Effect of Charcoal Type and Saccharose Concentration on The Growth of Abaca Banana Root (*Musa Textilis* Nee.)

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Abstract. Abaca banana (*Musa textilis* Nee.) is one of the economic fiber plants. The Abaca banana plantation can contribute to agricultural sustainability by efficiently managing inputs like soil, water, chemicals, and seedlings and adopting good practices for harvesting and postharvest activities. The lack of abaca banana supply has encouraged the expansion of abaca banana plantations. To support the availability of plant material, propagation by tissue culture technique becomes a reasonable alternative for mass production. This research aimed to study the responses of shoot production of abaca banana as a result of the effect of types of various charcoal and saccharose at Murashige and Skoog medium through *in vitro* method. Results showed that the addition of 2 g/l norit and 30 g/l saccharose significantly increased the number of leaves, the length of the planlet, and the number and length of the roots.
Keywords: Abaca Banana, Charcoal, *In Vitro*, Saccharose, Tissue Culture.

1 Introduction

The Abaca plant is a species of banana with high product value and general uses, such as being used as a textile material and a material for papers for important documents. Abaca is an emerging commodity with limited availability to meet the market demand, necessitating extensive cultivation [1,2,3]. The economic value of the Abaca plant lies in its stem, which contains fibers as the raw materials of textiles and papers. The fibers have a strong physique and are resistant to humidity and saltwater, making them suitable to be utilized as the raw material of durable, quality paper (such as money, document paper, cheque paper), filter paper, tea bags, clothes, underwater cable wrapper, and ropes [2]. Due to its multipurpose fibers and promising potential, the Abaca plant has garnered attention from stakeholders, including the private sector, state-owned enterprises, cooperatives, and farmers.

The key aspect of cultivating the Abaca banana plant lies in its contribution to agricultural sustainability by efficiently managing inputs like soil, water, chemicals, and seedlings and adopting good practices for harvesting and postharvest activities. Sustainable agriculture aims to maximize the use of natural resources for human needs while protecting

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the environment and minimizing reliance on external inputs. Moreover, it empowers farmers to enhance their knowledge and skills for more efficient practices [4].

The multiplication in the propagation using shoots is extremely low, and the planting materials are prone to damage during transportation, making them less durable and requiring significant space, resulting in high transportation costs. One of the alternatives to provide healthy seeds in large amounts and a fast manner is using seeds produced by tissue culture [5]. The tissue culture of banana plants is usually done using a medium that is supplemented with activated charcoal or carbon. These additives serve multiple functions, including absorbing toxic compounds in the medium and inhibitory substances secreted by the plantlet, stabilizing the medium's pH, promoting root growth by reducing light exposure, preventing or minimizing callus formation, and stimulating morphogenesis [6,7].

Activated charcoal can indirectly promote root growth by creating an environment that reduces light penetration into the media, thereby preventing the degradation of auxin required for root formation [8-11]. As activated charcoal accumulates more auxin in the medium, the frequency of root initiation increases, resulting in a greater number of roots formed. The addition of 2 g/l pro analysis and 2 g/l norit can boost the height of the plantlet, the size of the leaves, and the number of roots formed in the plantlet Oncidium [8,12].

Carbohydrates are vital components in the growth medium, providing the necessary nutrients for tissue culture development [13]. A carbon source is crucial since the explant being grown cannot perform photosynthesis independently. The plants can utilize some carbohydrates, such as monosaccharides (glucose, fructose, galactose, mannose, arabinose, ribose, and xylose), disaccharides (sucrose, maltose, lactose, cellobiose, and trehalose), trisaccharides (raffinose) and sugar alcohols (sorbitol, mannitol, and glycerol). The primary carbohydrate commonly utilized in tissue culture is saccharose [13,14]. Saccharose is a disaccharide that undergoes hydrolysis, splitting into two molecules of monosaccharides, namely glucose, and fructose. Based on this consideration, the effort to improve the growth of banana roots in this research was made by adding various types of activated charcoal and saccharose into the medium.

