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GROWTH PERFORMANCE AND CHLOROPHYL CONTENT OF MORINGA OLEIFERA CUTTING STEM AS AFFECTED BY PEAT SOIL-REDUCED GROWING MEDIA AND HORMONE

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ABSTRACT
Moringa oleifera (Lam.) has become highly valued. Vegetative propagation methods can address the limitations and make the cultivation process simpler. Our research aimed to determine the best conditions for early growth and chlorophyll content of *Moringa oleifera* cutting stems through the use of different planting media and hormone applications. A 3x3 factorial pot experiment design was used, with three types of growing media (100% peat soil (PS), 50% peat soil (PS) + 50% mushroom baglog waste (MBW), and 50% peat soil (PS) + 50% husk charcoal (HC) and three hormone treatments (coconut water, atonik (3 ml L^{-1} water), and 100 ppm rootone-F) being tested. The experiment was carried out in a completely randomized design with 3 replications. Results showed that the use of a 50% peat soil (PS) + 50% husk charcoal (HC) and the application of rootone-F significantly had a significant impact on the chlorophyll content. Neither the use of 50% peat soil (PS) + 50% husk charcoal (HC) nor the application of rootone-F, singly, was favorable for promoting bud length, root volume, and chlorophyll content. It is recommended to use 50% peat soil (PS) + 50% husk charcoal (HC) media and rootone-F for successful vegetative propagation and subsequent domestication.

KEY WORDS
Moringa, cutting, peat soil-reduced, growing media, hormone.

Moringa oleifera (Lam) is a well-known exotic multipurpose tree species with tremendous potential uses (Ezekiel et al. 2013). There is an excellent source of vitamins, carotenoids, essential amino acids, and minerals in the leaves of this plant (Zheng et al. 2019). It is a fast-regenerating native tree (Huda et al. 2017) in tropical and subtropical regions. Drumstick and horseradish trees belonging to the Moringaceae family (Devkota and Bhusal 2020) are commonly known as "Kelor" in Central Kalimantan, Indonesia, where they are traditionally planted as the backyard or farm forestry without fertilizer, pesticides, or artificial irrigation. *Moringa oleifera* grows best in a wide variety of soil types with neutral pH levels. It is most commonly grown in lowland areas, but it also grows well in slightly alkaline clay soils and sandy loam soils due to their favorable drainage (Gandji et al. 2018). Foliage biomass can be obtained through direct seeding, transplanting, or stem cutting (Huda et al. 2017).

Gandji et al. (2018) identified the following constraints in *Moringa oleifera* cultivating, one of them was a lack of knowledge of efficient production practices. Regarding the practice, the procurement of planting materials, nutrition, and hormone could be used as improved models. Akanbi et al. (2019) stated that to successfully vegetative propagation, it is essential to determine the role growth hormones, diameter magnitude play, and growth media. The growing media needed for *Moringa oleifera* is nutrient-enriched media (Zayed 2012; Zaku et al. 2015). Using peat soil, which is abundant in Central Kalimantan, as a growth medium is one of the challenges due to its poor physical, chemical, and biological properties. Peat has traditionally been used alone or in combination with inorganic (Méndez et al. 2015) and organic (Messiga et al. 2021) components due to the excellent combination of peat properties such as low pH, cation exchangeability, and porosity improvement. The encouraging results of (Ceglie et al. 2015) in organic seedling production showed three organic materials could maintain desirability >80% for all vegetable seedlings due to 31% peat reduction.

Moringa oleifera seedlings can be established quickly due to the physical, chemical, and biological properties of the media composition (Zayed 2012; Rufai et al. 2016; Akanbi et al. 2019; Ubaidillah et al. 2020). Growing media influences the extensive functional rooting system, seed germination, seedling growth, and vigor (Ubaidillah et al. 2020). A good growing media provide sufficient nutrient and water to allow oxygen diffusion and gas exchange between roots and their surroundings. Ibrahim et al. (2020) recommended the mixture of organic manure and topsoil to improve seed germination and early growth, similarly, Ede et al. (2015) proposed that sawdust plus poultry manure resulted in an improvement in the percentage of the survival seedling in the nursery in terms of seedling emergence velocity, plant height, girth stem, and leaf number. Even more, the combination media of (compost + NPK) could improve the biochemical, phytochemical, and antioxidant activities of *Moringa oleifera* (Sarwar et al. 2017).

