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Sucrose and coumarin effect on the growth and development of micro-cutting potato plant (*Solanum tuberosum*) variety Granola Kembang

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Abstract. *Manjaswari A, Pitoyo A, Sari SLA. 2018. Sucrose and coumarin effect on the growth and development of micro-cutting potato plant (Solanum tuberosum) variety Granola Kembang. Cell Biol Dev 2: 55-62.* This research aimed to determine the effect of various concentrations of sucrose and coumarin on the micro-cutting potato plant's growth and development. A completely Randomized Design (CRD) with a single concentration combination factor of sucrose and coumarin was treated well. Five levels of sucrose concentrations were 0, 30, 50, 70, and 90 g/L. While coumarin concentrations were 0, 20, 40, 60, 80 mg/L. Observation and data collection was made in the second month after the explant planting. Some parameters observed were morphological changes in explant characteristics, i.e., the percentage of explants response, the emergence of the shoot, shoot height, shoot number, leaf emergence time, number of leaves, the emergence of the root, root number, and root length. The data obtained were analyzed using ANOVA followed by DMRT at level test 5 % to determine if there is a significant difference between the treatments. The result showed that the higher sucrose concentration added into the media could increase the micro-cut growth and development of shoots and leaves; otherwise, it would negatively influence root development. The addition of coumarin in high concentration inhibited all the parameters of growth and development observed. Both combinations of sucrose and coumarin could increase the micro-cut development of shoot at 50 g/L of sucrose and 20 mg/L of coumarin concentration. Those concentrations could produce the best amount and higher shoot for micro-cut multiplications.

Keywords: Coumarin, in vitro, micro-cutting, potato, sucrose

Abbreviations: BAP: Benzyl Amino Purin; DAP: Days After Planting; GA: Gibberellin Acid; MS: Murashige and Skoog

INTRODUCTION

Potato (*Solanum tuberosum* Linn.) is a highland vegetable plant that belongs to the Solanaceae family. Potato is one of the world's main foodstuffs after rice, wheat, and corn (Rubatsky and Yamaguchi 1995) which contains high carbohydrates and relatively small amounts of fat, i.e., 1.0-1.5% (Bambang 1987). Therefore, potatoes as a national superior vegetable commodity are prioritized for development by the Indonesian government because they have prospects in supporting food diversification programs.

The Ministry of Agriculture (2015) stated that the number of imported potatoes, especially processed ones, has increased dramatically from 2012 until now. However, potato production is almost constant, as stated by the Indonesian Central Bureau of Statistics (2014); in 2014, it produced 1,347,815 tons of potatoes, while in 2015, it produced 1,219,269 tons of potatoes. In addition to the increasingly narrow land area, it is difficult for Indonesian potato farmers to obtain high-quality tubers. The local seeds used are generally degenerated and infected with various diseases caused by viruses. Therefore, it is necessary to produce high-quality potato seeding that produces seeds free of viruses and diseases (Mariani 2011).

One attempt to increase the productivity of quality potato seeds is in in-vitro culture techniques. In vitro plant propagation could be made, among others, through somatic embryogenesis, regeneration of adventitious organs, formation of axillary branches, and single-node culture (Pierik 1987). Single node culture is an in vitro culture technique in which one explant contains one node or only one leaf. The results of single node culture are generally referred to as micro cuttings/plantlets. Micro cuttings produced in-vitro can be used in the multiplication process, the formation of micro tubers, and mini tubers (Struik and Lommen 1990). With this in vitro culture technique, it is possible to produce potato seeds that are uniform and the same as the parent (true to type) in large quantities in a short time without depending on the season and free from pests, diseases, and viruses because they are maintained in aseptic conditions (Wattimena 1983).