2 Research method

This research was conducted in the Biotechnology Laboratory, Faculty of Agriculture, Universitas Pembangunan Nasional Veteran Yogyakarta, which was arranged following a two-factor completely randomized design. The first factor was the type of activated charcoal (coconut shell, norit, and pro analysis activated charcoals) with a concentration of 2 g/l, while the second factor was the saccharose concentration (20, 30, and 40 g/l). Each treatment was replicated five times, and each culture bottle contained one explant. The plantlets used in the experiment were produced through irradiation using Gamma 10 Grey rays, and the Murashige and Skoog medium was employed. The growth of the plants was manually assessed, considering the number and length of roots (in cm), the number of leaves, and the height of the plantlets (in cm). The data were presented as mean values, and the significance of differences was determined using one-way ANOVA, with differences at $p < 0.05$ considered significant.

3 Results and discussion

Plantlets obtained from the multiplication process typically exhibit a limited number of roots [6,7]. To facilitate the acclimatization process, root induction was carried out for four

weeks following the completion of the multiplication stage. Figure 1 illustrates the tissue culture of the Abaca banana with activated charcoal and saccharose treatments.

Fig.1. Root induction of Abaca banana plant with activated charcoal and saccharose treatment (S1: coconut shell, S2: norit, S3: activated charcoal, A1: 20 g/l saccharose, A2: 30 g/l saccharose, A3: 40 g/l saccharose).

For the plants to produce roots, the roots of explants obtained from the multiplication stage were separated, and the plants were planted in a medium that contained activated charcoal and saccharose [10-12,15]. Roots would grow after the plantlets produced shoots and new leaves. The shoot and new leaves were expected to be able to produce endogenous auxin, translocate it to basal, and induce the formation of roots [12,15]. The observation results revealed a significant interaction (mutual synergism) in all parameters, particularly in the treatment combination of 2 g/l norit and 30 g/l saccharose, which exhibited the highest number of roots and leaves, the longest roots, and the tallest plantlet (Table 1).

Table 1. The growth of the abaca banana plant in the rooting stage

Treatment	Number of roots	Length of roots (cm)	Number of leaves	Height of plantlets (cm)
Coconut shell and 20 g/l saccharose	7^{cd}	5.83^{d}	4^{bcd}	7.13^{cde}
Coconut shell and 30 g/l saccharose	5^{de}	5.93^{d}	3^{d}	7.53^{cd}
Coconut shell and 40 g/l saccharose	5^{de}	5.43^{d}	4^{bcd}	8.50^{c}
Norit and 20 g/l saccharose	12^{b}	12.20^{b}	6^{b}	14.13^{b}
Norit and 30 g/l saccharose	16^{a}	15.63^{a}	8^{a}	16.33^{a}
Norit and 40 g/l saccharose	6^{cd}	6.57^{c}	5^{bc}	11.57^{b}
Plant-derived activated charcoal and 20 g/l saccharose	3^{de}	5.03^{d}	4^{bcd}	7.50^{cd}
Plant-derived activated charcoal and 30 g/l saccharose	3^{de}	5.77^{d}	3^{d}	5.93^{de}
Plant-derived activated charcoal and 40 g/l saccharose	5^{de}	5.60^{d}	3^{d}	4.03^{e}
Interaction	(+)	(+)	(+)	(+)

Notes: The figures in one column that are followed by the same letters show insignificant differences in the 5% DMRT test.
(+) shows there was a positive interaction.

The positive effect of the activated charcoal on the ability of micro shoots to root can be attributed to two factors. Firstly, it reduces the light intensity at the base of the shoots, creating a suitable environment for the accumulation of auxin (a light-sensitive hormone) and its co-factors [6,7,12]. Auxin serves as an internal factor that has an effect when the roots come out [16,17]. Secondly, activated charcoal absorbs substances such as polyphenols, which inhibit root formation. By absorbing polyphenol compounds produced by the explants, activated charcoal alters the endogenous ratio between free polyphenol and conjugated polyphenol, in which the amount of free polyphenol decreases and the amount of non-active conjugated polyphenol increases, thereby enhancing the potential for root or shoot organogenesis [10,11,18].

The external factor influencing the formation of roots was the environment of the growing medium, such as light. Roots are negatively photoblastic, indicating that they thrive in a light-free environment. Light can degrade auxins needed for the growth and development of the roots. Consequently, activated charcoal can block light from entering the medium, thus promoting root growth. This finding aligns with the research conducted by Dumas and Monteuuis [12], which shows that light inhibits the formation of roots. The percentage of rooting of bananas in a dark condition is higher (66.7 percent to 100 percent) compared to the rooting when light is present (0 percent to 33.3 percent). Light hinders root initiation in certain plant species by breaking down indole-3-acetic acid (IAA), a hormone that supports root induction, and deactivating factors that drive rooting [5]. In the case of apple plants, the growth of the roots was improved when grown in a dark condition [19].

