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Research Article

Shoot multiplication growth of some apple cultivars with a combination of auxin and cytokinin hormones in vitro

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ABSTRACT

Apples (Malus sylvestris) have been grown in Indonesia, especially in highland areas such as Batu, Nongkojajar, and Poncokusumo. In order to propagate apple plants through in vitro, organ culture is performed in a controlled environment, free of pests and diseases. The research aimed to evaluate the effect of the composition of the growth regulator BAP-IAA on the growth and shoot multiplication of several apple cultivars in vitro. The research was carried out at the Indonesian Orchid Partners Laboratory Jl. Hasanudin 1 No 24 Junrejo District, Batu City, Indonesia. The four apple cultivars used were Fuji, Red Delicious, Gala, and Manalagi apples. Four types of growth regulator composition were the treatments: 3 mg L^{-1} BAP and 0.2 mg L^{-1} IAA, 6 mg L^{-1} BAP and 0.2 mg L^{-1} IAA, 3 mg L^{-1} BAP and 0.3 mg L^{-1} IAA, and 6 mg L^{-1} BAP and 0.3 mg L^{-1} IAA. The results showed that growth and yield were more precise in the combination of Red Delicious with the growth enhancer composition of 3 mg L^{-1} BAP and 0.3 mg L^{-1} IAA, as shown by the variable number of shoots and fresh weight of explants. The results showed that the combination of the Red Delicious apple cultivar with BAP 3 mg L^{-1}+IAA 0.3 mg L^{-1} can be recommended as the best treatment in mass shoot production/multiplication.

Keywords: composition; cytokinin-auxin; apple cultivars; shoot multiplication

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INTRODUCTION

Originating from West Asia and growing well in subtropical climates, apples (*Malus sylvestris*) are a type of fruit plant that grows yearly. Since 1935, apples have been grown in Indonesia, specifically in highland areas such as Batu, Nongkojajar, and Poncokusumo. According to data from the Directorate General of Horticulture in 2015, there was a significant increase in apple productivity, reaching 28.13% from 2012 to 2014. Despite this growth, the value of apple imports continues to increase every year, with the latest data in 2012 showing the amount of imports amounting to 214,245 tons. The lack of variety in local apple types is one of the reasons people prefer introduced apples (Directorate General of Horticulture, 2015).

The varieties Fuji and Red Delicious are the main introduced apple varieties in the market that are commonly consumed in Indonesia (Nurchayati & Hikmah, 2014). This can attract Indonesian people's interest in conventional propagation through vegetative methods, such as cuttings or grafting (attaching).

Apple plant varieties can be developed by bringing introduced apple plants to Indonesia. Apple plants are poorly adaptive, frequently perish, and have low multiplication rates because they require a difficult adaptation process from subtropical

to tropical climates (Ranaivozandriny et al., 2023). One method that can provide these plants is through embryo culture techniques and in vitro shoot multiplication with a high success rate (Bustaman et al., 2004).

In vitro multiplication of shoots has the benefit of multiplying shoots in a controlled environment (aseptic conditions), free from pests and diseases, and is effective for the production of thousands of cultivars in a restricted space and brief timeframe (Lestari, 2011; Zhang et al., 2020). The growth and development of shoots in vitro requires growth regulators such as the hormones auxin and cytokinin (Yuniastuti et al., 2016). The composition and concentration of growth regulators also need to be considered (Jumroh et al., 2014). Cytokinin and auxin compositions are useful in stimulating shoot formation and proliferation or root formation in apple buds, along with appropriate media for apple cultivar propagation and improvement (Shi et al., 2021; Abdalla & Dobránszki, 2024).

The use of growth regulators between auxin (Indoleacetic acid) and cytokinin (Benzylaminopurine) influences growth and shoot formation (Yusnita, 2015). The combination treatment of BAP 4 ppm and IAA 0.5 ppm can encourage an increase in the growth of the number of shoots by 87% compared to the control in pineapple shoots (Harahap & Nusyirwan, 2014). Syafii et al. (2013) reported that the combination of auxin and cytokinin growth regulators provides the best percentage of shoot emergence by selecting the right concentration of growth regulators. Therefore, the response of shoot multiplication of apple plant varieties to the combination of auxin and cytokinin hormones in vitro needs to be studied to increase the adaptation of apple plants, especially plants from outside Indonesia. This research aimed to determine the interaction of several combinations of BAP-IAA growth regulators on several apple cultivars in vitro.

