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Shoots induction of axillary buds in blueberries (*Vaccinium corymbosum* L.) in vitro with the addition of BAP and IBA

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ABSTRACT. Blueberry *Vaccinium corymbosum* L. is a fruit plant that has high economic value and many benefits. Blueberries in Indonesia are not yet in great demand so domestic needs are still met by imports. Conventional blueberry propagation is done using stem cuttings. The disadvantages of conventional propagation are that seedling growth is slow, requires a large source of planting material, and is ineffective. The aim of the research was to determine the effect of the combination of BAP and IBA hormones on the induction of axillary shoots in effective in vitro propagation of blueberries. The explants used were axillary buds of blueberry, sterilized using fungicide, bactericide, bayclin, sodium hypochlorite and alcohol. The research design used a completely randomized design (CRD) with a combination of BAP and IBA hormones. The BAP hormone consists of three concentrations, namely 0.5, 1.0, and 1.5 mg/L. The IBA hormone consists of three concentrations, namely 0, 0.1, and 0.2 mg/L. Based on research results, the combination treatment of BAP and IBA succeeded in inducing shoots, but was not able to induce roots. The BAP 0.5 mg/L treatment showed the best results with a shoot emergence rate of 4.33 weeks, shoot height of 1.68 cm, number of shoots 1.67, and number of leaves 12.67. The findings of this study highlight the potential of BAP and IBA hormone combinations in accelerating blueberry shoot induction, offering an alternative propagation method that could support domestic production and reduce dependence on imports.

Keywords: axillary bud; BAP; blueberries; IBA; in vitro propagation

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INTRODUCTION

Blueberry *Vaccinium corymbosum* L. is a fruit plant that has high economic value. Blueberries are used as fresh fruit, processed food, and flavoring. Blueberries have a high vitamin and antioxidant content. According to Soleha & Agungyudistira (2016), fruits from the genus *Vaccinium* contain high levels of flavonoids and anthocyanins. *Vaccinium corymbosum* has the largest flavonoid content compared to other berries, namely 9.01 mg/g (Soleha & Agungyudistira, 2016). The sources of antioxidants contained in blueberries are anthocyanins, phenols and flavonoids which are useful for improving cardiovascular conditions and preventing aging (Vescan *et al*., 2012). This is in line with Yang *et al.* (2022) that the anthocyanin content in blueberries is an antioxidant which can be anti-inflammatory and prevent cardiovascular disease. Blueberries contain bioactive compounds as agents for skin health (Ivarsson *et al*., 2023). The compounds contained in blueberries can be used as medicine (Patel, 2014).

The development of blueberry plants in Indonesia has not attracted much interest so domestic blueberry needs are still met by imports. Efforts to support the development of blueberries in Indonesia include providing a source of superior, quality and disease-free seedlings. Conventional blueberry propagation can be done using stem cuttings (Ruzic *et al*., 2012). The disadvantages of conventional propagation are slow seedlings growth, requiring a lot of planting material and labor. Apart from that, conventional propagation is less effective for growing plants in large numbers and the health of seedlings from pathogens is still quite low (Vescan *et al*., 2012). The lack of effective blueberry propagation methods makes it difficult to mass propagate blueberry seedlings (Schuch & Tomaz, 2018). The technological approach to plant propagation through tissue culture can meet the

needs of effective blueberry seedlings. Tissue culture can be used as a tool for rapid and efficient seed production (Ashar *et al*., 2023). Propagation of blueberries through tissue culture produces seedlings that are identical to the parents (Goyali & Igamberdiev, 2015). Success of micropropagation protocol depends upon the rate and number of multiple shoots (Bahadur *et al*., 2015).