Activated charcoal indirectly enhances the number of roots by creating a conducive environment for root growth by reducing light penetration into the medium. This prevents the degradation of auxin necessary for root formation. The more auxin accumulated by adding activated charcoal into the medium, the more frequently the root initiation occurs. Hence, the number of roots formed also increases. In addition, activated charcoal absorbs substances inhibiting the formation of roots, such as phenolic conjugate [5,15,18]. The research on *Pisum sativum* planted in the MS medium supplemented with 3 g/l activated charcoal has demonstrated that the absence of light results in the highest number of roots [5].

In terms of root length, the treatment using activated charcoal norit showed significantly longer roots than other kinds of activated charcoal because the activated charcoal in norit could reduce the light coming into the growing medium. Notably, pro-analysis activated charcoal and norit contained a higher percentage of activated charcoal, approximately 99 percent, while plant-derived activated charcoal contained 95-99 percent [1,8]. On the other hand, activated charcoal derived from coconut shells showed better overall performance than plant-derived activated charcoal, although it was still lower than norit. Activated charcoal made of coconut shell using a chemical activation method has advantages because it absorbs color/light or aroma [20,21]. The low intensity of light can stimulate endogenous growing substances to work more actively in the root initiation process. Overall, the dark condition stimulates more active cell division in the roots, improving root growth.

Saccharose comprises two monosaccharides, glucose and fructose, with covalent bonds. When plants or their tissues are cultivated *in vitro*, they cannot be autotrophs since the culture bottle environment does not support photosynthesis. Therefore, it is necessary to add a carbon source, and saccharose is commonly used for this purpose [13,14]. Saccharose is a disaccharide with molecular formula $C_{12}H_{22}O_{11}$. Sugar exists in large amounts in sweet fruits, stems, roots, tubers, and the sap of some higher plants, where saccharose is soluble in water. Saccharose functions in the thickening of cell walls, while auxin is a catalyst in the metabolism of carbohydrates and in regulating mitosis activity [1,18]. Wijayani and Muafi [11] conducted studies on Chrysanthemum plant tissue culture using ½ MS medium, and 40 g/l saccharose showed faster growth. Similarly, research on peanut plants using MS medium with various vitamin treatments and 40 g/l saccharose [6] indicated that the use of MS medium with 40 g/l saccharose resulted in the highest number of roots (8.3) in the shortest period (7.25 days).

4 Conclusion

The addition of active charcoal could significantly stimulate the tissue culture of Abaca. The best combination in the treatment used 2 g/l Norit and 30 g/l saccharose produced the highest number and the longest roots, the highest number of leaves, the highest plantlet, and the dry weight of the plantlet. This finding holds promise for advancing sustainable agriculture by optimizing input efficiency by applying activated charcoal and saccharose in the tissue culture of Abaca banana plants. Furthermore, it proposes a potential approach to achieving optimal acclimatization for the Abaca plant and other banana species in future research.

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References

[1] A. Wijayani and R. Srilestari, "Genetic diversity of plain medium resistant of Chrysanthemum plantlet produced of gamma-ray irradiation," Intl J. Biosci Biochem Bioinfo, vol. 6, no. 4, pp. 139-144, 2016.
[2] Badan Pusat Statistik (BPS), "Produksi pisang di Indonesia 2012-2022," http://www.DataIndonesia.id, April 5, 2023.
[3] Badan Pusat Statistik, "Tabel Produksi Buah-buahan di Indonesia," http://www.bps.go.id, 2023.