MATERIALS AND METHODS

Experimental site and design

The research was conducted at the Indonesian Orchid Partners Laboratory Jl. Hasanudin 1 No 24 Junrejo District, Batu City, Indonesia (-7.908931,112.558855; 739 masl). The planting medium was Murashige and Skoog (MS), with a pH of 5.6-5.8. The laboratory lighting used a 20-watt fluorescent lamp. The average laboratory temperature from the first to the eighth week was 20 ^{o}C.

The study used a randomized complete block design (RCBD) with two factors and three replications. The main factor used was apple cultivar (V), namely Fuji, Red Delicious, Gala, and Manalagi apples. The second factor was the composition of the growth enhancer (B), namely 3 mg L^{-1} BAP and 0.2 mg L^{-1} IAA, 6 mg L^{-1} BAP and 0.2 mg L^{-1} IAA, 3 mg L^{-1} BAP and 0.3 mg L^{-1} IAA, 6 mg L^{-1} BAP and 0.3 mg L^{-1} IAA. Each replication consisted of 3 bottle samples (explants). Fuji and Red Delicious apple explants were obtained from outside Indonesia, while Gala and Manalagi apple explants came from local cultivars.

The explants used came from embryo cultures already in the form of shoots and were 5 months old. The explants were subcultured four times. The first to third subcultures were on Murashige and Skoog (MS), while the fourth subculture was on MS medium with the addition of the growth enhancer Benzylaminopurine (BAP) 5 ppm and Naphthaleneacetic acid (NAA) 0.3 ppm.

The tools and materials were sterilized first before planting in the treatment medium. The tools used during planting were Petri dishes, Bunsen, scalpel blade, tweezers, filter paper, and sterile plastic and rubber. The materials used during planting were four explant cultivars in the form of shoots, Murashige and Skoog (MS) treatment medium in the form of balm bottles, growth regulators cytokinin Benzylaminopurine (BAP), and auxin Indoleacetic acid (IAA).

Tools and materials were sterilized with 70% alcohol and placed in laminar air flow (LAF). Next, the LAF was sterilized using an ultraviolet (UV) lamp and blower. The tools used, such as scalpels, tweezers, and Petri dishes, were sterilized again with Bunsen before planting explants into the treatment medium. When planting the explants into the treatment media, the explants were cleaned from parts that were yellow or brown and

weighed as the initial weight of the explants. Then, the explants were planted in sterilized treatment media and covered tightly using sterile plastic.

Observations

The number of shoots was observed by counting the shoots that had appeared ± 0.5 cm on the explant at 1-8 weeks after planting (WAP). The time of shoot emergence was observed when the shoots first grew, and the shoot initiation was determined from the number of explants from which shoots emerged. The total number of plants in each treatment was multiplied by 100%. Shoot height was observed from the surface of the media to the highest growing point using millimeter block paper at 8 WAP. Leafy explants percentages were counted by the number of leaf explants that had fully opened at 8 WAP. The explant fresh weight was obtained from the results of weighing the final weight of the plant at the end of the study at 8 WAP. Time of shoot emergence, shoot height, leafy explants percentage, and explant fresh weight did not show significant differences, so they were only shown at the end of the observation.

Statistical analysis

The data obtained was subjected to an analysis of variance (ANOVA). Variables that showed a significant effect due to treatment were further tested by Tukey's honestly significant difference (HSD) at α = 5% using SPSS version 9.0.

