

## EFFECT OF NAA AND BAP APPLICATION ON THE GROWTH RESPONSES OF *Mentha × piperita* L.

*Pengaruh Pemberian NAA dan BAP terhadap Respon Pertumbuhan  
Mentha × piperita L.*

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### ABSTRACT

*Mentha × piperita* L., also known as peppermint, is a plant with various uses, including medicine, cosmetics, and food. Numerous industries have a high need for peppermint products, but Indonesia is currently unable to meet this demand and should continue to import peppermint. One effort can be made to improve cultivation procedures, and tissue culture becomes one alternative. This research uses shoots as explants with Murashige & Skoog's basic media and growth regulators BAP and NAA. The research was conducted in two phases: six-week initial planting and seven-week subculture. The treatment of BAP 4 mg/L + NAA 0.5 mg/L provides better performance for the number of leaves, and BAP 3 mg/L produced the best response regarding the number of shoots. Furthermore, BAP 1 mg/L + NAA 1 mg/L produced the best response to shoot height and number of leaves, and BAP 3 mg/L + NAA 0.5 mg/L generated the best response to root length. Based on the research, BAP 3 mg/L is the optimal treatment.

**Keywords:** *Mentha × piperita* L., peppermint, Benzil Amino Purin, Naphthalene Acetic Acid

### ABSTRAK

*Mentha × piperita* L. atau peppermint merupakan salah satu tumbuhan yang memiliki berbagai manfaat dalam bidang, seperti untuk pengobatan, kosmetik ataupun pangan. Produk peppermint banyak dibutuhkan dalam berbagai industri, namun saat ini Indonesia masih belum dapat memenuhi kebutuhan peppermint sehingga masih mengandalkan import. Salah satu upaya yang dapat dilakukan adalah dengan meningkatkan teknik budidaya salah satunya melalui kultur jaringan. Penelitian ini menggunakan tunas sebagai eksplan dengan media dasar Murashige & Skoog dan zat pengatur tumbuh berupa BAP dan NAA. Penelitian dilakukan dalam 2 tahap yaitu penanaman awal selama 6 minggu dan subkultur selama 7 minggu. Hasil penelitian menunjukkan bahwa perlakuan BAP 4 mg/L + NAA 0,5 mg/L menghasilkan respon terbaik terhadap jumlah daun dan BAP 3 mg/L menghasilkan respon terbaik terhadap jumlah tunas. Selanjutnya, BAP 1 mg/L dan NAA 1 mg/L menghasilkan respon terbaik pada tinggi tunas dan jumlah daun, serta BAP 3 mg/L + NAA 0,5 mg/L menghasilkan respon terbaik terhadap panjang akar. Berdasarkan penelitian yang telah dilakukan diperoleh perlakuan terbaik yaitu BAP 3 mg/L.

**Kata kunci:** *Mentha × piperita* L., peppermint, Benzil Amino Purin, Naphthalene Acetic Acid

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## INTRODUCTION

*Mentha × piperita* L., popularly known as peppermint, is a member of the Lamiaceae family. This plant has several uses, such as flavoring, cosmetics, and medicinal. The leaves are helpful against fever, pharyngeal inflammation, nausea, vomiting, and diarrhea. *M. piperita* also possesses antioxidant, antibacterial, antiviral, anticarcinogenic, and anti-inflammatory properties (Trevisan et al., 2017). Moreover, *M. piperita* has a strong fragrance and biological components that have a role in dentistry and cosmetics (Mahendran & Rahman, 2020)

*M. piperita* contains various chemical compounds, including  $\alpha$ -pinene, sabinene, cineole, menthone, menthofurane, neomenthol, menthol, neomethyl acetate, methyl acetate, isomethyl acetate, and bourbonene (Saharkhiz et al., 2012). The main constituents include menthol, cinnamic acid, and flavonoid (Jurić et al., 2021). Native to Europe, this plant has already spread to the United States and Africa. Similarly, this plant has been progressively cultivated worldwide (Mahendran & Rahman, 2020)

Numerous industries have a high demand for peppermint products, but Indonesia is unable to meet its demand; thus, it remains reliant on imports. One effort can be made to improve *M. piperita*'s cultivation techniques (Hasanah et al., 2019). *M. piperita* is a plant that has not been extensively grown. Hybrid plants are typically sterile and yield few seeds; hence *M. piperita* reproduces through stolons (Dinu et al., 2021). Menthol import for the industry in Indonesia needs 16.10 tons/year. Therefore domestic production of menthol oil is needed for import substitution. Peppermint cultivation still faces low production and oil quality, with 11-52 kg/ha in the first harvest with a menthol level of 48% -53% (Trisilawati et al., 2020). It needs cultivation techniques by plant tissue culture to obtain large quantities of yield quickly.