Problems encountered in potato propagation in vitro are the time it takes to form roots and the formation of strong and healthy plantlets (Monnier 1990; Liz and Levith 1997). The composition of the culture media is very influential on the growth and development of explants. Sucrose is a carbon source commonly used under in vitro culture techniques and serves as a source of energy cells need to grow (Kimball 1994). Faria et al. (2004) stated that various treatments with a sucrose concentration of 60 g/L showed the highest plantlet growth compared to other treatments. Sucrose can also increase the formation of micro tubers of Dewa leaf plants. Sucrose in a concentration of 6% in the

media was able to increase the number of micro tubers significantly.

In addition to sucrose, media components that determine the success of tissue culture are the type and concentration of growth regulators (PGR) used (Ali et al. 2007). So it is because growth regulators have characteristics that can induce or inhibit plant physiological processes. Growth regulators that have inhibitory properties are also called retardants. One example of a retardant substance is coumarin which works by inhibiting the activity of GA, which plays a role in cell elongation and division. According to Sakya et al. (2003), the administration of coumarin can inhibit the emergence of branches in potato plants. Conversely, the treatment without coumarin showed more branches than the coumarin treatment at a concentration of 45 mg/L.

Research on the effect of sucrose and coumarin on potato micropropagation results has been done previously. However, research on the effect of the combination of the two on the yield of potato plant propagation has never been done. Based on the description above, it is necessary to research the effect of giving various concentrations of sucrose and coumarin or their combination on the growth and development of potato micro-cuttings in vitro to produce high-quality and high-quantity seeds. One of the potential potato varieties is the Granola Kembang variety, which has not been widely studied for its in vitro propagation.

MATERIALS AND METHODS

Materials

This research was carried out for three months, from December 2015 to March 2016. This research was conducted at the Biology Laboratory, the Faculty of Mathematics, Sebelas Maret University, Surakarta. The plant materials were derived from plantlets produced by single shoot multiplication of potato tuber (*Solanum tuberosum* Lin.) Granola Kembang variety aged four weeks after planting in MS medium without growth regulators (MS 0) collection from the garden at the Kledung Horticultural Seed Garden, Temanggung. The materials for making the treatment media were Murashige Skoog (MS) instant basal medium with vitamins (Phyto Technology Laboratories), sucrose, standard coumarin HPLC (High-Performance Liquid Chromatography), 99% (Sigma), aquadest, and 96% alcohol.

Procedure

In this study, a single factor Completely Randomized Design (CRD) was used, namely a combination of sucrose and coumarin concentrations. The sucrose concentration consisted of 5 levels, namely 0, 30, 50, 70, and 90 g/L and 80 mg/L and the coumarin concentration consisted of 5 levels, namely 0, 20, 40, 60, and 80 mg/L with 3 times replications.

Tools such as culture bottles, measuring cups, beakers, Petri plates, Erlenmeyer, spatula, tweezers, and dropper pipettes were washed thoroughly, then sterilized using an autoclave at 121°C, under 1.5 atm pressure for 15 minutes.

The treatment media was stocked with 1000 mL for each treatment. Instant MS media (PhytoTechnology Laboratory) in powder form was weighed using an analytical balance of as much as 4.43 g. After that, 4.43 g of instant MS was put into an Erlenmeyer. Sucrose and coumarin were added according to the variation of the concentration used. For example, to make S4K4 treatment, 90 g of sucrose and 80 mg of coumarin are needed. 500 mL of aquadest was added to an Erlenmeyer glass containing instant MS, sucrose, and coumarin, then heated on a hot plate with the help of a magnetic stirrer. The medium was adjusted until the pH reached 5.5 - 5.7. If the pH was too low, 1 N NaOH was added, while if the pH was too high, 1 N HCl was added. Next, 8 g of agar was added to the media solution, then distilled water was added until the volume reached 1,000 mL. After boiling, the media was poured into sterile culture bottles with a volume of approximately 25 mL per bottle. Then the bottle was immediately covered with aluminum foil and glued with plastic wrap. The media in the bottle was then re-sterilized by autoclave at a temperature of 121ºC and steam pressure of 1.5 atm for 2 hours. The sterilized media bottles are cooled at room temperature and stored in the culture room.