Several researchers indicated that hormones or growth regulators influence the performance of *Moringa oleifera* sprouting and early growth by enhancing their rooting abilities that were difficult to root under ordinary circumstances (Antwi-Boasiako and Enninful 2011; Katoriya et al. 2021). (Muazzinah and Nurbaiti 2017; Ajie et al. 2019) have discovered that coconut water contains auxin, gibberellin, and cytokinin. Auxins can elongate plant cells, gibberellins can stimulate cell division and elongation, or both, and cytokinins can increase growth in plants by supporting cell division (Sembiring et al. 2017). Moreover, the use of coconut water as a plant growth regulator is affordable, easy to obtain, safe for health, and still effective (Zuhro and Hasanah 2017; Juanda et al. 2018; Mauguru et al. 2019; Rokhmah 2020; Andayani 2020; Azizah et al. 2021; Hasti et al. 2022; Lestaluhu et al. 2022). In 1000 mL of coconut water, there were 0.07 mg of auxin, 5.8 mg cytokinin, and a trace of gibberellin among other compounds (Rokhmah 2020; Ningsih and Nugroho 2021). Rootone-F and atonik are synthetic commercial auxin (Amelia et al. 2020; Marpaung et al. 2022; Najoan et al. 2022; Siswanto et al. 2022).

By encouraging the formation of adventitious roots in stem cuttings, auxin is the most effective rooting aid for *Moringa oleifera* (Bukar et al. 2015; Katoriya et al. 2021). Additionally, Krishnan (2019) argued that plant hormones could stimulate and modify the system of natural growth by preventing the flower and fruit drop, resulting in yield increases. Consequently, this study focused on evaluating how peat soil-reduced media and hormone influence *Moringa oleifera* stem-cutting growth performance and chlorophyll content to ensure growth availability.

MATERIALS AND METHODS OF RESEARCH

A pot experiment was performed at the Agronomy Department, University of Palangka Raya, Central Kalimantan, Indonesia located at latitude of S 2̊ 12’42” and longitude of E 113̊ 54’15” in March-May of 2021. Chlorophyll was measured using a spectrophotometer at the Agronomy Department of the University of Palangka Raya. The initial peat soil properties were evaluated at the Integrated Laboratory of the University of Palangka Raya.

The experiment consisted of two treatments under a completely randomized design with three replications. The first treatment included different planting media of 100% peat soil; 50% peat soil + 50% baglog mushroom waste; 50% peat soil + 50% husk charcoal, while, the second treatment was hormone application including coconut water, atonik (3 ml L^{-1}), and 100 ppm rootone-F.

Several fresh healthy cuttings (25 cm long, 2 cm diameter) were taken from a local accession mother tree aged 4 years in Palangka Raya. Coconut water was obtained from the local area. Rootone-F is a white flour containing Indole-3-Butyric Acid (IBA), Naphthalene Acetic Acid (NAA), and Indole Acetic Acid (IAA) (Meilawati and Purwiyanti 2020), while, the active substances in atonik are sodium arthonitrophenol, sodium paranitrophenol, sodium 2.4 dinitrophenol, IBA (0.057%), and sodium nitrogulacol (Siswanto et al. 2022).

The undisturbed peat soil was taken from Kalampangan village at a depth of 20 cm. Before being used as a growing media, the peat soil was wind-dried for a week and then filtered through 2 mm mesh. Base ameliorants of chicken manure and dolomite were applied at dosages of 20 t ha^{-1} (50 g $polybag^{-1}$) and 2 t ha^{-1} (5 g $polybag^{-1}$), respectively.

These cuttings were rinsed with fungicide (1 mg furadan to 10 liters of water), dried, and then immersed in solutions of coconut water, 3 ml L^{-1} of atonik, and 100 ppm of rootone-F as determined by Total Dissolve Solutions for 30 minutes. They were then planted inside a polybag filled with three types of growth media of 100% peat soil (PS); 50% peat soil (PS) + 50% mushroom baglog waste (MBW); 50% peat soil (PS) + 50% husk charcoal (HC) at a depth of 1/3 cuttings into the soil. The treated polybags were spaced 20 cm apart. In a shade house, the cuttings were manually watered with 250 ml of uniformly distributed water each day. By pulling weeds manually, weeding was accomplished. A chlorophyll analysis was carried out by weighing 1g of *Moringa oleifera* leaves, grinding them, filtering the results, and pouring them into a test tube that already contained 9 ml of 85% acetone. The mixture was then analyzed with a Genesys 30 spectrophotometer wavelength 663 and 645 nm.

