



The Influence of Gibberellins and Smoke Water as a Stimulant for Germination and Vegetative Growth of *Syzygium aromaticum* (L.) Merr. & L. M. Perry

W. Muslihatin[†], R. P. D. Wahyudi^{id}, M. Iqbal^{id}, T. B. Saputro^{id} and T. Nurhidayati

Department of Biology, Faculty of Science and Data Analytics, Sepuluh Nopember Institute of Technology, Indonesia

[†]Corresponding author: Wirdhatul Muslihatin; wirdhatul.muslihatin@its.ac.id

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ABSTRACT

Clove or cengkeh (*Syzygium aromaticum*) is one of Indonesia's commodities with high domestic and international potential, considering that this plant is used as raw material for the cigarette industry. Therefore, it is necessary to optimize the production of Indonesian cloves, one of which is by using growth stimulators such as plant growth regulators (PGR). This study uses gibberellic acid (GA₃) and smoke water as exogenous growth triggers. The treatment given was soaking *S. aromaticum* seeds in gibberellic acid (GA₃) and liquid smoke for 24 h. The GA₃ concentrations used were 100 ppm, 75 ppm, 50 ppm, and 25 ppm. Smoke water was obtained from the pyrolysis of coconut shells, and the concentrations used were 0.5%, 1%, 2%, and 3%. Observations were conducted for 11 weeks and divided into two phases, namely the germination phase and the vegetative growth phase. Parameters measured included germination percentage, radicle, and plumula length in the first phase, root length, plant height, and number and area of leaves in the second phase. The best results were achieved with the soaking treatment using 0.5% smoke water, which showed a significant increase in all observed growth parameters. This is due to the content of karrikin in smoke water, which acts like a growth hormone and triggers the performance of other growth hormones. In addition, karrikin plays an active role in the germination process by changing the morphology of the seeds.

INTRODUCTION

Syzygium aromaticum, known as cloves or cengkeh, is a Dried Flower Bud that belongs to the family Myrtaceae and is native to the Maluku islands, Indonesia, and has spread and been cultivated around the world. The main part of the clove plant that is commercially valuable is its flowers, which are mostly used in the cigarette and food industry as spices or preservatives (Alfian et al. 2019, Batiha et al. 2020). In Indonesia, cloves are one of the superior commodities for plantations with high economic improvement potential (Zenti et al. 2021).

Based on data from the Ministry of Agriculture in 2020, Indonesia is the largest clove-producing country in the world. However, this does not make Indonesia the largest clove-exporting country in the world. Indonesia occupies the second position in clove exports after Madagascar, with an average export volume of 122.48 thousand tons (14.27%) (Zenti et al. 2021). This is because Indonesia uses most of its production to supply domestic industrial demand from the cigarette industry, which absorbs 85% of Indonesia's clove production (Hasibuan et al. 2022).

The increasing demand for cloves makes it necessary to optimize Indonesian clove production such as rehabilitation of non-producing or damaged plants and the introduction of technology that supports the plantation management process from pre-harvest to post-harvest. One of them is developing agricultural practices using growth stimulators such as plant growth regulators (PGRs) that can increase production. The use of PGRs can reduce the planting period and increase productivity in a shorter time (Matos et al. 2020).

Gibberellin (GA) is a phytohormone that regulates various aspects of plant growth and development through complex biosynthetic pathways. Specific roles of GA include seed germination, stem elongation, flower initiation, fruit, and seed development. Previous studies have shown that exogenous gibberellic acid (GA₃) increases the percentage of seed germination and subsequent seedling growth in different crops (Hasibuan et al. 2022). The study of Winarso et al. (2021) found that soaking oil palm seeds in gibberellic acid solution affects the rate of seed germination, percentage of seed germination, seed height, root length, wet weight, and dry weight of sprouts. In addition to using exogenous phytohormones, increasing productivity can also be done

through innovative cultivation techniques, such as giving smoke water solutions to plants. Smoke water is produced from the pyrolysis and condensation of smoke from the burning process of coconut shells. The compounds contained in smoke water are karrikin, acetic acid, and methanol, which function to accelerate plant growth and production (Murniati et al. 2020). Smoke water as a growth stimulant has been used on many species, including rice, pepper, celery, and lettuce seeds (Gama et al. 2021, Gupta et al. 2020, Zhou et al. 2014). Research Amiroh et al. (2022) state that the concentration of 2% smoke water provides significant interactions on all parameters of rice plant growth and yield.