The explants were planted aseptically in a Laminar Air Flow Cabinet (LAFC) Using a single nodule cutting technique. Plantlet collection of Kledung Horticultural Seed Garden, Temanggung, Explants of Granola Kembang variety aged four weeks after planting in MS medium without growth regulator (MS 0) were taken and cut into several parts. Each explant contains one node and one leaf blade. The explants were then planted in culture bottles using tweezers. Each culture bottle was planted with one explant. After the explants were planted in the media, the culture bottles were again covered with aluminum foil and glued with plastic wrap.

The explants planted on each treatment medium were incubated at 18-20°C and observed two months after planting. Observations were made on the percentage of explants responding to media, time of shoot emergence, length and number of shoots, time of leaf emergence, number of leaves, time of emergence of roots, length, and number of roots.

Data analysis

The data on changes in morphological characters were analyzed using Analysis Of Variance (ANOVA) and then continued with Duncan's Multiple Range Test (DMRT) at the 95% test level to determine if there was a significant difference between treatments using SPSS 16.0 software.

RESULTS AND DISCUSSION

Percentage of explants responding to media

In this study, combining sucrose and coumarin with different concentrations resulted in different growth responses of single-node explants. Explants showed a direct organogenesis response where explants grew to form shoots and roots without going through callus formation first (Dhaliwal et al., 2003). It is in line with the study where single-node explants were not found to have a callus. Single node explants grow to form shoots, leaves, and roots directly without callus formation first. The growth of single-node explants grown in media with a combination of sucrose and coumarin concentrations was observed up to 56 days after planting and is presented in Table 1.

Treatment without sucrose and coumarin addition in the media resulted in the percentage of explants responding to the media by 80%. The addition of sucrose to a concentration of 50 g/L increased the percentage of explants responding in a medium without coumarin to 100%. The addition of higher sucrose concentration caused a decrease in the percentage of explants responding in the media without coumarin. The percentage of responding to single-node explant media decreased at a concentration of 70 g/L and decreased at a different concentration of 90 g/L, without adding coumarin. According to Marlin (2005), the energy source (sucrose) available in high concentrations causes a decrease in osmotic pressure. In ginger (*Zingiber officinale* Rosc.), the low osmotic pressure in the media causes the explants to be unable to optimally absorb existing nutrients for growth so that their growth will be stunted.

The addition of coumarin 20 mg/L in the media resulted in an explant response with a percentage of 80%. Adding coumarin concentrations of up to 40 mg/L and 80 mg/L in media without sucrose caused a decrease in explants' response to media. The higher concentration of coumarin addition in the media without sucrose would inhibit the growth of single-node explants planted. The addition of coumarin concentrations of 40, 60, and 80 mg without sucrose showed no growth up to 56 days after planting. Hasni et al. (2014) stated that the higher the concentration of coumarin, the higher the percentage of dead plantlets. The coumarin addition at a concentration of 50 mg/L showed an increase in the percentage of dead potato plantlets and increased again at 75 mg/L. It is because the administration of coumarin in the media will inhibit the activity of the gibberellin hormone in the explants. According to Salisbury and Ross (1995), the gibberellin hormone promotes cell division, so if its activity is inhibited, it will inhibit the growth of the explant.

The formation of good micro cuttings can increase the multiplication of micro cuttings and the production of micro tubers in in-vitro conditions, and the production of mini tubers in the screen house. The combination of concentration of 50 g/L sucrose and 20 mg/L coumarin resulted in good explant growth due to the addition of sucrose with neither too high nor low concentration. Sucrose plays a role in cell elongation and expansion. If added in small amounts, it cannot provide an energy source, and if added too much, it will inhibit growth because it will inhibit the absorption of nutrients for plants (Marlin 2005). Furthermore, adding more than 20 mg/L of coumarin showed no growth because coumarin, as a retardant substance characteristic, reduces tissue metabolic activity and can inhibit the process of vegetative growth (Purnomo and Prahadini 1991).