Various studies on the propagation of *M. piperita* utilizing plant growth regulators (PGR) have been performed earlier, with the best shoot growth results at concentrations of 3 mg/L BAP (6-benzyloaminopurine) and 1.5 mg/L kinetin and the best root growth with indolyl-3-butyric acid (IBA) at 0.5 mg/L (Sharma et al., 2019). Khan et al. (2021) reported other research using MS media and the addition of PGR, including BAP (150  $\mu$ l/50 ml), 1-naphthaleneacetic acid (NAA) (20  $\mu$ l/50 ml), and indole-3-acetic acid (IAA) (20  $\mu$ l/50 ml), resulted in induction and multiplication of shoots in optimal condition. While research with others, PGR has not been done. Therefore, pursuing research with various PGRs to obtain optimum growth is essential. This research aims to figure out the optimal concentration of NAA and BAP for the tissue culture growth of *M. piperita*.

## MATERIAL AND METHODS

The research was conducted at the Research Center and Development of Medicinal Plants and Traditional Medicines in June 2020. The materials used in this study were *M. piperita* shoots obtained from Medicinal Plant and Traditional Medicine Research and Development Center, Tawangmangu. Instant Murashige & Skoog basal media (Phytotech Lab.), sucrose (Merck, Germany), BAP (Merck, Germany), NAA (Merck, Germany), and agrept WP 20 bactericidal (PT. Mastalin Mandiri, Jakarta), dithane M-45 fungicidal (Dow Agro Science, China), and bayclin desinfectan (SC Johnson Inc, US). The tools used are a shaker, autoclave, hotplate, and Laminar Air Flow (LAF).

The apical bud was used to initiate the culture as an explant. Before thorough disinfection, the explants were sterilized by soaking them for 3 minutes in a detergent solution and rinsing them three times in sterile distilled water. The proper disinfection method required a 7-minute soak in bactericide, followed by three times rinses with sterile distilled water. Then, the explants

were soaked in fungicide for 7 minutes and rinsed three times with aquadest. Next, the explants were transferred to LAF, soaked in 70% alcohol for 30 seconds, and then rinsed with sterile distilled water. The final sterilization consisted of a 1-minute soaking in sodium hypochlorite and three times washing with sterile distilled water (Wijaya & Sudrajad, 2019).

The sterilized explants were then cultured on media with a combination of PGR, as shown in Table 1. Explants were incubated for six weeks in a growth environment with controlled light, temperature, and humidity.

Table 1. The treatment combination of PGR on initiated cultured stage

Sample code	BAP (mg/L)	NAA (mg/L)
A1 (control)	0	0
A2	1	0
A3	2	0
A4	1	0.5
A5	1	1
A6	2	0.5
A7	0	0.5
A8	0	1

After six weeks of incubation, subcultures were performed with a combination of PGR, as shown in Table 2.

Table 2. The treatment combination of PGR in subcultured stage

Sample code	BAP (mg/L)	NAA (mg/L)
B1	1	0
B2	2	0
B3	3	0
B4	1	0.5
B5	1	1
B6	2	0.5
B7	2	1
B8	3	0.5
B9	3	1
B10	4	0.5
B11	4	1

## RESULTS AND DISCUSSION

This study was carried out in two stages: initial planting and subculture. Incubation was carried out six weeks during the initial planting, followed by subculturing for seven weeks. Murashige and Skoog (MS) are the basic media for initial and subcultured plantings. The MS media has a wide range of uses due to its high potassium, ammonium, nitrate, and potassium content. Although these components are required for plant growth, high salt levels do not always result in optimal growth in explant development (Setiawati et al., 2018). According to Khan et al. (2021), MS can easily produce axillary shoot proliferation (Khan et al., 2021). In this research, BAP and NAA were utilized as PGR because BAP is a form of cytokinin resistant to degradation, and NAA is a type of auxin that is more stable than other auxins (Sari et al., 2018). The results of the research are shown in Tables 3 and 4.