So far, research related to the effect of gibberellin and smoke water on the growth and development of clove vegetation is limited. This study aims to study the effect of gibberellin and smoke water on the growth and development of clove to improve clove production and quality.

MATERIALS AND METHODS

Plant Material

The clove seeds used were the Zanzibar variety obtained from a private field in Wonosalam, Jombang, East Java, Indonesia. The seeds were selected from clove fruits with physiologically ripe criteria (purple-black), at least 2.5 cm long, 1-2 cm in diameter, free of pests and diseases, and no black marks (Noya et al. 2018, Setiawan & Rosman 2015). The skin of the clove fruit is carefully peeled off so as not to injure the seeds. The seeds are washed to remove mucus until clean. Then, the seeds are stored for 6 days before treatment.

Growth Stimulator Treatment

The treatments were soaking *S. aromaticum* seeds in gibberellic acid (GA₃) and liquid smoke for 24 h. The concentrations of GA₃ used were 100 ppm, 75 ppm, 50 ppm, and 25 ppm, while liquid smoke was 0.5%, 1%, 2%, and 3%. As a control, the seeds were soaked in distilled water at the same time.

Growing Media and Growth Parameters

Seeds that had been soaked in the growth stimulator were planted on cocopeat media to observe the germination phase for 4 weeks. In this phase, radicle length, plumula length, germination time, and germination percentage were measured with the following formula (Shah et al. 2021):

Germination rate (%) = (Number of normal sprouts/ Total number of sprouts) x 100%

In the second phase, seedlings of each treatment were taken and transplanted to a mixed medium of soil and organic fertilizer with a ratio of 2:1, and regular watering was done

in the morning. At the end of the phase, plant height, root length, number of leaves, and total leaf area were measured.

Data Analysis

This study used an experimental method of complete randomized design with 9 treatments, namely control, gibberellin 25 ppm (G1), 50 ppm (G2), 75 ppm (G3), 100 ppm (G4), liquid smoke 0.5% (A1), 1% (A2), 2% (A3) and 3% (A4). Each treatment had 5 replicates. The research data were analyzed using SPSS software with a one-way ANOVA test and continued with the Duncan Multiple Range Test (DMRT) test with a 5% test level.

RESULTS AND DISCUSSION

Germination Percentage

Seed germination or dormancy breaking is considered the initiation of the first phase of development in the life cycle of higher plants and is followed by the vegetative growth of seedlings. Seed germination begins with water absorption or imbibition and finishes with the appearance of radicles from the cotyledons (Wolny et al. 2018). This process can be accelerated by reducing skin thickness, immersion in water, or immersion in a growth stimulator solution (Wahyuni et al. 2021). In this study, exogenous growth stimulators used in soaking clove seeds were phytohormones, gibberellic acid (GA₃), and smoke water. The results showed differences in sprout morphology in each treatment that can be seen in Fig. 1. Normal sprouts of clove seeds in this study were determined based on the completeness of the essential structure of sprouts, namely roots, cotyledons, hypocots, epicots, and plumulae. The length of the sprout from the base of the root is at least 4 times the length of the seed. From all of the treatments, GA₃ 50 ppm and 75 ppm treatments and Smoked Water of 0.5%, 1%, and 2% showed the maximum germination percentage (Table 1). This is according to research by Abou El-Nour (2021), that low concentration of smoke water leads to an increase in the percentage and germination rate of seeds of pepper, celery, and lettuce.

The potential effect of smoke water in chemically breaking germination by the chemical interaction of smoke compounds with inhibitors in the seed coat, endosperm, or embryo, thereby enhancing seed germination. According to another study, the enhancing effect of smoke water may be related to changes in the synthesis/metabolism of endogenous hormones as a result of seed treatment with smoke. In addition, morphological factors where smoke particles can adhere, persist, and adsorb on the surface of plants and soil particles thus play an active role in the germination process by changing seed morphology and causing strong chemical

scarification on the seed surface, especially on seeds that have a hard shell. There is also the biological factor of the presence of a major active compound that has been identified as a butenolide recently named karrikin (KAR1); this compound is now recognized as initiating germination for many smoke-exposed and non-smoke-exposed species. KAR1 has effects similar to gibberellins and/or cytokinins, which can promote embryonic development and break seed dormancy in seeds so that seeds can germinate (Abou El-Nour 2021, Ghebrehiwot et al. 2008).