RESULTS AND DISCUSSION

Shoot number

The interaction of apple cultivar and the composition of the growth enhancer BAP-IAA on the number of apple plant shoots had a significant effect at 8 WAP (Table 1). The treatment combination of Red Delicious with BAP 3 mg L^{-1} +IAA 0.3 mg L^{-1} produced the highest number of shoots with an average value of 3.89 compared to the combination of Gala, Manalagi, and Fuji with BAP 3 mg L^{-1} +IAA 0.2 mg L^{-1}, BAP 6 mg L^{-1} +IAA 0.2 mg L^{-1}, and BAP 6 mg L^{-1} +IAA 0.3 mg L^{-1} at 8 WAP. Furthermore, Red Delicious apple shoots with a composition of BAP 3 mg $L^{-1}$and IAA 0.3 mg L^{-1} and Fuji with BAP 3 mg L^{-1} and IAA 0.3 mg L^{-1} had statistically the same number of shoots, 170% and 155% greater than Fuji apple shoots with a composition of BAP 6 mg L^{-1} and IAA 0.3 mg L^{-1} and Manalagi with BAP 6 mg L^{-1} and IAA 0.3 mg L^{-1} 8 WAP. The number of shoots produced was small in the combination treatment Fuji with BAP 6 mg L^{-1}+IAA 0.3 mg L^{-1} and combination treatment between Manalagi with BAP 6 mg L^{-1}+IAA 0.3 mg L^{-1}, an indication that Fuji and Manalagi cultivars were less optimal at too high concentrations of BAP-IAA growth regulators at 8 WAP. According to Yatim (2016), adding a BAP level of 3 ppm can generate high-shoot multiplication. Another finding, Geng et al. (2016) stated that only adding 2 mg L^{-1} was consistently effective in apple shoot proliferation.

There was an increase in the number of apple shoots in the combination of Fuji, Red Delicious, Manalagi, and Gala cultivars with the composition of BAP 3 mg L^{-1} + IAA 0.3 mg L^{-1} without any abnormalities (Figure 1C). When compared to the combination of Manalagi with the composition of BAP 6 mg L^{-1} and IAA 0.3 mg L^{-1}, it can be seen that the explants had a more limited shoot growth (Figure 1D). This is in line with Faisal et al. (2018), which revealed the synergistic influence of the hormones cytokinin and auxin to strengthen the rate of shoot proliferation and the best regeneration per explant of Ruta graveolens. The same results have been reported by Grzegorczyk-Karolak et al. (2022), explaining the effect of a concentration that is too high between 8 mg/L BAP can reduce the percentage of shoot growth and the number of shoots in explants. This illustrates that using the right concentration of cytokinin hormone (BAP) will give rise to or induce more shoot growth (Waseem et al., 2011).

This research showed that several apple cultivars and the composition of plant hormones BAP and IAA influenced the growth of the number of shoots, as seen in Red Delicious with a media composition of BAP 3 mg L^{-1} and IAA 0.3 mg L^{-1} was more effective in multiplying shoots compared to the Manalagi cultivar with media composition BAP 6

mg L^{-1} and IAA 0.3 mg L^{-1} at 8 WAP. There are several important things to note that the combination of apple cultivars with the composition of plant hormones can influence shoot number because shoot number is sensitive to the type of cultivar (Lizarraga et al., 2017), the type of explant (Avivi et al., 2022), the composition of the media (Furqoni & Efendi, 2018), type of hormone (Ratnasari et al., 2016), and type of explant (Azizi et al., 2017).

Table 1. Shoots number of apple cultivars in the growth regulators composition at 1-8 WAP.