Table 3. Six weeks initial planting results of *M. piperita*

Sample	Number of leaves	Number of shoots	Shoot length (cm)	Number of roots	Root length (cm)
A1	10	5 <sup>b</sup>	3.75 <sup>b</sup>	11	3.8
A2	2	2 <sup>a</sup>	1 <sup>a</sup>	0	0
A3	2	2 <sup>ab</sup>	2 <sup>ab</sup>	0	0
A4	10	2 <sup>ab</sup>	2.25 <sup>ab</sup>	1	0.25
A5	7	1 <sup>a</sup>	0.25 <sup>a</sup>	8	1.5
A6	2	2 <sup>ab</sup>	6.2 <sup>c</sup>	2	1.2
A7	5	1 <sup>a</sup>	0.9 <sup>a</sup>	4	1
A8	12	1 <sup>a</sup>	0.6 <sup>a</sup>	16	1.5

\*) Numbers within the same column with a different letter(s) are significantly different from each other according to the LSD test at P ≤ 0.05

Table 4. Seven weeks subcultured results of *M. piperita*

Sample	Number of leaves	Number of shoots	Shoot length (cm)	Number of roots	Root length (cm)
B1	11.31	9.67 <sup>ab</sup>	6.02	8.25	3.73 <sup>ab</sup>
B2	7.67	3.00 <sup>a</sup>	4.07	0.00	0.00 <sup>a</sup>
B3	12.16	26.25 <sup>b</sup>	8.22	21.50	4.24 <sup>ab</sup>
B4	13.22	10.00 <sup>ab</sup>	5.24	15.50	3.16 <sup>ab</sup>
B5	12.39	18.00 <sup>ab</sup>	8.58	23.50	4.19 <sup>ab</sup>
B6	7.35	23.00 <sup>ab</sup>	3.40	22.00	1.51 <sup>ab</sup>
B7	9.72	21.50 <sup>ab</sup>	6.47	10.50	3.13 <sup>ab</sup>
B8	10.59	23.00 <sup>ab</sup>	6.47	13.00	6.49 <sup>b</sup>
B9	9.66	13.50 <sup>ab</sup>	4.71	12.50	2.40 <sup>ab</sup>
B10	13.28	21.40 <sup>ab</sup>	6.73	9.20	3.38 <sup>ab</sup>
B11	11.20	14.50 <sup>ab</sup>	5.73	12.00	3.12 <sup>ab</sup>

\*) Numbers within the same column with different letter(s) are significantly different from each other according to the LSD test at P ≤ 0.05.

### Effect on The Number of Leaves

Explant growth is indicated by the number of leaves formed. Large amounts of leaves will produce many photosynthates, improving plant development (Nurhanis et al., 2019). The initial planting (Table 1) revealed that A8 (NAA 1 mg/L) produced the strongest growth with an average of 12 leaves, whereas A2 (BAP 1 mg/L), A3 (BAP 2 mg/L), and A6 (BAP 2 mg/L + NAA 0.5 mg/L) produced the fewest with two leaves. NAA given singularly was able to stimulate leaf growth, perhaps due to the presence of sufficient endogenous cytokinins. Exogenous auxins and endogenous cytokinins are suggested to stimulate leaf growth. The results contrasted with those of Kartiman et al. (2018), who used *Coelogyne pandurata* explants and found that using BAP at a concentration of 0.3-1 mg/L increased the number of leaves while using NAA at a concentration of 0.2-0.5 mg/L produced lower results than the control.

