tons/ha/year (Novarini & Sukardan, 2015). Additionally, the availability of sufficient and high-quality seeds is still very limited. Meanwhile, the need for ramie per hectare requires seeds with a minimum standard of 20000 - 25000 rhizomes per hectare. Currently, ramie development is limited to Central Java, West Java, Lampung, and South Sumatra, so the expansion of planting areas needs to be optimized. A sustainable supply of ramie seedlings is needed to support this. However, it is not in line with the current ramie seedling supply. This is because ramie seedlings can only be provided through conventional methods using rhizomes (Nuraini et al., 2022) from plants that are more than 2 years old. A rhizome is a part of the plant located below the soil surface, serving a different function than roots or radicles, and has horizontal growth (Genosko, 2020). So, producing ramie seedlings takes a long time and the rhizomes cannot be stored for long.

One of the efforts to propagate ramie seedlings can be done vegetatively by utilizing the plant stem cuttings. Ramie is a perennial plant that can be harvested every 60 days by cutting the base of the stem without uprooting the plant (Subandi, 2012). The appropriate stem-cutting method can produce seedlings at a low cost, with high viability, in large quantities, and uniformly (Apriani & Suhartanto, 2015).

Propagation of seedlings through stem cuttings can use the stem from the shoot, stem cuttings from the middle stem, and stem cuttings from the basal stem. The selected stems should have a uniform diameter and have turned brown (Bayan et al., 2018). It is hoped that all types of stem cuttings have the potential to be used as seedling sources of the same quality as rhizomes so that no part of the ramie plant stem is wasted.

The previous studies by Suherman, et al. (2016; 2017) often used rhizomes cut to a length of 10-15 cm from the Ramindo 1 variety in the propagation and production of ramie. Using stem cuttings from the shoot with a length of 7 cm is the best stem cutting that can be used as planting material. Shoot cuttings can produce vigorous *Plectranthus amboinicus Spreng* stem cuttings based on the success components of the cuttings and their growth (Apriani & Suhartanto, 2015). Stem cuttings from the basal stem can provide the best number of new leaves and root fresh weight in *Alstonia scholaris* plants, although not significantly different from other treatments (Putri et al., 2018). This study aims to determine the potential of effective stem cuttings that can be used as planting material for the propagation of seedlings of ramie.

Materials and Methods

Plant Material. The experiment was conducted at the experimental garden in Indonesian Instruments Standardization Testing Center for Sweetener and Fiber Crops, Malang, East Java, from July to December 2021. The ramie plants used were of the Ramindo 1 variety, which was 2 years old. Rhizome collection was carried out by digging up the roots of the ramie plants and washing them with water until clean. The planting material used for stem cuttings is rhizomes obtained from the root part of the plant that is 2 years old, characterized by having straight stems, visible buds, and a dark brown color with a diameter of about 0.5–0.8 cm and a rhizome length of 10 cm. Stem cuttings from the tip, middle, and base parts are obtained from ramie stems that are 2 months old, with a diameter of 0.3–0.5 cm and a stem length of 10 cm. The base stem cuttings have the criteria of a dark brown stem, the middle stem cuttings have a brownish-green color, and the tip cuttings are characterized by having growth points

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and retaining 2–3 leaves that are still growing (Figure 1).

Figure 1. Types of ramie stem cuttings whose basic explant sources come from: propagation from rhizomes (a), propagation from shoot stems (b), propagation from middle stem cuttings (c), and propagation from base stem cuttings (d).

Seedling sowing. The planting materials are collected and selected according to the criteria for the stem-cutting treatment used. Each type of stem cutting was sown in pot trays filled with a growing medium consisting of soil, compost, and mixed rice husk charcoal in a ratio of 1:1:1. Before planting, the stem cuttings were dipped in a *Rootone F* solution at a dose of 20 g per L for 5 minutes as a root primordia-stimulating hormone.

The pot trays sowed with ramie stem cuttings were then covered with a plastic polyethylene transparent cover to maintain temperature (20-30°C) and humidity (60 – 80%). The maintenance during the ramie cuttings nursery included watering, weeding, and pest control by spraying fungicides with the active ingredient *Mancozeb* at 2 g per L and insecticides with the active ingredient *Carbosulfan* at 5 g per L.