In vitro plant propagation is widely applied to various plant species. Optimizing blueberry propagation in vitro is important to produce effective seedlings. Blueberry propagation can be done through somatic embryogenesis or direct organogenesis. Previous research reported that propagation of blueberries through somatic embryogenesis with the addition of 2.3 µM TDZ hormone produced the highest maturation percentage of the blueberry embryos, 31% (Ghosh *et al.*, 2018). The addition of a combination of 0.5 mg/L zeatin with 0.25 mg/L dihydrozeatin to WPM media resulted in high blueberry proliferation of 4.26 and shoot length of 3.82 (Orsolya *et al*., 2019). In vitro propagation of blueberry plants requires appropriate culture media and the appropriate composition of plant growth regulators (PGR) (Cappelletti *et al*., 2016). Cytokinins are one of the major hormones that regulate growth and development of plants, auxin is the major phytohormone that induces root formation (Ahammed & Yu, 2016). Auxin and cytokinin are generally added to culture media for organogenesis and tissue growth *in vitro*. ZPT auxin and cytokinin work in interaction to trigger growth and morphology in cell, tissue and organ cultures (Anis & Ahmad, 2016). Cytokinins have a role in stimulating cell division, stimulating the formation of shoots, influencing cell metabolism, and stimulating dormant cells, while auxin triggers root growth. According to Dwiyani (2015), the combination ratio of auxin and cytokinin can influence the direction of morphogenesis and growth and development of plants in tissue culture. Optimization of blueberry micropropagation is essential to increase the blueberry industry and the demand for blueberry seedlings (Wang *et al*., 2023). The ratio of using a greater concentration of cytokinin than auxin can stimulate the growth of shoots and leaves. This study aimed to determine the effect of adding a combination of BAP and IBA hormones on the shoot induction of axillary bud explant in blueberry propagation in vitro. The findings suggest that optimizing the combination of BAP and IBA hormones could enhance in vitro blueberry propagation efficiency, potentially reducing reliance on conventional methods and supporting sustainable domestic cultivation.

MATERIALS AND METHODS

Research design. The research design was carried out using a completely randomized design (CRD) with a combination of BAP and IBA hormone treatment. The BAP hormone factor consisted of three concentration levels, namely 0.5, 1.0, and 1.5 mg/L. The IBA hormone factor consisted of three concentration levels, namely 0, 0.1, and 0.2 mg/L. The treatment was repeated 3 replicates.

Culture media. Culture media was made by mixing WPM media nutrient solution, 30 g/L sugar, 8 g/L agar gel, and distilled water. The media solution was added with hormones according to the treatment and homogenized with a magnetic stirrer. The pH of the medium was adjusted to 5.4. The media solution was cooked until it boiled and poured into bottles at a rate of 15 mL/bottle. The culture medium was sterilized using an autoclave at 121℃.

Sterilization and planting of explants. The explant used was the axillary bud of blueberry. The axillary buds were washed with running water, soaked in a fungicide solution for 30 minutes and soaked in a bactericide solution for 30 minutes. Sterilization of explants was continued with sterilization in laminar. Explants were sterilized by soaking in 70% alcohol solution for 1 minute and soaking in 1% NaOCl sodium hypochlorite solution for 10 minutes (Arencibia *et al.,* 2013). The explants were rinsed with sterile distilled water solution 3 times. The sterilized explants were then cut into pieces about 2.5-3 cm long with one axillary bud. Explants that had been planted were stored in an incubation room at a temperature of 25℃ and illuminated with LED lights for 8 hours/day, with a light intensity of 2000 lx.

Observation. Observation variables included the early emergence of shoots, plant height, number of shoots and number of leaves. Observations of the early emergence of shoots were carried

out from the beginning of inoculation until they showed a response. The early emergence of shoots was observed by recording the length of time the explants showed a shoot formation response. Observations of plant height, number of shoots and number of leaves were observed at the end of the observation in the 8th week.

Data analysis. Data obtained from observations were analyzed using analysis of variance (ANOVA), if the results obtained showed significantly different, they would be further analyzed using the Duncan multiple range test (DMRT) at a 95% confidence level. Data analysis was carried out using the SPSS version 26 statistical application.