This study also shows a decrease in the percentage of germination of cloves with soaking GA_3 and smoke water with increasing concentration. Mulik et al. (2021) explained that increased concentrations could cause toxicity in plants because of the phenolic content that can suppress the growth of radicles and increase free radical compounds in plants if given in high concentrations.

Time of Germination

In this study, each treatment caused a difference in the germination time, as shown in Table 1. Time of germination is the length of time required for one seed to germinate (Al-

Ansari & Ksiksi 2016). Overall, soaking seeds with GA_3 and smoke water had no significant effect ($P>0.05$). The treatment with the fastest germination time is found in 0.5% smoke water treatment, 6 days, while the longest germination time is found in 3% smoke water treatment, 20 days. This is caused by acetic acid, methanol, and phenol compounds in smoke water, which help break down seed dormancy and stimulate embryonic growth (Gupta et al. 2020, Murniati et al. 2020, Sreekissoon et al. 2021, Wolny et al. 2018).

Radicle and Plumula Length

Sprouts from seed soaking treatment in GA_3 solution and smoke water for 24 hours can be seen in Fig. 2. Each treatment produces different radicles and plumules lengths (Table 1). Overall, both growth stimulators had a significant influence on radicle and lead length ($p<0.05$). Compared to controls, 0.5% smoke water produced the highest radicle and plumula length. The results showed that the effect of smoke water on radicle length was very significant ($p<0.05$). This is in line with research by Bhardwaj (2012), where low concentrations of smoked water (0.1% and 0.2%, V/V) applied to papaya seeds stimulate endosperm stretching, causing the seed coat

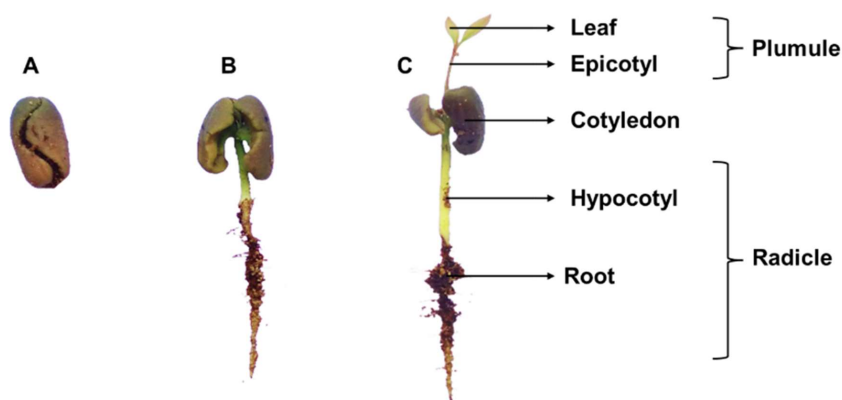


Fig. 1: Sprout type and morphology induced by soaking treatment of clove seeds in GA_3 and smoke water solution. (A) non-germinated seeds, (B) abnormal sprouts, (C) normal sprouts.

Table 1: The effect of soaking seeds in GA_3 solution and smoke water in the germination phase of cloves.

Treatment	Long Radicles [cm]	Plumule Length [cm]	Time of germination [day]	Rate of Germination [%]
Control	2.23 ^b	0.20 ^b	10.00 ^a	33%
GA_3 25ppm	4.70 ^{ab}	0.85 ^{ab}	9.33 ^a	67%
GA_3 50ppm	5.30 ^{ab}	0.60 ^{ab}	7.33 ^a	100%
GA_3 75ppm	3.97 ^{ab}	0.33 ^{ab}	11.33 ^a	100%
GA_3 100ppm	4.27 ^{ab}	0.20 ^b	11.33 ^a	67%
Smoke water 0.5%	6.03 ^a	1.30 ^a	6.00 ^a	100%
Smoke water 1%	5.37 ^{ab}	0.47 ^{ab}	9.67 ^a	100%
Smoke water 2%	4.73 ^{ab}	0.07 ^{ab}	11.33 ^a	100%
Smoke water 3 %	4.13 ^{ab}	0.17 ^b	20.33 ^a	67%

Note: Numbers were followed by different letters showing a significant difference based on the DMRT test ($\alpha=0.05$).