Apple cultivars (V)	Composition of growth regulators (B)	Shoots number (WAP)							
		1	2	3	4	5	6	7	8
Fuji	MS and 3 mg L^{-1} BAP and 0.2 mg L^{-1} IAA	1.22a	1.22a	1.22a	1.33a	1.44a	1.78a	2.22a	3.00 abc
	MS and 6 mg L^{-1} BAP and 0.2 mg L^{-1} IAA	1.22a	1.22a	1.22a	1.33a	1.44a	1.56a	1.67a	1.67 ab
	MS and 3 mg L^{-1} BAP and 0.3 mg L^{-1} IAA	1.00a	1.00a	1.11a	1.44a	1.44a	1.89a	2.33a	3.11 bc
	MS and 6 mg L^{-1} BAP and 0.3 mg L^{-1} IAA	1.00a	1.00a	1.00a	1.11a	1.11a	1.22a	1.22a	1.44 ab
Red Delicious	MS and 3 mg L^{-1} BAP and 0.2 mg L^{-1} IAA	1.44a	1.44a	1.44a	1.67a	1.67a	1.78a	1.78a	1.89 ab
	MS and 6 mg L^{-1} BAP and 0.2 mg L^{-1} IAA	1.11a	1.11a	1.11a	1.22a	1.22a	1.33a	1.33a	1.44 ab
	MS and 3 mg L^{-1} BAP and 0.3 mg L^{-1} IAA	1.11a	1.11a	1.11a	1.44a	1.89a	2.56a	2.78a	3.89 c
	MS and 6 mg L^{-1} BAP and 0.3 mg L^{-1} IAA	1.22a	1.22a	1.22a	1.33a	1.33a	1.33a	1.33a	1.44 ab
Gala	MS and 3 mg L^{-1} BAP and 0.2 mg L^{-1} IAA	1.22a	1.22a	1.22a	1.44a	1.67a	1.67a	1.67a	1.89 ab
	MS and 6 mg L^{-1} BAP and 0.2 mg L^{-1} IAA	1.22a	1.22a	1.22a	1.22a	1.33a	1.44a	1.67a	1.78 ab
	MS and 3 mg L^{-1} BAP and 0.3 mg L^{-1} IAA	1.00a	1.00a	1.00a	1.11a	1.44a	1.67a	1.89a	2.44 abc
	MS and 6 mg L^{-1} BAP and 0.3 mg L^{-1} IAA	1.00a	1.00a	1.00a	1.00a	1.00a	1.33a	1.56a	1.78 ab
Manalagi	MS and 3 mg L^{-1} BAP and 0.2 mg L^{-1} IAA	1.11a	1.11a	1.11a	1.22a	1.28a	1.50a	1.61a	1.83 ab
	MS and 6 mg L^{-1} BAP and 0.2 mg L^{-1} IAA	1.00a	1.00a	1.00a	1.22a	1.22a	1.22a	1.33a	1.33 ab
	MS and 3 mg L^{-1} BAP and 0.3 mg L^{-1} IAA	1.11a	1.11a	1.11a	1.33a	1.67a	1.89a	2.00a	2.44 abc
	MS and 6 mg L^{-1} BAP and 0.3 mg L^{-1} IAA	1.11a	1.11a	1.11a	1.11a	1.11a	1.11a	1.11a	1.22 a
	V factors	ns	ns	ns	ns	ns	ns	ns	*
	B factors	ns	ns	ns	ns	ns	*	*	*
	V*B	ns	ns	ns	ns	ns	ns	ns	*

Note: Values with the same letters are not significantly different based on the Tukey test ($p \leq 0.05$); ns = not significant; * = significant at α = 5% level.

Explant fresh weight

Apple cultivar, the composition of the BAP-IAA growth regulator, and their interactions had a significant effect on the fresh weight of apple shoots at 8 WAP (Table 2). The composition of BAP 3 mg L^{-1} and IAA 0.3 mg L^{-1} demonstrated the highest average fresh weight values, 1.6 and 1.5 g, for the Red Delicious and Gala apple cultivars, respectively compared with the composition of BAP 6 mg L^{-1} and IAA 0.3 mg L^{-1} as a similar IAA concentration. The composition of BAP 3 mg L^{-1} and IAA 0.3 mg L^{-1} generated explant fresh weight of 93%, 154%, 146%, and 167%, respectively, for the Fuji, Red Delicious, Gala, and Manalagi apple shoot cultivars. Interestingly, in the combination of Fuji and

Manalagi apple cultivars, the composition of BAP 3 mg L^{-1} and IAA 0.2 mg L^{-1} produced the same fresh weight (1.1 and 0.9 g), not different from BAP 3 mg L^{-1} and IAA 0.3 mg L^{-1} (Table 2). This observation aligns with Kodad et al. (2021), who explained that BAP has a role in cell division and accelerating shoot development/multiplication so that it increases the fresh weight of shoots. With the addition of the optimal concentration of BAP 3 mg L^{-1} +IAA 0.3 mg L^{-1}, in vitro shoot propagation can be carried out more quickly and efficiently, which allows the production of apple seedlings in large quantities (Chmielarz et al., 2023), free of pests and diseases (Delgado-Paredes et al., 2021), and stability in the supply of quality seedlings (Faisal et al., 2018).