RESULTS AND DISCUSSION

The axillary bud explants showed a shoot formation response with signs of swollen axillary buds and a light green color. This is in line with Samir & Arigundam (2020), proliferation in axillary shoots, shoots reproduce through branching of axillary shoots from explants. The addition of the combination hormone BAP and IBA showed significant results on the early emergence of blueberry shoots. The BAP 0.5 mg/L (P1) treatment showed the best results with early emergence of shoots, namely 4.33 days after planting. The combination treatment of BAP 1.0 mg/L + IBA 0.1 mg/L (P5) and BAP 1.5 mg/L + IBA 0.1 mg/L (P6) showed the earliest time for shoot emergence, namely 9 days after planting. Early timing of rapid shoot emergence can accelerate the formation of leaf organs and shoot elongation. The 0.5 mg/L BAP treatment produced the highest number of shoots, namely 1.67 shoots. Other concentration treatments, either BAP alone or a combination of BAP and IBA, were only able to produce 1 shoot/explant. The addition of a combination of BAP and IBA hormones showed significant results on shoot height. The BAP 0.5 mg/L (P1) treatment showed the highest shoots, namely 1.68 cm. The combination treatment of BAP hormone (0.5, 1.0, and 1.5 mg/L) with IBA 0.1 mg/L showed no significant difference in shoot height. The BAP 1.5 mg/L + IBA 0.1 mg/L (P6) treatment showed the shortest shoot height, namely 0.34 cm. Shoots in treatment P6 showed stunted and stunted growth until the 8th week of observation. The longer the shoots that form produce the greater the number of leaves. The BAP treatment of 0.5 mg/L (P1) produced the highest number of leaves, namely 12.67. The BAP 1.5 mg/L + IBA 0.2 mg/L (P9) treatment produced the lowest number of leaves. Based on the results of this study, it shows that the combination of BAP and IBA produces a lower number of shoots and shoot length compared to the combination of zeatin and dihydrozeatin. The combination of 0.5 mg/L zeatin with 0.25 mg/L dihydrozeatin produces high blueberry proliferation of 4.26 and shoot length of 3.82 (Orsolya *et al*., 2019).

Table 1. Observation of the early emergence of shoots, shoot height, number of shoots, and number of leaves on shoots induction of blueberries in the 8th week

Treatment	Early emergence of shoots (days)	Shoot height (cm)	Number of shoots	Number of leaves
P1 (BAP 0.5 mg/L)	4.33 ± 2.31 a	1.68 ± 0.95 a	1.67 ± 0.58	12.67 ± 1.53 a
P2 (BAP 1.0 mg/L)	4.67 ± 1.15 a	1.18 ± 0.23 ab	1.00 ± 0.00	11.00 ± 1.00 b
P3 (BAP 1.5 mg/L)	6.33 ± 1.53 ab	0.90 ± 0.09 bc	1.00 ± 0.00	10.33 ± 0.58 b
P4 (BAP 0.5 mg/L+ IBA 0.1 mg/L)	7.67 ± 0.58 bc	0.44 ± 0.06 c	1.00 ± 0.00	4.67 ± 1.15 de
P5 (BAP 1.0 mg/L+ IBA 0.1 mg/L)	9.00 ± 2.00 c	0.37 ± 0.05 c	1.00 ± 0.00	5.33 ± 0.58 cd
P6 (BAP 1.5 mg/L+ IBA 0.1 mg/L)	9.00 ± 1.00 c	0.34 ± 0.04 c	1.00 ± 0.00	4.00 ± 0.00 de
P7 (BAP 0.5 mg/L+ IBA 0.2 mg/L)	5.67 ± 0.58 ab	0.78 ± 0.15 bc	1.00 ± 0.00	6.33 ± 1.15 c
P8 (BAP 1.0 mg/L+ IBA 0.2 mg/L)	5.33 ± 1.53 ab	0.68 ± 0.16 bc	1.00 ± 0.00	5.67 ± 0.58 cd
P9 (BAP 1.5 mg/L+ IBA 0.2 mg/L)	8.67 ± 1.53 c	0.46 ± 0.05 c	1.00 ± 0.00	3.67 ± 0.58 e

Note: Numbers followed by the same lowercases are insignificantly different based on Duncan multiple range test at a level of α 0.05