to break and allow the radicle to elongate and emerge. The pyrolytic compound karrikin found in smoke water interacts directly or indirectly with important phytohormones, namely gibberellic acid, and auxin, in plants to trigger germination. Smoke-derived signaling molecules, known as karrikins, are composed of carbon, hydrogen, and oxygen. These low-molecular-weight compounds exhibit a characteristic bicyclic structure featuring a pyran and a butenolide-containing lactone ring. The first identified karrikin, 3-methyl-2H-furo[2,3-c] pyran-2-one (KAR1), demonstrates the highest abundance and efficacy in stimulating seed germination. Karrikins are to interact, either directly or indirectly, with key phytohormones such as abscisic acid, gibberellic acid, auxins, and ethylene. Remarkably, karrikins can induce germination in some plant species at exceptionally low concentrations, exhibiting potency comparable to established plant hormones (Chiwocha et al. 2009, Garrido et al. 2023, Waters & Nelson, 2023, Zhou et al. 2014). Germination begins with gibberellins that diffuse into the aleurone layer and stimulate the cell to synthesize amylase, which then hydrolyzes starch in the endosperm, producing maltose

molecules that are converted into glucose and transported to the embryo as the energy needed to grow and give rise to radicles (Lee et al. 2022). In addition, gibberellic acid and auxins also enhance the process of cell division and elongation, resulting in large seedlings compared to controls (AL-Hade & Alselawy 2019).

Plant Height and Root Length

After germination, the plant will enter the vegetative growth phase, marked by the bud formation and the expansion of leaves, for initiating photosynthesis. Additionally, the roots and stems of the plant also grow and develop over time over time (Wei et al. 2023). The vegetative growth phase of the clove plant (*Syzygium aromaticum*) after 11 weeks is shown in Fig. 3.

In this study, data on root length and plant height taken at the end of the study were presented in Table 2. Overall, soaking seeds in GA₃ solution and smoke water had no significant effect on root length and clove plant height ($P > 0.05$). However, soaking seeds in 25 ppm gibberellic acid

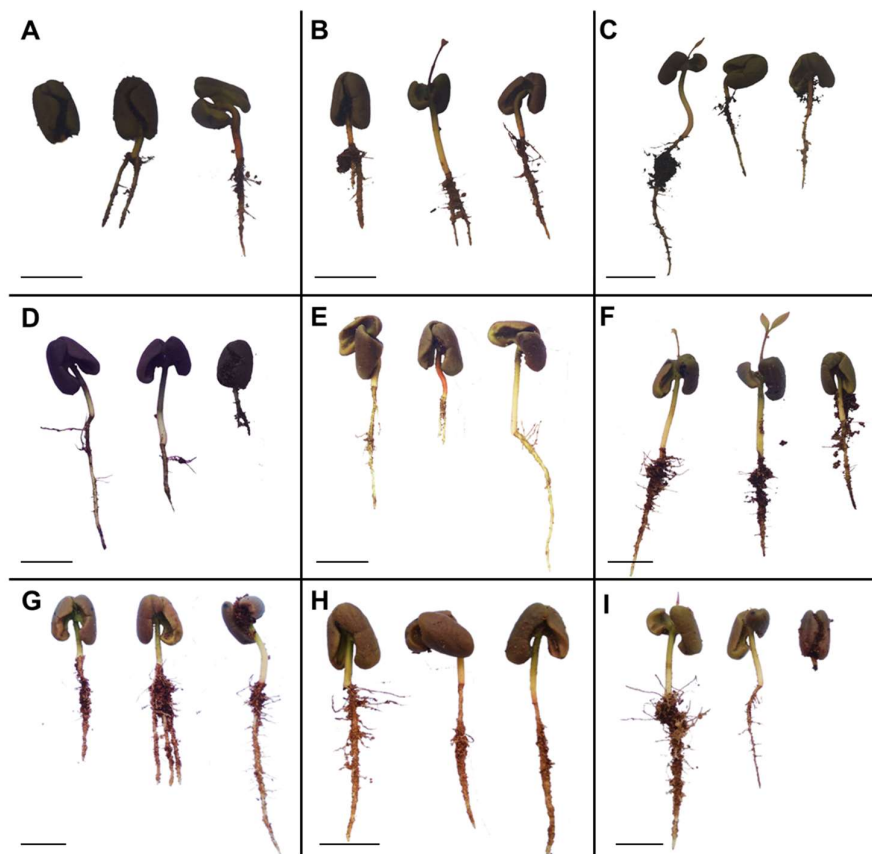


Fig. 2: Germination phase of cloves (*Syzygium aromaticum*) after soaking treatment of GA₃ and smoke water. Scale bar 2cm. (A) Control, (B) GA₃ 25ppm, (C) GA₃ 50ppm, (D) GA₃ 75ppm, (E) GA₃ 100ppm, (F) Smoke water 0.5% (G) Smoke water 1% (H) Smoke water 2% (I) Smoke water 3%.