During a two-month trial, growth parameters (bud length and bud number) were measured. Bud length was measured weekly for eight weeks from the initial sprouting bud to the bud tip. A weekly count of buds was conducted for 8 weeks. During harvest, data were collected on root volume and chlorophyll content. To determine root volume, a volumetric flask was filled with water and the change in the amount of water displacement due to the root was measured. Spectrophotometers were used to determine the chlorophyll content. Qualitative observations were made regarding root patterns.

Quantitative data recorded were statistically analyzed using MS excel, and then the results were subjected to ANOVA. The treatment differences were tested for significance using the LSD test at a 0.05 probability level. The quantitative data was presented in both figure and table, while qualitative data was delivered in a figure and explained.

RESULTS OF STUDY

Peat used for growing media had the following properties (Table 1). Peat soil has a high soil acidity (pH 3.36). Nitrogen is a vital macronutrient for crops, but it is often lacking in agricultural soils as indicated by the low N content in Table 1 (0.53%), conversely, the C-org is very high due to peat soils are formed from various litters. Maftu’ah et al., (2019) reported a substantial decrease in nitrogen levels of around 0.6% in peatlands that have undergone extensive cultivation.

Table 1 – Chemical properties of peat soils used as growing media

Chemical properties	Value	Category*
pH H2O	3.36	Very acid
C-org (Walkley & Black)	1674%	Very high
Total N (Kjeldahl)	0.53%	Low
P_2O_5 (Bray I)	90.05 ppm	High
CEC (*N* NH4OAc pH 7.0)	13.30 $cmol_c\ kg^{-1}$	Low
Base saturation	55.94%	Medium
Ca-available	1.44 $cmol_c\ kg^{-1}$	Very low
Mg-available	4.95 $cmol_c\ kg^{-1}$	High
K-available	1.04 $cmol_c\ kg^{-1}$	Very low
Na -available	0.15 $cmol_c\ kg^{-1}$	Low

**Institute of Soil Research, 2009.*

There is a high of available P, suggests that the peat soil being used has matured. The availability of P in the soil depends on the quantity of P in the adsorption complex, which is influenced by the pH and the amount of organic matter present, regulating its availability. The reason for the low CEC value is due to the presence of ombrogenous peat soil with low pH.

Furthermore, the CEC value is significantly influenced by the ratio of lignin and humic substances present in the soil. The cationic bases (K, Ca, Mg, and Na) are essential for promoting plant growth. According to Table 1, the available K was determined to be quite low. The amount of K present in peat soils can differ depending on the extent of peat decomposition and mineralization, as noted by (Maftu'ah et al., 2019).