In this study, the addition of the cytokinin single hormone namely BAP showed a faster shoot emergence time, taller shoots, and a greater number of leaves. Lower BAP concentrations resulted in better growth. This is in line with previous research, the addition of a single cytokinin hormone 2iP 5 mg/L to WPM media resulted in blueberry proliferation of 3.66 (Orsolya *et al*., 2019). By adding the single cytokinin hormone zeatin 2 mg/L to half strength of MS media, stem segment explants can

produce more than 4 shoots (Gao *et al*., 2018). Zeatin was efficient in direct regeneration of blueberry shoots (Cappelletti *et al*., 2016).

Our results showed that the addition of the single hormone namely BAP showed better results compared to the combination of BAP and IBA. On the contrary, Cappelletti *et al.* (2016) reported that regeneration efficiency for blueberry cultivar *sveva* was obtained in WPM medium with a combination of BA 3 mg/L and IBA 0.2 mg/L. The combination of the BAP hormone (0.5, 1.0, and 1.5 mg/L) with IBA 0.2 mg/L showed faster shoot emergence, higher shoots, and a greater number of leaves compared to the combination of the BAP hormone (0.5, 1.0, and 1.5 mg/L) with an IBA of 0.1 mg/L. Based on previous research, propagation of *bluecrop* and *berkeley* varieties of blueberries grown on WPM media with a combination of Zeatin and 0.1 mg/L IBA hormone produced a greater number of shoots than without the addition of IBA hormone (Ruzic *et al*., 2012). Different with this study, propagation of blueberries planted on WPM media containing BAP without being combined with the IBA hormone produced the highest number of shoots. The type of hormone and concentration added or combined influence the response of explants in vitro regeneration. Differences in growth responses can be caused by media composition with different concentrations of cytokinin and auxin. The combination of hormones IBA 0.1 mg/L, TDZ 0.0005 mg/L, and Zeatin 1 mg/L on WPM media exhibited the highest shoot fresh weight 304.21 mg, shoot length of 50.98 mm, number of shoots 17.17 shoots per blueberry plantlets (Wang *et al*., 2023). According to Cuce & Sokmen (2015), the combination of 1 mg/L zeatin hormone with 0.1 mg/L IBA produced better shoot multiplication compared to the combination of 1 mg/L zeatin hormone with 0.1 NAA.

The type of media can also influence the growth of blueberries in vitro. According to Cuce & Sokmen (2015), multiplication of blueberry shoots on WPM media showed the best results compared to MS and AN media. Hung *et al*. (2016) reported that a combination of 50% WPM and 50% MS media with the addition of 1 mg/L zeatin hormone showed the best blueberry propagation results with the number of shoots 5.9, shoot length 53 mm, number of leaves 65.3, number of nodes 33.2. *In vitro* propagation of blueberries is optimal on WPM media, while the proliferation stage is optimal on WPM media formulated by replacing NH_4NO_3 in WPM with $(NH_4)_2SO_4$ (Wang *et al*., 2023). Wang *et al.* (2019) reported that MS and WPM media can induce both callus production, shoots multiplication and shoots proliferation.

The addition of the combination hormone BAP and IBA was able to induce shoots in axillary buds of blueberry, but the explants were unable to produce a large number of shoots. The influence of auxin and cytokinin in appropriate amounts will determine the direction of cell differentiation. The suitability of auxin and cytokinin enzymes can result in the desired direction of cell differentiation, such as the formation of shoots, which influences the number of leaves produced. Auxin and cytokinin work in interaction to direct the growth and morphology of cell cultures, tissues and plant organs (Dwiyani, 2015). The growth of blueberry shoots was able to produce a single shoot, only treatment P1 produced more than one shoot.