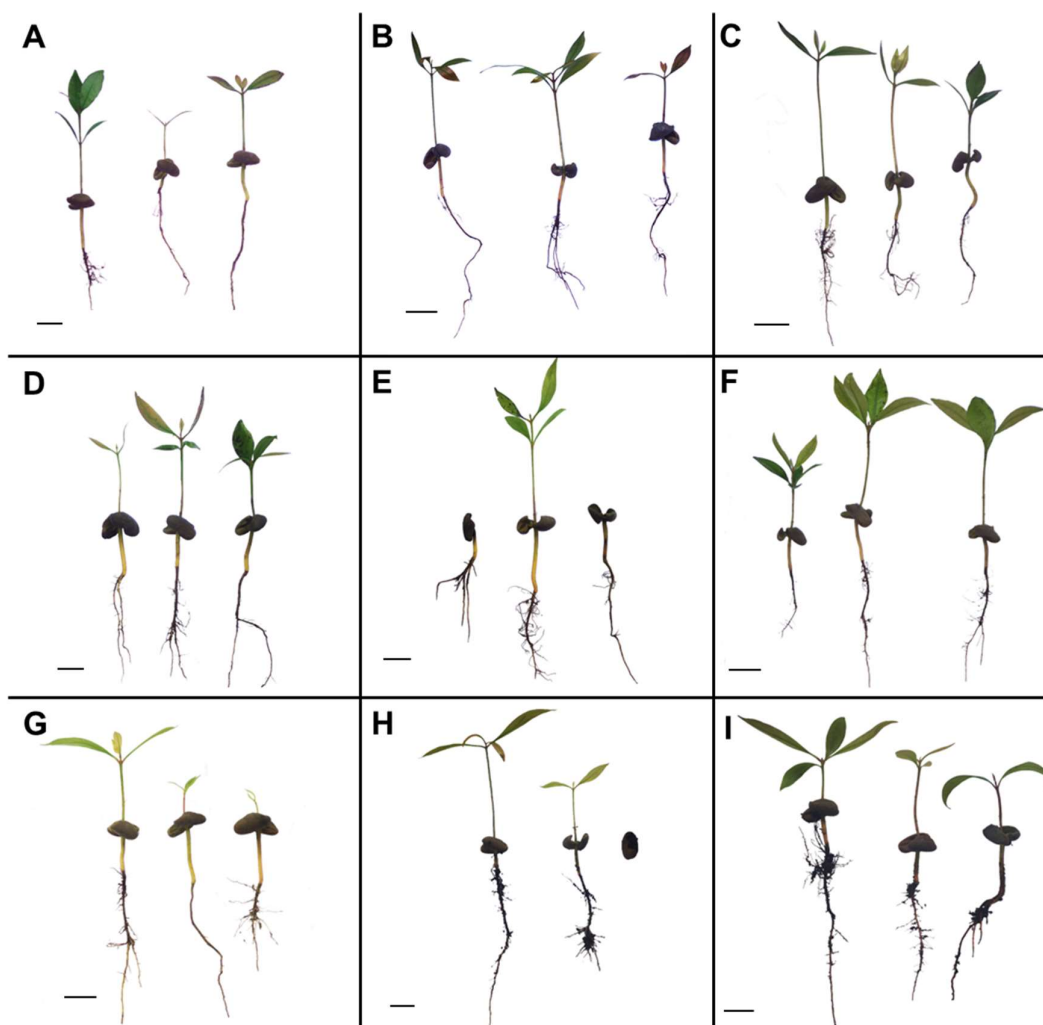


Fig. 3: Clove plant (*Syzygium aromaticum*) after soaking treatment of GA₃ and smoke water. Scale bar 2cm. (A) Control, (B) GA₃ 25ppm, (C) GA₃ 50ppm, (D) GA₃ 75ppm, (E) GA₃ 100ppm, (F) Smoke water 0.5% (G) Smoke water 1% (H) Smoke water 2% (I) Smoke water 3%.