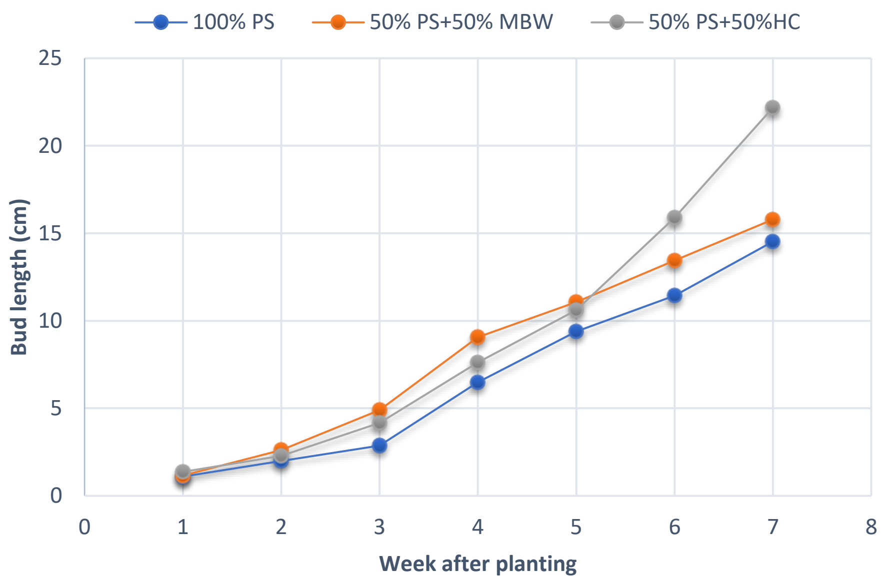


Figure 1 – Effect of growing media on the bud length at 1-7 WAP

Substituting of 50% peat soils by 50% HC resulted in the highest bud length, followed by 50%PS+50% MBW, and 100%PS. During the first five weeks, the 50% peat soils-reduced adding by 50% MBW exhibited the most rapid growth in bud length. However, during the sixth and seventh weeks, the growing medium consisting of 50%PS+50%HC promoted the greatest growth in bud length.

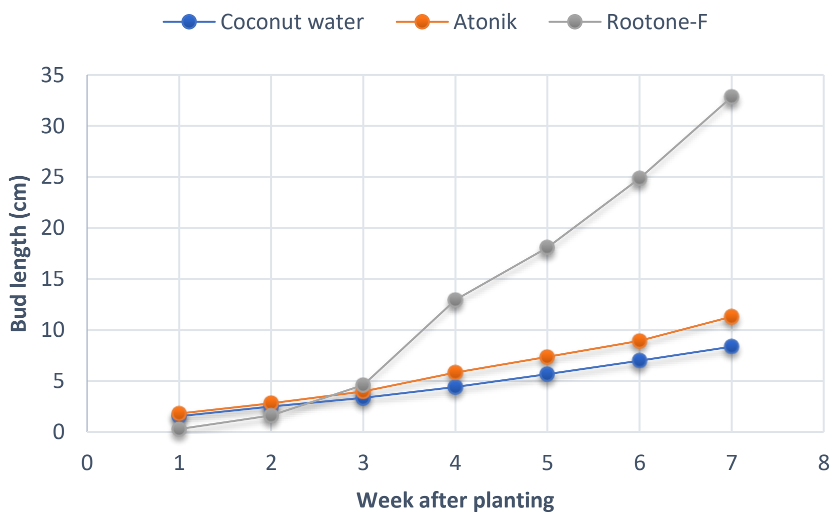


Figure 2 – Effect of hormones on the bud length at 1-7 WAP

In figure 2, the impact of rootone-F on bud length appears distinct compared to the effects of atonik and coconut water. Rootone-F was observed to promote the growth of bud length from the third week up to the seventh week. Figure 3 demonstrates that 50% PS + 50% HC yielded a greater quantity of buds compared to the other treatments, meanwhile, coconut water was a highly effective treatment for promoting an increased number of buds in Moringa at 1-7 weeks (Figure 4).