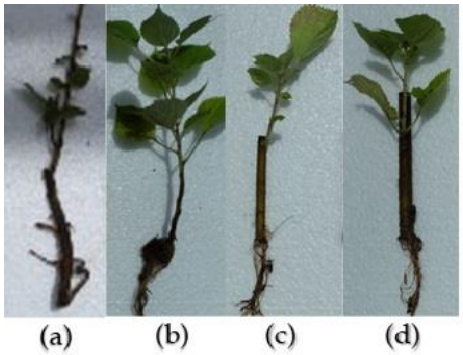

Figure 2. Ramie seedling 4 weeks after planting: rhizome (a) Shoot (b), middle stem cutting (c), and basal stem cutting (d).

Seedling planting. Transplanting was carried out when the ramie seedlings were 4 weeks old (Figure 2) into polybags measuring 20 cm x 20 cm filled with a growing medium consisting of a 1:1 mixture of soil and rice husk charcoal. Transplanting was done in the afternoon, and the ramie plants were watered until the polybags reached field capacity.

Plant maintenance included watering, manual weeding, and pest and disease control by spraying fungicides with the active ingredient *Mancozeb* at 2 g per L and insecticides with the active ingredient *Carbosulfan* at 5 g per L in 6, 8, and 10 weeks after planting (WAP). Fertilization was also carried out by applying NPK Mutiara fertilizer at 3 grams per plant at 2 and 6 WAP.

Observations. Observations were made on the growth percentage (%) by counting the number of seedlings that grow, dividing it by the number of seedlings planted, and multiplying by 100% at 4 weeks after planting (WAP), shoot length (cm) at 4 WAP, while plant height (cm), number of leaves, root length (cm), number of roots, stem diameter (mm), and plant fresh weight (g) were observed at 12 WAP.

Data analysis. The experimental design used a Completely Randomized Design (CRD) with a single factor, which was the type of stem cutting consisting of four types: rhizome, shoot cuttings, middle stem cuttings, and basal stem cuttings. Each treatment was repeated 5 times, resulting in 20 experimental units. Data were analyzed using analysis of variance (ANOVA) with SAS version 9.4 for Windows (SAS Institute Inc, 2023). The significant difference in the mean value of the treatment was further tested using Tukey's Honestly Significant Difference (HSD) at a significant level of 5%.

Results and Discussion

Growth percentage and shoot length. Plant growth can begin with germination, marked by the emergence of the radicle, which produces roots, and the plumule, which forms shoots. The germination process affects the survival rate of seedlings and plant growth, which is also influenced by the climate (Haj Sghaier et al., 2023). The treatment of different types of ramie stem cuttings showed a significant effect on the growth percentage of ramie stem cuttings (Table 1). Ramie stem cuttings from the basal stem showed the highest growth percentage, reaching 92.4%, but this was not significantly different from the rhizome cuttings, which had a growth percentage of 87.8%.

Table 1. Effect of stem cutting types on growth percentage and shoot length ramie seedlings

Type of stem cuttings	Growth percentage (%)	Shoot length (cm)
Rhizome	87.8 ab	15.0 a
Shoot cuttings	85.8 b	11.0 b
Middle stem cuttings	80.4 c	11.0 b
Basal stem cuttings	92.4 a	7.2 c
Mean	86.6	11.1
Coefficient variance	3.9	13.9
P-value	0.0009**	<.0001**

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Tukey's Honestly Significant Difference (HSD) test at the level of 5 %.

The high growth ability of ramie seedlings originating from the basal stem indicates that the basal stem has more mature tissues ready to support root growth compared to the upper or middle stem cuttings. The basal stem cutting also often has more carbohydrate reserves and nutrients, such as hormones that can stimulate the growth of new shoots and roots. This is consistent with the findings of Husen (2012), which stated that carbohydrate reserves in the stem promote rooting when combined with effective auxin treatment. Additionally, the average germination percentage of seedlings reached 86.6%, categorizing ramie stem cuttings as high-quality seeds. On the whole, a seed germination percentage rate above 80% is considered quite high and indicates superior seed quality (Bobokalonov & Cheryomushkina, 2018). This result also aligns with the research by Lodhiyal, et al. (2023) which found that vegetative propagation of some plants through stem cuttings showed a success rate of over 80%.