had the greatest effect among other treatments and resulted in the greatest root length and plant height. Followed by Smoke water 3% for root length and gibberellic acid 50 ppm for plant height. Shtin et al. (2022) stated that gibberellin, or gibberellic acid, has an important role in the elongation of roots and stems. Gibberellin, together with auxins concentrated in the root elongation zone, will act as positive regulators of cell division. GA₃ also increases the activity of enzymes such as hydrolase enzymes that function to break down cell walls so that cells can develop and lengthen. Smoke water plays a role in cell elongation in the presence of karrikin, which triggers auxin synthesis and encourages cell elongation (Chumpookam et al. 2012). Some studies report statistically significant increases in both plant height and root length following Coconut shell smoke water application. Karrikin in smoke water might influence

the balance of key plant hormones like auxins, gibberellins, and cytokinins. These hormones play a crucial role in cell division, elongation, and root development. However, the specific hormonal interactions and their impact on height and root length remain unclear (Gama et al. 2021, Mulyawanti et al. 2019).

Number and Area of Leaves

Data related to the number and average leaf area of each treatment can be seen in Table 2. Overall, seed soaking treatment in GA₃ solution and smoke water had a significant effect on leaf count and leaf area ($P < 0.005$). 0.5% smoke water treatment resulted in the highest number and area of leaves compared to the entire treatment. While the treatment with the lowest number of leaves and leaf area is GA₃100ppm. This is in line with Chumpookam et al. (2012), where low

Table 2: Effect of soaking seeds in GA₃ solution and smoke water on the vegetative phase of clove plants.

Treatment	Root Length [cm]	Plant Height [cm]	Number of leaves	Leaf Area [cm ²]
Control	6.50 ^a	4.03 ^a	3.33 ^{ab}	144.33 ^{ab}
GA ₃ 25ppm	12.97 ^a	5.67 ^a	5.33 ^{ab}	197.67 ^{ab}
GA ₃ 50ppm	9.50 ^a	5.57 ^a	6.00 ^a	138.67 ^{ab}
GA ₃ 75ppm	9.60 ^a	4.43 ^a	5.33 ^{ab}	134.67 ^{ab}
GA ₃ 100ppm	5.37 ^a	2.03 ^a	2.00 ^b	43.67 ^b
Smoke water 0.5%	9.93 ^a	5.33 ^a	6.33 ^a	275.33 ^a
Smoke water 1%	10.20 ^a	2.83 ^a	3.00 ^{ab}	125.00 ^{ab}
Smoke water 2%	7.80 ^a	3.63 ^a	2.67 ^{ab}	148.67 ^{ab}
Smoke water 3 %	10.33 ^a	3.23 ^a	3.33 ^{ab}	212.33 ^{ab}

Note: Numbers were followed by different letters showing a significant difference based on the DMRT test ($\alpha=0.05$).

concentrations help in the process of leaf formation in papaya seedlings by increasing the performance of IAA synthesis in the apical meristem of shoots containing leaf primordia can spur leaf formation. While gibberellins trigger leaf growth by stimulating cell division and elongation. An increase in the number of leaves also increases the amount of chlorophyll and photosynthesis so that more energy is obtained by the plant by maximizing its development. Leaf dilation is also triggered by plant hormones such as auxin (IAA) that stimulate leaf dilation by stimulating cell growth on the developing side of the leaf. Cytokinins can also stimulate cell division in growing leaves, which supports leaf dilation. The synthesis of these two hormones can be increased by the presence of Karrikin in smoke water (Chumpookam et al. 2012, Mulik et al. 2021, Ren et al. 2019).

CONCLUSION

Based on the research conducted, soaking clove seeds in GA₃ solution and smoke water affects various aspects of early plant growth, especially germination percentage, radicle and plumula length, root length, plant height, and number and area of leaves. The best results were achieved by immersion treatment using 0.5% smoke water, which showed a significant improvement in all observed parameters. This is due to the karrikin content in smoke water, which can trigger the synthesis of growth hormones in plants. Despite this, it was found that an increase in the concentration of growth stimulators can reduce their effectiveness or even harm plant growth, as it increases phytotoxicity.

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ORCID DETAILS OF THE AUTHORS

- W. Muslihatin: <https://orcid.org/0000-0001-7122-6785>
 R. P. D. Wahyudi: <https://orcid.org/0009-0007-5635-1087>
 M. Iqbal: <https://orcid.org/0009-0007-3043-8790>
 T. B. Saputro: <https://orcid.org/0000-0002-8414-1438>