Number of shoots

The first shoot on each explant is an elongation of the bud or node. In this study, the average shoots appeared on the 14th day after planting. Treatment with 50 g/L sucrose and 60 mg/L coumarins showed the earliest shoot emergence 7 days after planting. That also applies to treating 70 g/L sucrose with 20 mg/L coumarins or 40 mg/L coumarins. The last emergence of the shoot in the treatment of 0 mg/L coumarins and 90 g/L sucrose was at 28 days after planting. The number of shoots indicates success in in-vitro propagation techniques. The more shoots formed, the higher the multiplication rate. The number of shoots formed ranged from 1–3 per explant planted in the media (Table 2, Figure 1).

Table 1. The effect of variations in the concentration of sucrose and coumarin on the percentage of success of explants responding to media (%)

Coumarin (mg/L)	Percentage of explants responding to media (%)				
Sucrose (g/L)	0	20	40	60	80
0	80	80	0	0	0
30	100	80	0	0	0
50	100	100	40	60	0
70	80	100	80	0	0
90	60	100	0	0	0

Table 2. The average number of shoots on variations in the concentration of sucrose and coumarin

Coumarin (mg/L)	Number of shoots (shoots/explants)				
Sucrose (g/L)	0	20	40	60	80
0	1,3^{ab}	1,0^{a}	*	*	*
30	1,0^{a}	1,0^{a}	*	*	*
50	2,0^{b}	1,0^{a}	1,0^{a}	1,0^{a}	*
70	1,0^{a}	1,0^{a}	1,0^{a}	*	*
90	1,3^{ab}	2,0^{b}	*	*	*

Note: * shoots do not grow up to 56 DAP

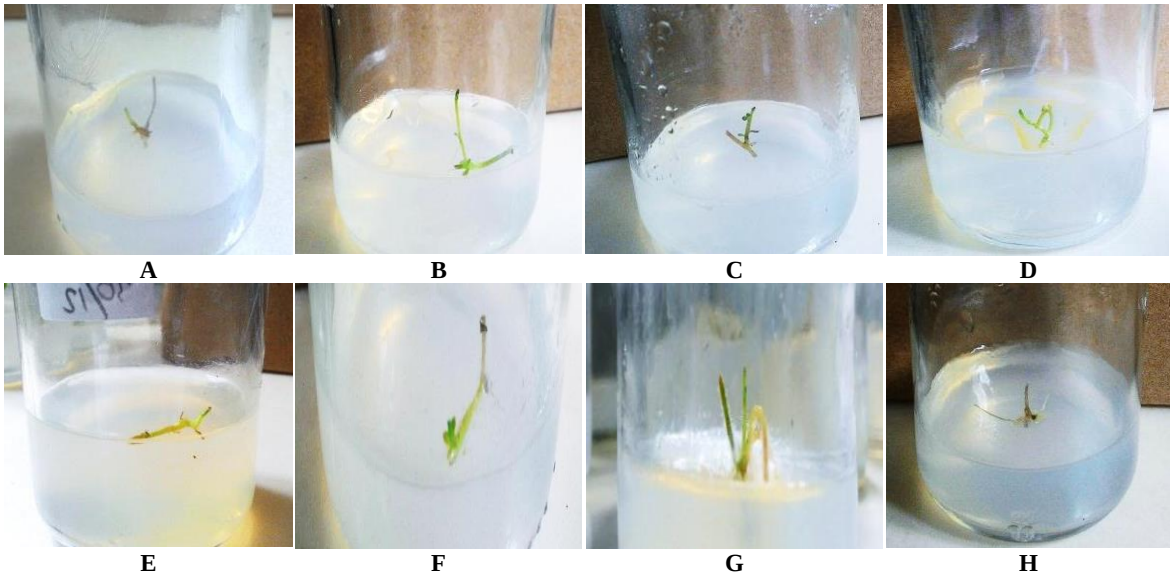

Figure 1. Shoots growing on the 7th day after planting in treatment: A. without sucrose and coumarins; B. sucrose 30g/L without coumarin; C. sucrose 70 g/L without coumarin; D. sucrose 90g/L without coumarin; E. coumarin 20 mg/L without sucrose; F. sucrose 70 g/L and coumarin 20 mg/L; G. sucrose 90 g/L and coumarin 20 mg/L; H. sucrose 90 g/L and coumarin 80 g/L