The type of ramie cutting showed a significant effect on the shoot length of ramie at 4 WAP. Stem cutting using rhizomes showed the best shoot length, reaching 15 cm, which was significantly different from the other stem cutting treatments. The shoot length resulting from stem cuttings can significantly affect the growth and development of the plant. Cuttings from longer rhizomes usually have more energy reserves in the form of starch, which can form shoots and elongate roots. Similarly, highland plants can store fructans as carbohydrate reserves in their roots during winter to produce tuna in the following season (Yoshida, 2021). The photosynthesis results obtained by each plant will affect the amount of photosynthesis in the form of carbohydrates. These carbohydrates are also abundant in rhizomes, where the rhizome is the main factor influencing shoot development (Suherman et al., 2017).

Plant height and number of leaves. Table 2 shows that the type of ramie stem cutting has a significant effect on plant height and the number of leaves. Shoot cuttings showed the highest plant height, reaching 60.8 cm, but this was not significantly different from the middle stem and basal stem cuttings. The same pattern was observed in the number of leaves, with shoot cuttings having the highest number of leaves, 41.4 leaves, which was significantly different from the other stem-cutting types.

Increased plant height and the number of leaves can have a significant impact on the overall growth of ramie plants. The highest plants with more leaves often have better access to light and can utilize more nutrients from the growing medium. According to Tanaka et al., (2006) in *Pisum sativum L*, auxin plays a role in inhibiting cytokinin biosynthesis in the nodal stem to suppress the growth of axillary buds and enhance apical dominance. Auxin is the main hormone that controls the occurrence of apical dominance, primarily playing a role in the long-distance polar auxin transport system and local auxin biosynthesis in modulating shoot branching (Thelander et al., 2022). The research by Apriani & Suhartanto (2015) showed that shoot cuttings also produced the best plant height and number of shoots compared to cuttings from the middle and basal stems in *Plectranthus amboinicus Spreng* plants.

Root length and number of roots. The plant's ability to absorb nutrients and water can be determined by root growth. The elongation of plant cells, involving the cell wall and turgor pressure on the cell wall, can lead to root emergence. The ability of leaves to photosynthesize actively can influence the length and number of plant roots (Rayburn & Sharpe, 2019; Ye et al., 2023). The type of stem-cutting treatment showed a significant effect on root length and the number of roots (Table 3).

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Table 2. Effect of stem cutting types on plant height and number of leaves ramie seedlings.

Type of stem cuttings	Plant height (cm)	Number of leaves
Rhizome	43.8 b	29.4 c
Shoot cuttings	60.8 a	41.4 a
Middle stem cuttings	58.0 a	30.4 bc
Basal stem cuttings	60.2 a	33.8 b
Mean	55.7	33.7
Coefficient variance	3.9	8.1
P-value	<.0001**	<.0001**

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Tukey's Honestly Significant Difference (HSD) test at the level of 5 %.

Table 3. Effect of stem cutting types on root length and number of roots ramie seedlings.

Type of stem cuttings	Root length (cm)	Number of roots
Rhizome	11.0 a	30.4 a
Shoot cuttings	4.2 b	31.6 a
Middle stem cuttings	5.4 b	12.8 b
Basal stem cuttings	5.2 b	15.2 b
Mean	6.5	22.5
Coefficient variance	23.0	19.0
P-value	<.0001**	<.0001**

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Tukey's Honestly Significant Difference (HSD) test at the level of 5 %.

Rhizome cuttings showed the best root length, which was 11 cm, and this was significantly different from the other stem-cutting treatments. Meanwhile, for the number of roots parameter, the shoot cuttings showed the highest number of roots, reaching 31.6, but this was not significantly different from the rhizome cuttings.