The composition of growth regulators in apple shoot culture influenced the rise in fresh mass of explants in all apple cultivars (Figure 1). The Fuji apple cultivar had an increased number of shoots, thereby increasing the fresh weight of the explants, especially in the growth enhancer composition BAP 3 mg L^{-1} and IAA 0.3 mg L^{-1} (Figure 1C) compared to the composition BAP 6 mg L^{-1} and IAA 0.2 mg L^{-1} (Figure 1D). Meanwhile, the fresh weight of explants at the composition of BAP 6 mg L^{-1} and IAA 0.3 mg L^{-1} showed a relatively insignificant rise in each apple cultivar (Figures 1B and D). This aligns with Britto et al. (2021) that adding the hormone BAP concentration of 3 mg/L can increase the development of orchid explants in a proliferative direction.

Table 2. Explant fresh weight of apple cultivars in the growth regulators composition at 8 WAP.

Apple cultivars (V)	Fresh weight of explant (g)			
	MS, 3 mg L^{-1} BAP, 0.2 mg L^{-1} IAA	MS, 6 mg L^{-1} BAP, 0.2 mg L^{-1} IAA	MS, 3 mg L^{-1} BAP, 0.3 mg L^{-1} IAA	MS, 6 mg L^{-1} BAP, 0.3 mg L^{-1} IAA
Fuji	1.10bcde	0.79abc	1.43de	0.74ab
Red Delicious	0.67ab	0.64ab	1.60e	0.63ab
Gala	0.58ab	0.52ab	1.50e	0.61ab
Manalagi	0.90abcd	0.62ab	1.31cde	0.49a
V*B	*			

Note: Values with the same letters were not significantly different based on the Tukey test ($p \leq 0.05$). ns = not significant; * = significant at α = 5% level.

Shoot initiation

The percentage of apple sprouting was influenced by the composition of the growth enhancer BAP-IAA (Table 3). The lower the concentration of BAP, regardless of the growth enhancer IAA, the higher the percentage of apple sprouting. The composition of BAP 3 mg L^{-1} and IAA 0,3 mg L^{-1} can increase the percentage of germination by up to 48% compared to BAP 6 mg L^{-1} and IAA 0,2 mg L^{-1}. In contrast, the composition of the growth modifier BAP 3 mg L^{-1} and IAA 0,2 mg L^{-1} was not different from the composition of the growth modifier BAP 3 mg L^{-1} and IAA 0,3 mg L^{-1}, although there was a difference in IAA concentration difference of 0.1 mg L^{-1}. The Fuji and Red Delicious apple cultivars produced a germination percentage of 29%, while the Gala apple cultivar only produced a germination percentage of 19%. The increase in shoot proliferation can be influenced by the plant type, the explant's origin, and the concentration given (Syafii et al., 2013). Regarding the concentration of the BAP hormone, it has been reported by Meneguzzi et al. (2017) that using a concentration of 2-3 mg L^{-1} was more successful in increasing the number of sprouting explants 3 times compared to the control. According to Yasmin et al. (2022), the effective combination and concentration for increasing shoot proliferation is 4.0 mg L^{-1} BAP (cytokinin) + 0.5 mg L^{-1} NAA (auxin) by 58% compared to a concentration of 1.0 mg L^{-1} BAP (cytokinin) + 0.5 mg L^{-1} NAA (auxin).