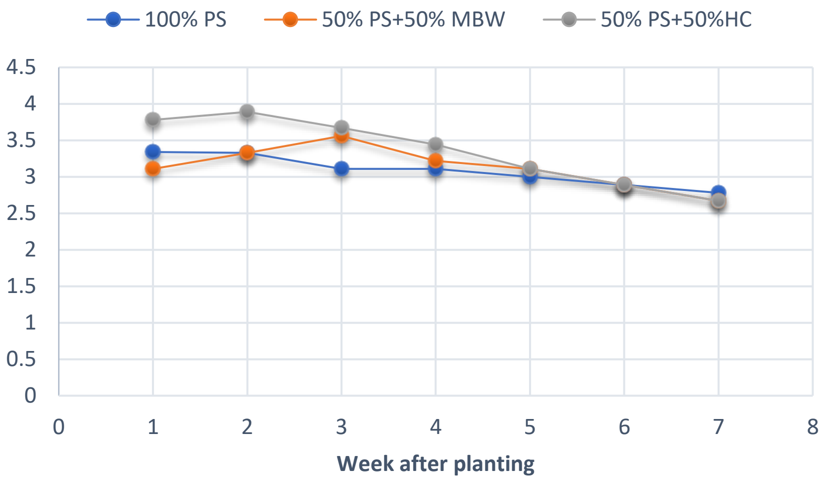


Figure 3 – Effects of growing media on bud number at 1-7 weeks

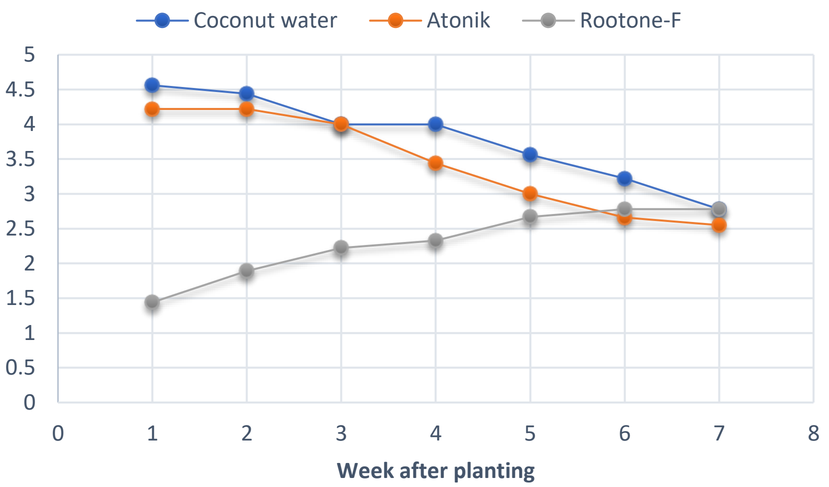


Figure 4 – Effects of hormones on bud number at 1-7 weeks

These differences in growth may be due to the varying organic components in the growth media. The presence of half-HC in peat soil led to more favorable growth than the use of only 100% PS, since it led to maximum bud length and root volume. The significant improvement in terms of bud length and root volume was about 70.44% and 47.39% respectively. Additionally, the use of half-MBW resulted in an 8.65% increase in bud length compared to using only 100% PS. However, an unexpected decrease in the number of buds was observed as a result of both organic matter applications (Table 2). Despite having no statistical significance, the combination of 50% PS + 50% HC produced the highest root volume followed by 100% PS and the combination of 50% PS + 50% MBW (Table 2).

Table 2 – Effects of growing media composition on the growth of Moringa oleifera L.

Treatment	Bud length (cm)	Bud number	Root volume (ml)
100% PS	16.54 a	2.78	2.11
50% PS + 50% MBW	17.97 a	2.67	1.78
50% PS + 50% HC	28.19 b	2.67	3.11

Note: Means along the column with the same letter are statistically non-significant according to LSD test at α=0.05.

The ANOVA results revealed that the single treatment of planting media has no significant effect ($p>0.05$) on root volume, and vice versa in the case of hormones. Among the treatments, rootone-F produced the significantly highest root volume and bud length with an increase of 210% and 89% compared to coconut water and atonik, respectively (Table 3). In comparison with coconut water, atonik resulted in 30.58% and 63.93% increased bud length and root volume, but not significantly. Contrarily, coconut water works as a natural hormone, resulting in the shortest roots volume and bud length but not the number of buds (Table 3).

Table 3 – Effects of hormone on the growth of Moringa oleifera L.

Treatment	Bud length (cm)	Bud number	Root volume (ml)
Coconut water	9.81 a	2.78	1.22 a
Atonik (3ml L^{-1} water)	12.81 a	2.44	2.00 ab
Rootone-F (100 ppm)	40.08 b	2.89	3.78 b

Note: Means along the column with the same letter are statistically non-significant according to LSD test at α=0.05.

The amount of chlorophyll in biochemical reactions, which play a principal role in photosynthesis, is significantly affected by a combination of planting media and hormone treatment ($p<0.05$) when using a diameter of 2 cm and a length of 25 cm of cuttings (Table 4).