The treatment without sucrose and coumarin produced an average of 1.3 shoots in each explant planted. Adding a certain amount of sucrose in a medium without coumarin resulted in the number of shoots formed being unstable. The number of shoots formed decreased at a concentration of 30 g/L and increased at 50g/L. At the addition of 50 g/L sucrose in media without coumarin, the shoots formed were at the peak, that an average of 2 shoots per explant planted. The more sucrose added to the media resulted in a higher number of shoots up to a sucrose concentration of 50 g/L. Some of the sucrose in the media lifted into the cells will be converted into energy, and some will be converted into materials needed to stimulate growth (Salisbury and Ross 1995).

The treatment of 20 mg/L coumarins without sucrose produced an average of 1 shoot per explant planted. After adding 20 mg/L, shoots were not able to form. The more coumarins added to the medium without sucrose, the more shoots formed decreased until no shoots were formed at all, namely in the treatment of coumarin concentrations of 40, 60, and 80 mg/L without the addition of sucrose. Paclobutrazol is a retardant substance with an inhibitory function like coumarins, inhibiting the synthesis of gibberellins. Paclobutrazol inhibits the sequence of oxidation reactions from ent-kaurene to ent-kaurenoic acid in the formation of gibberellin acid (GA). When the formation of GA is inhibited, cell division can still occur, but the cell will not elongate, causing the nodes or shoots to become short (Arteca 1996).

The combination of sucrose and coumarin produced several shoots at the concentration of 90 g/L sucrose and 20 mg/L coumarins. With the addition of 20 mg/L coumarins, the more sucrose added was able to increase the number of shoots formed, which reached an average of 2 shoots per explant planted. It is because sucrose is the primary energy source and carbon in in-vitro culture and plays a crucial role in the cell cycle (Tyas et al., 2013). Therefore, more sucrose added could encourage cell division and formation (Dewitte and Murray 2003).

Shoot height

The height of the shoots formed was observed at the end of the observation (56 days after planting). The height of the shoots formed was measured from the node's base to the tip of the shoot. The resulting shoot height reached 8 cm. The average shoot height in the treatment of the combination of sucrose and coumarin concentrations can be seen in Table 3.

Treatment without adding sucrose and coumarin resulted in an average shoot height of 7.13 cm and tended to decrease with the addition of sucrose up to 50 g/L. The addition of 70 g/L sucrose in media without coumarin resulted in the highest shoots reaching 7.5 cm. The addition of sucrose concentration up to 70 g/L increased shoot height and decreased drastically at the addition of 90 g/L sucrose. As a carbon source, sucrose, when absorbed, can affect osmotic pressure, causing cell elongation (Krook et al. 1998).

Table 3. Average shoot height to variations in coumarin and sucrose concentrations

Coumarin (mg/L)	Shoot height (cm)				
Sucrose (g/L)	0	20	40	60	80
0	7,13^{d}	1,03^{a}	*	*	*
30	6,53^{cd}	1,93^{ab}	*	*	*
50	4,23^{bc}	4,43^{bc}	1,93^{ab}	1,76^{ab}	*
70	7,5^{d}	1,7^{ab}	2,73^{ab}	*	*
90	2,46^{ab}	8^{d}	*	*	*

Note: * shoots do not grow up to 56 DAP. Numbers followed by the same letter show no significant difference in DMRT 5%

In the administration of 20 mg/L coumarins without sucrose, there was a decrease in shoot height to an average

of 1.03. The more coumarin added in the medium without sucrose, the lower the shoot height. The formation of shoots is inseparable from dividing active and differentiated meristem tissue and is supported by the presence of organic and inorganic compounds in the media. Administration of coumarins as phenolic compounds can inhibit the work of GAs which function in cell expansion and division (Wattimena 1988).