Table 2 shows the subcultured results, which revealed that the B10 (BAP 4 mg/L +NAA 0.5 mg/L) produced the most leaves, whereas the B7 (BAP 2 mg/L +NAA 0.5 mg/L) produced the fewest. Cytokinins play an important role in leaf formation. Because BAP stimulates cell division, increasing the PGR concentration enhances cellular division, increasing the number of leaves (Budisantosa et al., 2018). A higher cytokinin-to-auxin ratio can promote shoot and leaf growth. The addition of cytokinins interacts with the auxin. It shows that in vitro plant growth controlled by balance and interaction of growth regulators contained the explant (endogenous) or absorbed

from medium (exogenous) (Nurhanis et al., 2019). (Nurhanis et al., 2019). According to the LSD test, there was no significant variation in the number of leaves between the initial and the subcultured planting.

### **Effect on The Number of Shoots**

The number of shoots is the most important factor in determining the effectiveness of tissue culture, as this parameter reflects the success of plant multiplication. The greater the number of shoots grow, the more multiplication can be performed to produce a growing number of plants (Rezaldi et al., 2022). In the initial planting, the A1 (control) produced the greatest number of shoots, with an average plant height of five. It is possibly due to endogenous hormone regulators, which cause plants to produce the best shoot growth. The treatments with the least effective results were A5 (BAP 1 mg/L + NAA 1 mg/L), A7 (NAA 0.5 mg/L), and A8 (NAA 1 mg/L). These results were probably due to the presence of endogenous auxin, given that the addition of exogenous auxin in significant quantities inhibited shoot growth. Low auxin concentrations promote optimal shoot growth, whereas excess auxin concentrations inhibit growth (Latifa et al., 2022; Pamungkas & Puspitasari, 2018). According to research published by Widiyatmanto et al. (2012), growth began to decrease at a concentration of 1 mg/L NAA. NAA concentrations below 1 mg/L are expected to yield superior results. Latifa et al. (2022) research was supported by the discovery that using BAP 1.5 ppm + NAA 0.25 ppm to achieve the best shoot growth and by research conducted by Adugna et al. (2020) using kinetin 0.5 mg/L + NAA 0.01mg/L produced the greatest number of shoots.

The B3 (BAP 3 mg/L) treatment yielded the highest number of shoots in the subcultured results, with 26. The combined treatment of BAP and NAA produced a significant number of shoots, ranging from 10 to 23. Three shoots were produced by the B2 (BAP 2 mg/l), which yielded the least amount. Louw et al. (2018), BAP is a kind of cytokinin with strong activity in cell division and bud formation. In the interaction between cytokinins and auxins, the action of cytokinins cannot be separated from that of auxins, as the effects of blocking or stimulating cell division depend on the phytohormones interacting. Concerning shoot induction, their functions are complementary (Kartiman et al., 2018). High levels of cytokinins and low levels of auxins can also enhance shoot formation morphogenesis, hence increasing the growth of apical shoots (Louw et al., 2018; Nofiyanti et al., 2021). LSD results indicated that at the time of the initial planting, treatments A2, A5, A7, and A8 had a significant effect on the control.

### **Effect on Shoot Length**

At the site of meristem tissue growth, cell division and elongation influence the increase in plant height. Auxins and cytokinins affect both processes (Nurhanis et al., 2019). Auxin is found in the apical meristem, where it promotes the elongation of shoots, whereas cytokinins are responsible for cell elongation. The A6 (BAP 2 mg/L + NAA 0.5 mg/L) resulted in the highest shoots from the initial planting, as measured by the shoot height. Compared to controls and other treatments, this treatment yielded significant results. The statement of Lestari et al. (2019) that BAP can stimulate shoot growth more efficiently in the presence of auxin in the culture medium supports these results. An NAA concentration of 0.5-1 mg/L decreased shoot size. It is likely due to the absence of BAP. The PGR promotes protein synthesis and cell division to induce budding (Erawati et al., 2020). Hiswan et al. (2020) showed different results using *Bambusa vulgaris* shoot explants to demonstrate that NAA 0.5 mg/L produced the greatest plant height compared to a combination of BAP and NAA.

B6 (BAP 1 mg/L + NAA 1 mg/L) produced the tallest shoots in subcultures, while B7 (BAP 2 mg/L + NAA 0.5 mg/L) produced the shortest shoots. It is possibly the presence of endogenous cytokinins in the explants at 1 mg/l BAP and 1 mg/l NAA that produces shoot growth. The statement of Alqamari et al. (2020) balance conditions between auxins and cytokinins result in callus growth. It is possibly due to sufficient endogenous cytokinins inducing shoot growth. Varied growth responses were seen when BAP and NAA were given at different concentrations. In addition to the availability of sufficient phytohormones, internal and external environmental variables during the incubation stage also affect plant growth (Erawati et al., 2020). The initial planting in the A6 produced the maximum yield, which was significantly greater than the control and other treatments. Still, the subcultured yielded no significant differences in the shoot length parameter.