[4] N. V. Thanh and C. Yapwattanaphun, "Banana Farmers' Adoption of Sustainable Agriculture Practices in the Vietnam Uplands: the Case of Quang Tri Province," Agric Agric Sci Procedia, vol. 5, pp. 67-74, 2015.
[5] P. S. Kumar and V. I. Mathur, "Chromosomal Unstability in Callus Culture of Pisum sativum," J. Plant Cell. Tiss.Org.Cult., vol. 78, pp. 267-271, 2004.
[6] R. Srilestari, "Induksi embrio somatik kacang tanah pada berbagai macam vitamin dan sukrosa," J. Ilmu Pertanian, vol. 12, no. 1, pp. 43-50, 2005.
[7] R. Srilestari and Suwardi, "Induksi akar pisang abaka secara in vitro dengan menggunakan macam media dan thiamin," J. Agrivet, vol. 26, no. 1, pp. 1-7, 2020.
[8] D. E. Peck and B. G. Cumming, "Beneficial effect of activated charcoal on bulblet production in tissue cultures of Muscari armeniacum," J. Plant Cell Tiss. Org Cult., vol. 6, pp. 9-14, 2006.
[9] I. Mariska and D. Sukmajaya, "Perbanyakan bibit Abaca melalui kultur jaringan," Balai Penelitian Bioteknologi dan Sumber Daya Genetik Pertanian, Bogor, Indonesia, 2003.
[10] A. Wijayani, Muafi, E. Wahyurini, and R. Srilestari, "Regeneration callus of chrysant after irradiated ray gamma for the resilience of plain medium," Journal Techno, vol. 1, pp. 61-67, 2015.
[11] A. Wijayani and Muafi, "In vitro regeneration of chrysanthemum callus after gamma-ray irradiation for its resistance to medium plains," J. Information, vol. 19, no. 6A, pp. 1813-1817, 2016.
[12] E. Dumas and O. Monteuuis, "In vitro rooting of micropropagated shoots from juvenile and mature Pinnus pinaster explants: influence of activated charcoal," Plant Cell Tissue Organ Cult, vol. 40, pp. 231-235, 1995.
[13] M. Yaseen, T. Ahmad, G. Sablok, A. Standardi, and I. A. Hafiz, "Role of carbon sources for in vitro plant growth and development," Mol Biol Rep, vol. 40, pp. 2837–2849, 2013.
[14] T. Ahmad, N. A. Abbasi, I. A. Hafiz, and A. Ali, "Comparison of sucrose and sorbitol as the main carbon energy source in morphogenesis of peach rootstock GF-677," Pak J Bot, vol. 39, no. 4, pp. 1264–1275, 2007.
[15] M. A. Rahman and J. Blake, "Factors Affecting in vitro proliferation and rooting of shoots of jackfruits," Plant Cell Tissue Organ Cult, vol. 13, pp. 179-187, 2008.
[16] N. Unsong, W. Tilaar, and B. R. A. Sumayku, "Sterilisasi Dan Penggunaan Zat Pengatur Tumbuh BAP (Benzile Amino Purin) Terhadap Pertumbuhan Eksplan Tunas Pisang Abaka (Musa textilis Nee) Melalui Teknik In Vitro," J. Agri-Sosio Ekonomi, vol. 18, no. 3, pp. 717–724, 2022.
[17] R. R. D. Putri, Suwirmen, and N. Nasril, "Pengaruh Naphthalene Asam Asetat (NAA) pada Pertumbuhan Akar Pisang Raja Kinalun Secara In Vitro," Jurnal Biologi Universitas Andalas, vol. 6, no. 1, pp. 1-5, 2018.
[18] A. K. Dwivedi, B. K. Banerji, D. Chakrabarty, A. K. A. Mandal, and S. K. Datta, "Gamma-ray induced new flower color chimera and its management through tissue culture," Ind J Agric Sci, vol. 70, pp. 853-855, 2000.
[19] R. H. Zimmerman and I. Fordham, "Simplified method for rooting apple cultivars in vitro," J Am Soc Hortic Sci, vol. 110, pp. 461-462, 1985.
[20] M. Lutfi, Hanafi, B. Susilo, J. Prasetyo, and Sandra, "Characteristics of activated carbon from coconut shell (Cocos nucifera) through chemical activation process," IOP Conf. Series: Earth and Environmental Science, vol. 733, p. 012134, 2021.
[21] E. H. Sujiono, D. Zabrian, Zurnansyah, Mulyati, V. Zharvan, Samnur, and N. A. Humairah, "Fabrication and characterization of coconut shell activated carbon using variation chemical activation for wastewater treatment application," Results Chem, vol. 4, p. 100291, 2022.