A rhizome is a horizontally oriented stem (some species have vertical positions) that grows underground and can produce roots and shoots at its nodes. Rhizomes function as nutrient storage for the vegetative propagation of the parent plant and the distribution of plant species (Li et al., 2022; Stedeford, 2023; Petruzzello, 2024). The root length and number of roots in stem cuttings derived from rhizomes are quite good compared to other stem cuttings because the surface of the rhizome has many thin meristematic tissues capable of forming roots. According to Guo, et al. (2021), rhizomes have apical meristem tissues with thick epidermal surfaces to protect the tissues and aid in pushing through the soil. The nodes on ramie rhizomes produce additional roots to expand the plant's root system.

The roots of shoot cuttings are fibrous and have younger tissues, making it easier for roots to emerge and resulting in a higher number of roots than other stem cuttings. The research by Apriani & Suhartanto (2015) showed that stem cuttings taken from the shoot had better root systems in *Plectranthus amboinicus Spreng* plants compared to cuttings taken from the middle and basal stem. In *Gyrinops versteegii* plants, the best rooting was also observed in shoot cuttings with soil media and the use of IBA at a concentration of 200 ppm (Setyayudi, 2018).

Stem diameter and plant fresh weight. A uniform stem diameter can provide better structural stability and support yield production without the risk of damage. Ohta & Makino (2019) stated that a uniform and upright stem diameter can enhance plant production in supplying fruit to meet market demand. The type of cutting treatment showed a significant effect on increasing the stem diameter of ramie plants. Stem cuttings from the basal stem provided the highest stem diameter, which was 4.8 mm, although it was not significantly different from the other treatments except for the rhizome. The basal stem has a slightly larger diameter compared to other stem cuttings. The research by Suherman, et al. (2016) showed that the average stem diameter of Ramindo 1 ranged from 34-53 mm at 12 WAP. The length and diameter of stem cuttings can affect the efficiency of rooting emergence (Lebedev, 2019). Producing the best stem cuttings is also influenced by the cutting method and the diameter of the stems used (Kumar et al., 2023).

Table 4. Effect of stem cutting types on Stem diameter and plant fresh weight ramie seedlings.

Type of stem cuttings	Stem diameter (mm)	Plant fresh weight (g)
Rhizome	4.2 b	37.8 b
Shoot cuttings	4.6 ab	38.8 b
Middle stem cuttings	4.6 ab	39.4 b
Basal stem cuttings	4.8 a	44.6 a
Mean	4.6	40.2
Coefficient variance	7.2	5.8
P-value	0.0087**	0.0027**

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Tukey's Honestly Significant Difference (HSD) test at the level of 5 %.

The high fresh weight of the plant indicates greater biomass production and correlates with increased yield obtained from the photosynthates produced and allocated for the growth of fruit or seeds. Du, et al. (2021) stated that the fresh weight and dry weight of the plant can illustrate the overall accumulation of plant biomass and serve as good indicators of plant growth. Table 4 shows that the type of stem cuttings has a significant effect on the fresh weight of the plant. Stem cuttings from the basal stem showed the best plant fresh weight, which was 44.6 g, and this was significantly different from other types of cuttings.

The larger the stem diameter, the higher the plant's fresh weight, and this relationship is linear. The potential plant's fresh weight derived from basal stem cuttings tends to be higher because the diameter of the basal stem gradually decreases from the lower base toward the top of the plant. The level of plant fresh weight accumulation in each stage of plant growth shows an increasing trend (Bai et al., 2020). This is thought to be due to the presence of organic or chemical substances in plant tissue. In the chemical analysis of fiber, 71.75% cellulose, 12.11% hemicellulose, 1.06% lignin, and 1.70% ash were found (Marinho et al., 2018). Research by Suherman et al. (2017) showed that Ramindo 1 had the best fresh stem and leaf weight and was not significantly different from Bandung A clone ramie. Research by Putri, et al. (2018) also showed that stem cuttings from the base of the stem had a high fresh root weight in Alstonia Scholaris and were not significantly different from other treatments.

Conclusion

The type of stem cutting from the basal stem showed the best growth percentage (92.4%), stem diameter (4.8 mm), and plant fresh weight (44.6 g). Shoot cuttings showed the best number of roots (31.6), plant height (60.8 cm), and number of leaves (41.4). Cuttings from shoot and basal stems can potentially be used as planting material for the production of ramie seeds other than rhizomes.

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