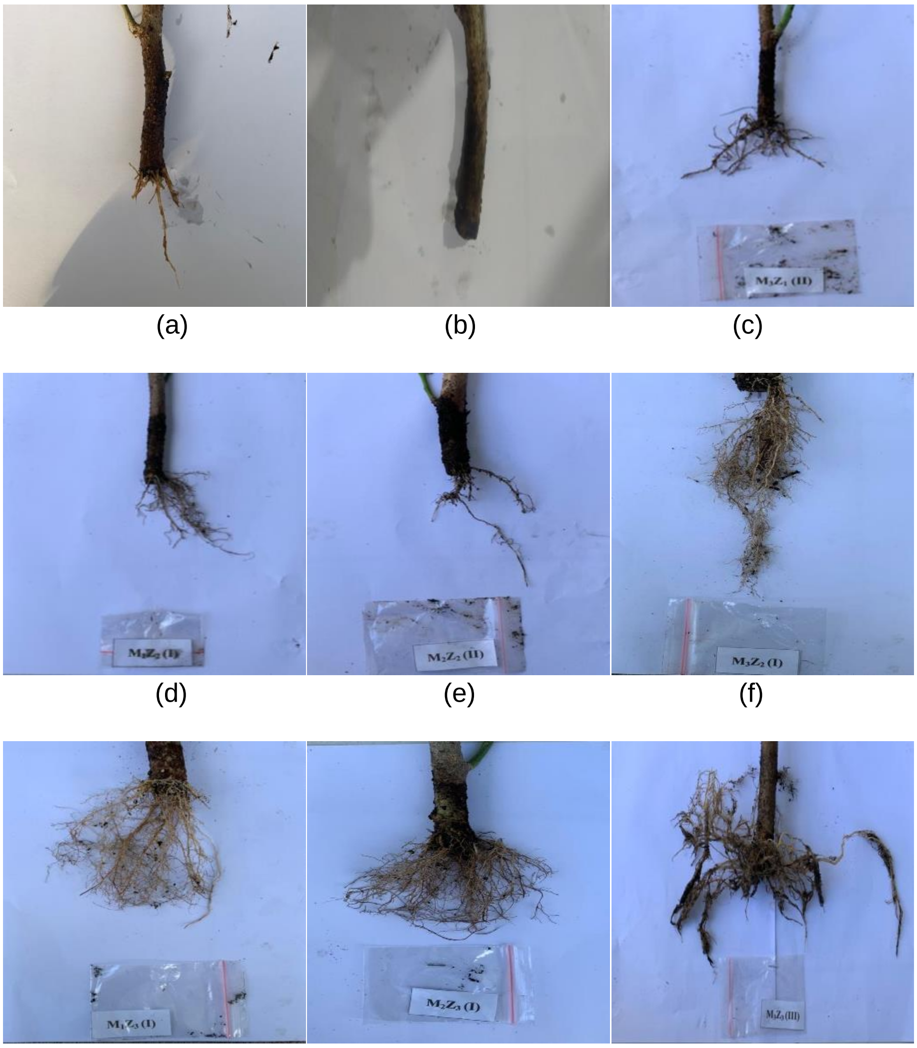
Table 4 – Effects of planting media composition and hormone on chlorophyll contain (mg g-1) of Moringa oleifera L.

Planting media	Hormones			Average
	Coconut water	Atonik	Rootone-F	
100% PS	0.43 a	1.13 abc	3.07 d	1.39
50% PS + 50% MBW	0.72 abc	0.54 ab	1.31 abc	0.86
50% PS + 50% HC	1.73 bc	1.85 c	3.45 d	2.34
Average	0.96	1.17	2.61	
LSD 0.05	1.20			

Note: Means along the row and column with the same letter are statistically non-significant according to LSD test at α=0.05.

Neither planting media nor hormones affected the chlorophyll number of *Moringa oleifera* stem cuttings ($p<0.05$). The most effective planting media used was 50% PS + 50% HC followed by 100% PS and 50% PS + 50% MBW, respectively. Commercial hormones rootone-F and atonik outperformed natural hormones (coconut water) in terms of chlorophyll value. The maximum chlorophyll content is achieved by applying 50% PS + 50% HC combined with rootone-F, conversely, the lowest is obtained by using 100% PS added to coconut water (Table 4).

Figure 5 shows a rooting pattern visualization of *Moringa oleifera* treated with media variation and hormone. Rootone-F combined with all planting media was more effective in promoting root growth compared to the other hormones in combination with the planting media, as evidenced by higher tap and lateral roots. The roots treated with rootone-F tended to grow horizontally, while roots treated with atonik grew vertically and roots treated with coconut water disappeared. Most root system characteristics, including the growth of the primary root as well as lateral roots and root hairs, are regulated by hormones.