Propagation of blueberries on WPM media with the addition of BAP and IBA was only able to induce shoots and form leaf organs, but the shoots were not able to form root organs. This is because the ratio of hormones given, the ratio of cytokinin is greater than auxin, triggering shoot growth. Shoots proliferation in media with the addition of optimal hormones can increase the number of shoots formed. Root induction needs to be done by sub-culture into root media by adding auxin single hormone. According to Guo *et al.* (2019), root induction of the *ozarkblue* cultivar blueberry was successfully carried out on half strength (½ WPM) woody plant media with the addition of 0.1 mg/L IBA hormone resulting in a rooting percentage of 97% after 45 days with an average number of roots and root length of 6.5 roots and 3.5 cm. The *sierra* cultivar showed the highest root percentage, namely 100%, with an average number of roots and root length of 5.9 roots and 3.9 cm. The success of *in vitro* regeneration also depends on the response of each species and cultivar to the type of growth regulator given (Samir & Arigundam, 2020). Rooting *bilberries* in WPM media with the addition of

IBA 0.5 mg/L or 1.0 mg/L with activated charcoal produces the best rooting and a root percentage of 60% (Cuce & Sokmen, 2015).

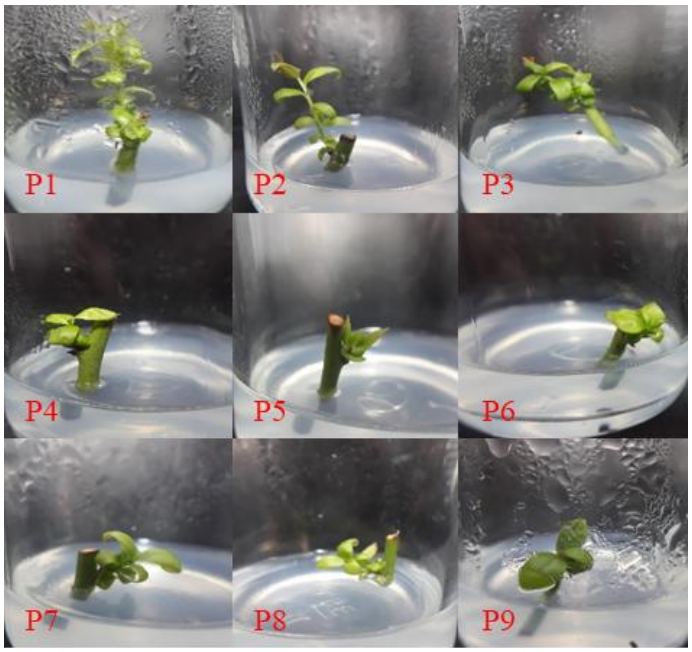

Fig. 1. Growth of blueberry shoots in the treatment with the addition of BAP hormone and the combination of BAP with IBA

Fig. 2. Shoot growth in the best treatment was BAP 0.5 mg/L

In this study, the shoots that formed also did not develop significantly. Type of cytokinin has a significant influence on the shoot length of blueberries (Schuchovski & Biasi, 2019). Generally reduction of PGR in the subculture medium is favorable for elongation and proliferation (Bahadur *et al*., 2015). According to Samir & Arigundam (2020), bud meristems do not develop until the stem elongates and grows, due to apical dominance. Stem elongation in shoot proliferation blueberry cultivar *duke* were carried out in a WPM medium with addition of 15 mg/L of 2iP (Cappelletti *et al*., 2016).

Optimization of blueberry propagation in vitro is very important as an initial reference in producing superior seeds on a large scale. This study has shown success in the induction of shoot organs, although it has not been successful in producing root organs. The findings of this study are a good first step in vitro blueberry shoot regeneration. Basically, each type of culture media and combination of hormones given will provide a different response in blueberry regeneration in vitro.

Therefore, further research is needed regarding the right culture media along with a combination of hormones with optimal concentrations as an effort to induce root organs.

CONCLUSION

Based on research results, the combination of BAP and IBA can induce blueberry shoots, but until the 8th week the shoots are not able to produce roots. The combination of BAP and IBA was not effective in producing a lot of shoot multiplication in blueberry micropropagation. The 0.5 mg/L BAP treatment showed the best results with the fastest early shoot emergence parameters, namely 4.33 days, shoot height 1.68 cm, number of shoots 1.67, and number of leaves 12.67.

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