A good combination of sucrose and coumarin treatments in producing shoot height was the combination of a concentration of 90 g/L sucrose and 20 mg/L coumarins. At 90 g/L sucrose in 20 mg/L coumarin medium, the highest shoot height was 8 cm on average. Krook et al. (1998) stated that sucrose absorbed by cells would be rapidly hydrolyzed to hexose by the cell wall invertase enzyme. Glucose and fructose from the hydrolysis of sucrose enter the cells for metabolism and are then used as a carbon source and energy source for cell division and formation.

Number of leaves

Leaves are essential to plant organs, especially for photosynthesis, so that plants can produce food and experience optimum growth (Arimarsetiowati and Ardiyani 2012). The sucrose and coumarin combination treatment resulted in leaf emergence time and leaf number varying from 7 to 28 days after planting. The first leaf appeared in the treatment of the combination of concentrations of 70 g/L sucrose and 20 mg/L coumarins 7 days after planting. The appearance of the last leaf under observation was in the treatment of the combination of 90 g/L sucrose without coumarin. The number of leaves on the growth of a plant plays a significant role. It is related to vegetative growth, plants' ability to carry out photosynthesis, and various other metabolisms. The combination of sucrose and coumarin concentrations in the media resulted in a different number of leaves for each combination up to 56 days after planting (Table 4).

Table 4. The average number of leaves on variations in coumarin and sucrose concentrations

Coumarin (mg/L) / Sucrose (g/L)	Number of leaves (leaf/explant)				
	0	**20**	**40**	**60**	**80**
0	9,33^{cd}	4,0^{ab}	*	*	*
30	6,67^{bcd}	3,6^{ab}	*	*	*
50	7,33^{bcd}	9,33^{cd}	0,33^{a}	3^{ab}	*
70	19,66^{e}	4,0^{ab}	3^{ab}	*	*
90	5,33^{bc}	10.67^{d}	*	*	*

Note: * no leaves appear until the end of observation (56 DAP). Numbers followed by the same letter show no significant difference in DMRT 5%

Without sucrose and coumarin addition, the number of leaves produced from each explant planted was 9.33. Furthermore, adding sucrose up to 50 g/L in media without coumarin reduced the number of leaves formed. At a concentration of 70 g/L sucrose without coumarin in the media, the highest number of leaves formed was 19.66 on average per explant planted. The more sucrose was added to the media without coumarins, the number of leaves formed increased to a concentration of 70 g/L and decreased when sucrose was given at a concentration of 90 g/L. Carbohydrates are a source of carbon and energy needed when shoot meristem cells divide and enlarge to form new tissue to form leaf primordia (Haryanti et al. 1998).

The administration of coumarin with a concentration of 20 mg/L without adding sucrose in the media showed a decrease in the number of leaves formed compared to without coumarin and sucrose. The more coumarins added to the media without sucrose, the lower the number of leaves. However, adding coumarin with a 40, 60, and 80 mg/L concentration did not result in leaf formation in the planted explants. The addition of coumarin in the media made the number of leaves formed less than the treatment without sucrose. The application of coumarin, a phenolic compound, can inhibit plant growth (Prawiranata et al. 1981), as shown in Figure 2.