### Effect on The Number of Roots

The quantity of roots indicates the range of nutrient uptake by the plant. The more roots a plant has, the wider it may spread and collect more nutrients (Pratama et al., 2022). Observing the number of roots in the initial planting revealed that A8 (NAA 1 mg/L) resulted in the greatest root growth, whereas A2 (BAP 1 mg/L) and A3 (BAP 2 mg/L) did not affect root growth. Auxin was provided at sufficient concentrations for root growth in the A8 treatment, resulting in optimal growth. Auxins are involved in stimulating root growth, whereas cytokinins promote shoot growth. Auxin at higher concentrations than cytokinins will stimulate root growth, whereas cytokinins at higher concentrations than auxin stimulate shoot growth (Nofiyanti et al., 2021). An excessive concentration of auxin can inhibit root growth, although it stimulates root growth. There is a maximum concentration at which these hormones can disrupt the hydrogen cross-links between the cellulose molecule chains in plant cell walls. Additionally, excessive auxin in plants inhibits root elongation, as seen by a surge in ethylene at the root tips (Debitama et al., 2022).

Subculture B6 (BAP 1 mg/L + NAA 1 mg/L) produced the greatest number of roots, but subculture B2 (BAP 2 mg/L) caused no root growth. It related to the fact that B6 could stimulate root growth by adding exogenous auxin, whereas B2 could not induce root growth without adding auxin. Budisantosa et al. (2018) discovered that BAP treatment and MS base medium could not induce root growth due to the necessity of auxin and cytokinins for root initiation. B1 (1 mg/l BAP) was able to generate roots, probably due to the existence of endogenous auxin that could stimulate root production without adding exogenous auxin. The analysis results provide an overview of the initial planting and subcultures, revealing no statistically significant differences between the various treatments.

### Effect on Root Length

In the initial planting, the A1 (control) produced the best growth compared to the other. The explants probably possessed significant endogenous auxin levels, allowing for rapid root development without adding exogenous auxin. A2 (1 mg/L BAP) and A3 (2 mg/L BAP) exhibited the lowest performance. It is likely since no auxin was given to the medium and the addition of BAP inhibited root growth. Heckmann et al. (2011) revealed in their research that the exogenous application of cytokinins at values of  $10^{-6}$ M to  $10^{-8}$ M inhibited the root growth of *Lotus japonicus*. It is because root elongation is sensitive to elevated BAP concentrations and is inhibited at high concentrations. Sibyan et al. (2018) and (Rezaldi et al., 2022) also reported that BAP inhibits root growth even though it stimulates shoot growth.

B8 (BAP 3 mg/L + NAA 0.5 mg/L) produced the longest roots compared to other subculture treatments. High concentrations of cytokinins and low concentrations of auxin interact

synergistically in cell division, root growth, and plant regeneration in vitro (Ilham & Prayoga, 2019). These two hormones play a role in the elongation and division of root tip meristems in addition to their role in root growth. The B2 (BAP 2 mg/L) yielded the lowest results, possibly due to BAP's suppression of root growth in the absence of auxin, which inhibited root extension. In the initial planting, no significant results were obtained for any treatments. Still, the subculture results for B8 (BAP 3 mg/L +NAA 0.5 mg/L), which produced the highest yield, were significantly different from B2 (BAP 2 mg/L), which produced the lowest yield.

## CONCLUSION

Based on the research conducted, it can be concluded that BAP 4 mg/L + NAA 0.5 mg/L produced the best response for the number of leaves, BAP 3 mg/L produced the for the number of shoots, BAP 1 mg/L, and NAA 1 mg/L produced the best response on shoot length and the number of leaves. Next, BAP 3 mg/L + NAA 0.5 mg/L produced the best response in terms of root length. BAP 3 mg/L produced the best results for the main parameter, the number of shoots.

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