(a) (b) (c) (d) (e) (f) (g) (h) (i)

Figure 5 – Rooting pattern of *Moringa oleifera*

Note: (a) 100% PS and coconut water; (b) 50% PS+50% MBW and coconut water; (c) 50% PS +50% HC and coconut water; (d) 100% PS and atonik; (e) 50% PS+50% MBW and atonik ; (f) 50% PS+50% HC and atonik; (g) 100% PS and rootone-F; (h). 50% PS+50% MBW and rootone-F; (i). 50% PS+50% HC and rootone-F.

Substituting husk charcoal for peat soil produced more lateral roots and root hairs (Figure 1) supported by the highest root volume data (Table 2).

DISCUSSION OF RESULTS

The variation in growth performance may be attributed to differences in the organic component present in the growth media. Peat soil media containing half-HC improved plant growth in terms of bud length and root volume. In addition, a half-MBW increased bud length (Table 2). This could be attributed to the fact that MBW and HC added could enhance the vegetative growth of the *Moringa oleifera* seedlings. Both MBW and HC are organic materials that serve as soil improvers. Organic fertilizer is recommended for *Moringa oleifera* due to the results in enhanced plant height, stem width, chlorophyll content, and index of leaf area (Murwa et al. 2016), equally, Akanbi et al. (2019) demonstrated that organic matter leads to more efficient vegetative growth, as well as enhancing water holding capacity, aeration, and germination. At 70% water holding capacity, shoot length, leaf number, and branch number responded favorably (Azam et al. 2020). Despite *Moringa oleifera* being adaptable to a wide range of soil pH (5.0-9.0), usually it prefers a neutral to slightly acidic (pH 6.3-7.0) (Domenico et al. 2019). Better root volume caused by substituting husk charcoal could be attributed to improving nutrient uptake and availability in the growth media is higher than others. Thus, nutrients were readily accessible and used. In addition, the combination of charcoal + compost provided the most successful results for *Moringa oleifera* cuttings (Rokhmah and Ikarwati 2022), similarly, when growing media (soil: charcoal: compost) was combined with rootone-F, it was more effective for *Citrus aurantifolia* (Rungeo 2022). However, Azwin (2018) claimed that shoot cuttings of *Shorea uliginosa* Foxw could not grow well using rootone-F combined by peat soil and sand media. Similarly, Febriandy et al. (2022) found no significantly effects of rootone-F and planting media on all cajuput parameters observed (*Melaleuca cajuputi*).

Rootone-F is a source of auxin (Mulyani and Ismail 2015; Setiawati et al. 2018; Ellya et al. 2023). Auxin is a group of growth-regulating substances that are crucial in plant growth and development processes (Bukar et al. 2015) such as acceleration of the formation of root primordia (Mulyani and Ismail 2015; Setiawati et al. 2018). The optimal concentration of auxin will loosen epidermis cells, facilitating root exit, increasing root length and number (Efendi and Supriyanto 2021). Additionally, auxin increases osmotic pressure, cell permeability, protein synthesis, plasticity, and cell wall development, including cell growth and cell enlargement (Efendi and Supriyanto 2021).

It is common to use auxins such as IAA, IBA, and NAA as a method for propagating stem cuttings by rooting them (Bukar et al. 2015). Mashamaite et al. (2020) reported similar responses on roots of *Moringa oleifera* subjected to auxin at low concentrations. As a rooting aid, auxins are most effective at promoting adventitious root formation in stem cuttings. A treated cutting rapidly develops a uniform and extensive root system, which survives better when transplanted than an untreated cutting (Katoriya et al. 2021). Root formation from stem cuttings was connected to hormonal balance within the cuttings. A delicate balance exists between auxins, which regulate root production and growth, and cytokinins which control shoot growth. Root calluses form as a result of the interaction of these two hormones. A disruption in hormonal balance may have severe consequences for plant growth (Rufai et al. 2016).