Figure 2. Leaves growing in treatment: A. without sucrose and coumarins; B. sucrose 30 g/L without coumarin; C. sucrose 50 g/L without coumarin; D. sucrose 70 g/L without coumarin; E. coumarin 20 mg/L without sucrose; F. sucrose 50 g/L and coumarin 40 mg/L; G. sucrose 70 g/L and coumarin 20 mg/L

The best combination of sucrose and coumarin concentrations in producing the number of leaves was the treatment of the combination of sucrose concentration at 70

g/L and without coumarin, in which in this treatment, the highest number of leaves was 19.66 with a significant difference from other treatments when viewed from the results of one way ANOVA statistical test. The number of leaves increased due to increased cell division in leaf primordial. Fatima et al. (2004) stated that in vitro propagation of potato cultivar PARS 70 with MS medium with 8% sucrose resulted in an average of 5.71 leaves formed.

Administration of higher concentrations of coumarin with the same sucrose concentration showed a decrease in the number of leaves formed. As shown in Figure 2, in Figure 2.C, the treatment of 50 g/L sucrose and 0 mg/L coumarins showed more leaves formed than the treatment of 50 g/L sucrose and 40 mg/L coumarins (Figure 2.F). It is because although sucrose was added in the same two media when combined with other substances, such as coumarin, in different concentrations, it showed a difference in the number of leaves formed. The more coumarin added would inhibit the number of leaves formed.

Number of roots

Root formation in plantlets is vital because it can increase growth during the in-vitro propagation process. The combination of the concentration of sucrose and coumarin in the media showed the beginning of the emergence of different roots in each treatment, where the roots that appeared ranged from 7 to 35 days after plants. In this study, the highest number of roots that appeared until the end of the observation was 42.33 roots, namely at the treatment of sucrose concentration of 70 g/L without coumarin. On the other hand, the number of roots was the least in treating the combination of concentrations of 70 g/L sucrose and 40 mg/L coumarins (Table 5).

In treating media without sucrose and coumarin, the average number of roots that grew was 5.66. However, adding sucrose to the media increased the number of roots that appeared. The addition of sucrose resulted in the number of roots increasing until the addition of sucrose at a concentration of 70 g/L was an average of 42.33 roots/explant. Still, there was a decrease in the addition of 90 g/L sucrose. In addition, according to Kazemiani et al. (2012), an increase in sucrose concentration above 30%, namely 40%, increased the percentage of root formation in the MS medium without the addition of growth regulators such as BAP.

With the addition of coumarin concentration of 20 mg/L, the number of roots formed increased to 9.33 roots/explant. However, at higher concentrations, there was a decrease in the number of roots formed. The more coumarin added to the media without sucrose, the lower the number of roots formed. In the treatment of coumarin concentrations of 40, 60, and 80 mg/L without sucrose, no roots were formed until the end of the observation. According to Sakya et al. (2003), coumarin as a growth inhibitor effectively inhibits or suppresses gibberellin activity in plants. Inhibition of gibberellins by coumarins will accelerate the entry of plants into the generative phase so that energy for the process of the branch, node, and root growth will be diverted for tuber formation.

Better treatment of sucrose and coumarin combination for increasing the number of roots formed in explants planted in the media was the addition of sucrose at 70 g/L without the addition of coumarin, with the average number of roots per explant being 42.33. On the other hand, administering paclobutrazol, the same retardant as coumarin, as much as 5.0 mgl/l, actually produces fewer roots than the control. The administration of paclobutrazol at a concentration of 5.0 mg/L reduces the number of roots produced (6.80) when compared to the control (13.6) (Shahid 2007).

Root length

Root length results from the extension of the cells behind the tip meristem; the longer the root, the wider the area of nutrient absorption is expected. This study measured root length from the base where the roots grew (single-node explants) to the tip in mm. The longest roots were formed without sucrose and coumarin treatment, while the shortest leaves were formed in the 50 g/L sucrose and 40 mg/L coumarin treatment (Table 6).