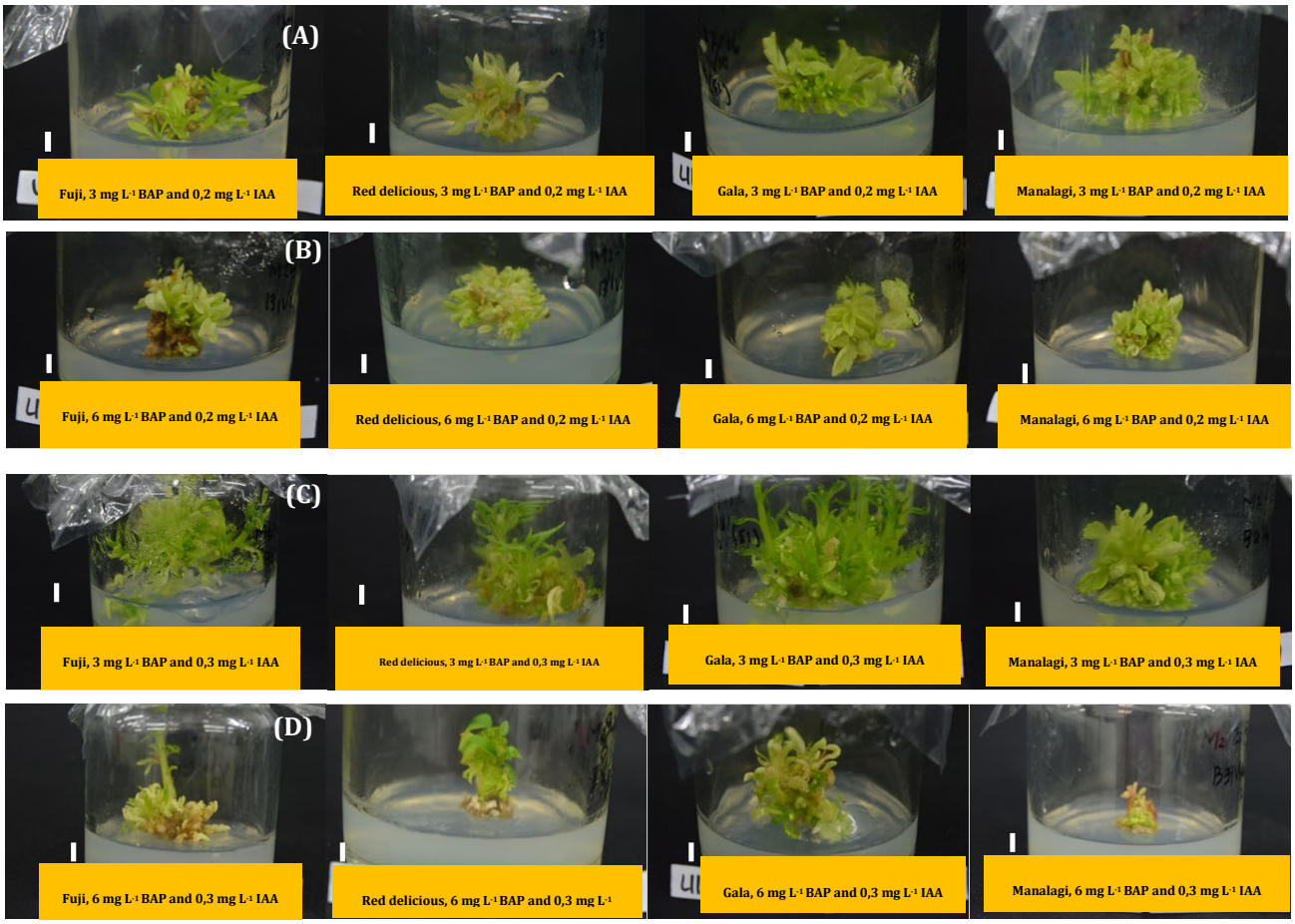

Figure 1. Number of shoots on explants of Fuji, Red Delicious, Gala and Manalagi apple cultivars at 8 WAP. a) Appearance of shoots on media with a composition of 3 mg L^{-1} BAP and 0.2 mg L^{-1} IAA; b) Appearance of shoots on media with a composition of 6 mg L^{-1} BAP and 0.2 mg L^{-1} IAA; c) Appearance of shoots on media with 3 mg L^{-1} BAP and 0.3 mg L^{-1} IAA; d) Appearance of shoots on media with 6 mg L^{-1} BAP and 0.3 mg L^{-1} IAA.

Plant height

The composition of the growth enhancer BAP-IAA for several apple plant cultivars did not differ significantly in shoot height (Table 3). Apple cultivars had a similar average shoot height, 1.06-1.11 cm. The same thing happened with the BAP-IAA growth regulator composition treatment, which had a height ranging from 0.99-1.88 cm at the age of 8 WAP. This aligns with Guo et al. (2011), who explained that the growth enhancer cytokinin is important in inducing shoot proliferation, not plant height growth. The same results have been reported by Salfiani and Paserang (2021) that the combination of BAP-IAA hormones in all treatments did not affect the shoot length of vanilla explants. This was confirmed by Resmi & Nair (2007), who reported that shoot height growth was inhibited due to the increased activity of triploid cultivar shoot multiplication in media employing a combination of Benzylaminopurine and Indoleacetic acid.