In addition, in comparison to coconut water (Table 3), rootone-F exhibits an increase in bud length of 308.56% and an increase in atonik of 30.58%. As a consequence, the increased synthesis of auxin in the root system and their transport to the axillary buds contribute to the acceleration of vegetative growth. These findings could be probably linked to the understanding that *Moringa oleifera* is higher with 20 ppm NAA-treated (Krishnan 2019). Furthermore, rootone-F at a concentration of 200 mg L^{-1} resulted in superior bud length and leaf number of water apple (Mulyani and Ismail 2015).

Coconut water as a growth regulator has been widely used and has been proven to have a significant effect on plant growth (Zuhro and Hasanah 2017; Juanda et al. 2018; Mauguru et al. 2019; Meilawati and Purwiyanti 2020). This study, however, found that coconut water reduced roots and bud length but not buds number (Table 3). These results are consistent with those (Khoriady 2022), who found that coconut water application did not affect all *Moringa oleifera* parameters, suggesting both combination treatments and immersion time may be more effective. According to Meilawati and Purwiyanti (2020), the root and shoot length of green and red betel could be propagated more effectively with the optimal dosage of rootone-F and coconut water. Moreover, Devitriano and Syarifuddin (2021) suggested that longer immersion times had a significant effect on the germination, vigor, and dry weight of *Moringa oleifera*. Mulyani and Ismail (2015) also stated that 3 hours of immersion was the appropriate soaking time for root length, root number, and root weight of water apple. In this recent study, cuttings were immersed for 30 minutes. At the same time, the lowest bud number was observed when atonik was applied (Table 3) suggesting that exogenous auxin from atonik could not intervene cell division process in *Moringa oleifera,* which was found to be enriched with secondary metabolites (Rufai et al. 2016).

The combination of growing media and hormones significantly affects the contains of chlorophyll involved in biochemical reactions that play a key role in photosynthesis. Chlorophyll is a pigment found in chloroplasts that converts CO_2 into carbohydrates through photosynthesis by capturing light energy (Wasonowati et al. 2019). Furthermore, (Imoro et al.

2012; Wasonowati et al. 2019; Ya et al. 2019; Farooq et al. 2022) found that environmental influence leaf chlorophyll formation resulting in chlorophyll concentration in *Moringa oleifera*. The response of leaf chlorophyll can serve as an indicator of a plant's tolerance to stress conditions. The chlorophyll content response was highest with 50% PS + 50% HC, and the preferred hormone was the commercial hormone (Table 4). Krishnan (2019) also found that a 20ppm auxin improved growth parameters such as tree height, leaf area, and chlorophyll content, as well as boric acid 0.2%'s effect on fruit set.

The root system is affected by three major processes. First, it increases the exploratory capacity of the root system by division at its primary root meristem. Second, lateral roots form; and third, root hairs form which increases primary and lateral root surface (López-Bucio et al. 2016). Additionally, roots exist in a hostile environment where they can lose their roots due to biotic or abiotic stress (Li et al. 2016). Rootone-F was the most effective hormone in promoting of both tap and lateral roots, even when combined with all growing media as shown in figure 1. Exogenous auxins generate lateral roots in many plants, while auxins and ethylene can both increase the number and length of root hairs (López-Bucio et al. 2016). Auxin and ethylene could coordinate root pattern changes at different levels of root organization (Li et al. 2016) suggesting that a hormonal signal may be involved in root responses to nutrients *(López-Bucio* et al. 2016). With rootone-F, roots grew horizontally while with atonik, roots grew vertically and with coconut water, roots disappeared (Fig. 1). In a clayed-platy soil, (Valdés-Rodríguez et al. 2018) found that more than 50% of *Moringa oleifera* tap and first lateral roots are tuberous with horizontally, while only 19% are vertically oriented. Additionally, its lateral roots are on average 13°. With its longer, lower inclination roots, *Moringa oleifera* covers a longer horizontal distance. Thus, nutrient-rich upper layers can be explored more widely and more nutrients can be obtained.

CONCLUSION

Considering these findings, it can be concluded that peat soil-reduced by substituting organic matter of husk charcoal provide favorable media for budding and rooting, which it produces maximum bud length and root volume. Rootone-F and atonik both increase bud length and root volume, but rootone-F significantly increases both. Rootone-F produces the highest root volume with 210% and 89% increment compared to coconut water and atonik. A combination of 50% peat soil+50% husk charcoal and rootone-F significantly increases chlorophyl content for stem cuttings of *Moringa oleifera*.

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