Table 5. The average number of roots to variations in sucrose and coumarin concentrations

Coumarin (mg/L)	Number of roots (root/explant)				
Sucrose (g/L)	0	20	40	60	80
0	5.66^{a}	9.33^{ab}	*	*	*
30	12.33^{abc}	8.33^{ab}	*	*	*
50	25.33^{abc}	3.9^{bc}	4^{a}	28.66^{abc}	*
70	42.33^{c}	24.66^{abc}	3.66^{a}	*	*
90	4.6^{a}	29.33^{abc}	*	*	*

Note: * no roots appear until the end of the observation (56 DAP)

Table 6. The average root length against variations in the concentration of sucrose and coumarin

Coumarin (mg/L)	Root length (mm)				
Sucrose (g/L)	0	20	40	60	80
0	$26,94^{d}$	$9,14^{abc}$	*	*	*
30	$15,38^{bc}$	$7,80^{ab}$	*	*	*
50	$17,56^{c}$	$6,75^{ab}$	$5,02^{a}$	$8,37^{ab}$	*
70	$10,42^{abc}$	$10,44^{abc}$	$9,43^{abc}$	*	*
90	$10,12^{abc}$	$6,34^{ab}$	*	*	*

Note: * no roots appear until the end of the observation (56 DAP)

Figure 3. Roots formed in treatment: A. without coumarin and sucrose; B. sucrose 30 g/L without coumarin; C. sucrose 70 g/L without coumarin; D. sucrose 50 g/L and coumarin 20 mg/L; E. sucrose 70 g/L and coumarin 20 mg/L; F. sucrose 90 g/L and coumarin 20 mg/L.

In the treatment without adding sucrose and coumarin, the average length of the roots formed reached 26.94 mm. In the sucrose addition of 30, the root length decreased to 15.38 mm and increased again in the sucrose addition of 50 to 17.56 mm. After adding 50 g/L sucrose, there was a decrease in the length of the roots formed. The more sucrose added to the medium, the less the length of the roots formed. With the addition of 70 g/L sucrose, the roots formed reached 10.42 mm and decreased again with the addition of 90 g/L sucrose to 10.12 mm. Root length results from the extension of the cells behind the tip meristem (Dewi 2007). The longer the roots, it is expected that the area of nutrient absorption will be wider so that the distribution of nutrients from the media to plants can run smoothly. If the root length is inhibited by increasing the amount of sucrose added, it will affect the subsequent acclimatization after the in-vitro culture process.

The addition of 20 mg/L coumarins without the addition of sucrose in the media caused a decrease in the length of the formed roots to 9.1 mm. The more coumarin added in the medium without sucrose, the shorter the roots. In the treatment of 40, 60, and 80 mg/L coumarins without sucrose, no root formation occurred until the end of the observation. One of the physiological effects of coumarin is to inhibit growth and. root extension (Wattimena, 1988). Prawiranata et al. (1981) suggested that the most common effect of administering phenolics (coumarins) inhibits growth, such as cell division and elongation.

The combination of sucrose and coumarin did not affect the roots because the longest roots occurred in the control medium without sucrose and coumarin. Tyas (2013) said that the highest number of roots was found in media with a concentration of 1/2 MS plus 1% sucrose. The limited concentration of MS media with sucrose affects the number of nutrients pummelo plantlets obtain in culture. The reduction of nutrients in the media caused the explants to grow slowly; leaf formation and shoot length decreased, while root formation and elongation increased as a means of nutrient absorption. Roots formed in the treatment of the combination of concentration of 70 g/L sucrose without the addition of coumarin (Figure 3.C) spread throughout the media. In comparison, in the treatment of the combination of concentration of 70 g/L sucrose and 20 mg/L coumarins (Figure 3.E), the roots formed tended to gather around the mother plant.

In conclusion, adding sucrose could increase the growth and development of micro cuttings on shoots and leaves but has a negative effect on root development. Coumarin in high concentrations in the media would inhibit the growth and development of shoots, leaves, and roots. While the combination would increase the growth and development of micro cuttings shoots. The combination of the concentration of 50 g/L sucrose and 20 mg/L coumarins is the best combination to get the micro cuttings with good average results on each parameter.

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