Leafy explants percentage

In apple plant cultivars, the composition of the growth enhancer BAP-IAA did not significantly affect the leafy explants (%) (Table 5). Apple cultivars had a percentage of leafy explants ranging from 70% to 81%, while the composition of the growth enhancer BAP-IAA was 75%-79%. Linked to Table 5, the same shoot height and leaf percentage in all treatments strongly indicated the growth enhancer BAP-IAA on shoot proliferation. The same results as Rosmaina (2011) revealed that increased high shoot proliferation reduced the number of leaves. Wulandari et al. (2017) stated that administering BAP and IAA has similar effects on leafy explants across all treatments. This is due to an imbalance

between the cytokinin and auxin hormones and the explants' ability to absorb these hormones effectively.

Table 3. Shoot initiation, plant height, leafy explants percentage, and time of shoots emergence of apple cultivars in the growth regulators composition at 8 WAP.

Treatment	Shoots initiation (%)	Plant height (cm) [t]	Leafy explants percentage (%) [t]	Time of shoot emergence (days)
Apple cultivars (V)				
Fuji	29a	1.09a	78a	37.71a
Red Delicious	29a	1.11a	81a	37.35a
Gala	19a	1.06a	78a	39.67a
Manalagi	23a	1.06a	70a	38.77a
Composition of growth regulators (B)				
MS, 3 mg L-1 BAP and 0.2 mg L-1 IAA	25ab	1.08a	78a	37.38a
MS, 6 mg L-1 BAP and 0.2 mg L-1 IAA	8a	0.99a	74a	40.15a
MS, 3 mg L-1 BAP and 0.3 mg L-1 IAA	56b	1.19a	79a	33.88a
MS, 6 mg L-1 BAP and 0.3 mg L-1 IAA	10a	1.06a	75a	42.10a
V factors	ns	ns	ns	ns
B factors	*	ns	ns	ns
V*B	ns	ns	ns	ns

Note: Values with the same letters were not significantly different based on the Tukey test (p≤0.05). ns = not significant; * = significant at α = 5% level; t = data were transformed with $\sqrt{x+0.5}$ before analysis

Time of shoot emergence

In apple plant cultivars, the growth enhancer BAP-IAA composition did not significantly affect the time of shoot emergence (Table 3). Local apple cultivars (Gala and Manalagi) needed shoot emergence times of 39.67 and 38.77 days after planting (DAP), respectively. Similarly, the Fuji apple cultivar required a shoot emergence time of 37.71 DAP. The composition of the growth modifier BAP 3 mg L^{-1} and IAA 0.3 mg L^{-1} needed a shorter time of 33.88 days for apple shoots to appear after planting. Meanwhile, for the composition of the regulator grows BAP 6 mg L^{-1} and IAA 0.3 mg L^{-1}, the time for shoots to appear was relatively long (42.10 DAP). According to Syafii et al. (2013), the interaction of growth regulators between cytokinin and auxin at a concentration of 0.2 ppm TDZ and 0.3 ppm IBA played a crucial role in the speed of pineapple shoot induction. Other research also states that using MS media with the addition of the BAP hormone 3 mg L^{-1} was the best concentration for inducing shoot emergence (Nazihah et al., 2023). This aligns with the findings of Restanto et al. (2024) to speed up the emergence of shoots because the BAP hormone plays an active role in cell division.

In general, combining apple shoot cultivars with the composition of the growth enhancer BAP-IAA affects shoot multiplication. The magnitude of the effect of BAP-IAA growth regulator treatment on explants is also influenced by the cultivar. The adaptation mechanism of apple cultivars observed was due to the use of the BAP-IAA growth regulator composition, namely the time of shoot emergence (Table 3), differences in the explants percentage that sprouted (Table 3), shoots number and fresh weight of explants (Tables 1 and 2). The research results align with those of Guo et al. (2011), who state that the growth regulators cytokinin and auxin, with the right composition, can increase and induce shoot multiplication effectively.

The morphological response of apple cultivars observed due to the use of the growth enhancer composition BAP 3 mg L^{-1} and IAA 0.3 mg L^{-1} showed superiority with a higher percentage of explants sprouting compared to BAP 6 mg L^{-1} and IAA 0.3 mg L^{-1} (Table 3). The research results follow Harahap and Nusyirwan (2014), a BAP composition of less than 5 mg L^{-1} and appropriate IAA can produce more chrysanthemum shoot induction. Regardless of cultivar, apple plants showed a low percentage of sprouting results at BAP 6 mg L^{-1} and IAA 0.2 mg L^{-1} and BAP 6 mg L^{-1} and IAA 0.3 mg L^{-1}. Interestingly, the higher concentration of growth regulator BAP 6 mg L^{-1} and IAA 0,3 mg L^{-1} gave a relatively longer

shoot emergence time compared to growing BAP 3 mg L^{-1} and IAA 0.3 mg L^{-1} against apple cultivars (Table 3). The key functions of cytokinin are primarily attributed to its involvement in DNA synthesis, cell division, shoot growth, and the mechanism that governs the formation of the mitotic spindle, which includes the regulation of protein synthesis (Govindaraju & Aruselvi, 2018).

The research has implications for determining the growing environment with the correct composition of BAP-IAA growth regulators in several apple cultivars. The composition of the growth modifier BAP 3 mg L^{-1} and IAA 0.3 mg L^{-1} showed a high shoot multiplication growth rate and shoot fresh weight yield in Fuji, Red Delicious, and Gala apples (Tables 1 and 2). According to Sagai et al. (2016), the function of cytokinins (BAP) plays an important role in the process of cell division. Besides, it can also stimulate and activate enzymes to support cell division, resulting in shoot proliferation. Meanwhile, if the BAP concentration was 6 mg L^{-1}, it decreased the percentage of germination and shoot weight yield. The same thing also happened to pineapple shoot explants (Syafii et al., 2013). On the other hand, when IAA was increased to 1 mg L^{-1}, apple shoot proliferation was 66% higher compared to without IAA hormone (Abdella et al., 2023). Among the cytokinins tested in the study, BAP was the most effective in promoting shoot multiplication and metabolite production (Jezyna et al., 2018).

Research also opens up opportunities to increase shoot multiplication using the right composition of growth regulators. The use of a regulatory substance composition appropriate to the explant growing environment can be based on reviewing the variables in the number of shoots, the percentage of germination, and the fresh weight of the explant. Accurate yield estimation can be done by considering the number of shoots and the percentage of leaves. Meanwhile, the variables of plant height and number of leaves cannot be used as yield estimates because there was no significant growth (Table 3). According to Syafii et al. (2013) leaves number was not influenced by the administration of the hormones cytokinin (TDZ) and auxin (IBA) to pineapple plants until 6 weeks after incubation.

However, plant height variables as predictors of multiplication and fresh weight of explants must be used carefully. In research, apple cultivars with a suitable growing environment media BAP 3 mg L^{-1} and IAA 0.3 mg L^{-1} produced the same number of shoots and explant weight. Further studies are needed to see the quantitative variables of apple plants on more cultivars. According to Sriskandarajah et al. (1990) and Sedlak and Paprstein (2016), apple cultivars (Gala, Royal Gala, and Jonagold) caused an enhancement in shoot emergence time and shoot number.

CONCLUSIONS

The treatment combination between apple cultivars with BAP-IAA hormone composition had a real interaction on the multiplication and fresh weight of shoots at 8 weeks after planting. The treatment combination between Red Delicious with the composition of BAP 3 mg L^{-1} + IAA 0.3 mg L^{-1} was the best treatment compared to the treatment combination between apple cultivars Fuji, Red Delicious, Gala, and Manalagi with the composition of BAP 6 mg L^{-1} +IAA 0.2 mg L^{-1}, and BAP 6 mg L^{-1} +IAA 0.3 mg L^{-1} at 8 weeks after planting. The composition of the BAP-IAA growth regulator also induced the percentage of explants sprouting 8 weeks after planting. The results showed that the combination of the Red Delicious apple cultivar with BAP 3 mg L^{-1}+IAA 0.3 mg L^{-1} can be recommended as the best treatment in mass shoot production/multiplication.

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