

INDUCTION OF POTATOES (*Solanum tuberosum* L.) MICRO TUBER AT SOME COUMARIN CONCENTRATIONS AND INCUBATION TEMPERATURES

WARNITA WARNITA* RENI MAYERNI, NILLA KRISTINA
AND NUR ELLIA NADILA

Agronomy Department Faculty of Agriculture, Andalas University, Padang, Indonesia
[WW, RM, NK, NEN].

[*For Correspondence: E-mail: warnita@agr.unand.ac.id]

Article Information

Editor(s):

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Reviewers:

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(2) Fabio Vogelaar Carlucci, Universidade de Sao Paulo, Brazil.

Received: 02 February 2021

Accepted: 08 April 2021

Published: 26 April 2021

Original Research Article

ABSTRACT

Potatoes are a priority horticultural crop to be developed because they can be used for food diversification. Increasing the production of micro tubers is needed for the provision of potato seeds. The purpose of this study was to obtain the interaction between coumarin concentration and incubation room temperature on the induction of potato micro tubers. This research was conducted at the Plant Tissue Culture Laboratory, Faculty of Agriculture, Andalas University, Padang from September 2019 to January 2020. The study was a two-factor factorial with 4 replications in a completely randomized design (CRD). The first factor is the concentration of coumarin which consists of 5 levels, namely 0, 25, 50, 75 and 100 mg / l. The second factor is the temperature of the incubation room which consists of 3 levels, namely: 20, 25 and 30 ° C. The data were analyzed by using the F test and followed by the Tukey's test. The research data showed that there was an interaction between the application of several coumarin concentrations and the incubation room temperature to time of tubers emergence, the fastest tubers appeared 13.26 days after the addition of the tuber medium (DAT) at 100 ppm of coumarin and a temperature of 30 ° C. The best application of 100 ppm coumarin for time of tubers emergence, number of tuber and fresh weight of root. Incubation room temperature affected time of tubers emergence, but did not affect diameter of tuber, fresh weight of tuber and fresh weight of shoot.

Keywords: Coumarin; temperature; induction; micro tubers; potato.

INTRODUCTION

The potato plant (*Solanum tuberosum* L.) is an important horticultural crop because it contains a balanced nutrition. Potatoes can be consumed by patients with diabetes because of their low sugar content. In line with the modern lifestyle in urban areas and the proliferation of industrial foodstuffs made from potatoes, the demand for potatoes continues to increase. The increasing demand for potatoes is not in line with the availability of domestically produced potatoes so that they are still imported.

The need for potatoes continues to increase every year in line with the increasing population and the development of industries that require raw materials for potatoes. Potato production in 2018 was 1,284,760 tons with a productivity of 18.71 tons / ha and in 2019 potato production increased to 1,314,657 tons with a productivity of 19.27 tons / ha [1].

The high demand for potatoes needs to be balanced with an increase in production, where the success of production is largely determined by the availability of quality seeds. Efforts to procure potato seeds with good quality and quality continue to be made, such as with tissue culture techniques. This technique can produce uniform, pathogen-free, season-independent micro shoots. Furthermore, from micro shoots can produce micro tubers, mini cuttings and mini tubers [2].

In the seed potato seed system, the use of in vitro technology is an important part of the production of certified seeds based on pathogen-free seeds as source seeds [3]. Micro potato tubers are miniature potato seeds produced through plant tissue culture techniques [4].

The growth of explants in tissue culture is determined by the medium used. The response of plants or plant parts of a cultivar (variety) varies with the composition of the plant growth media, especially growth regulators. This variation is due to the growth phase and the ability of plants to absorb and translocate growth regulators, as well as the ability of different endogenous growth substances so that the response to plantlet growth

and tuber formation will be different. Warnita [2] report that MS media is a medium that is widely used for potato tissue culture.

The part of the plant used as a propagation material is explants. Basically every part of the plant can be used as an explant. Warnita [2] used single node explants for in vitro potato propagation. Sapura [5] used two node cuttings for the induction of micro shoots and the formation of potato plantlets. Micro tubers are free of pests and diseases and have the potential to become superior seeds [6].

Tissue culture plants cannot produce sufficient amounts of their own carbohydrates, so they need to be added to the media. Sources of carbohydrates that are commonly used are glucose and sucrose with a concentration of 2-3% [7,8]. Pierick [9] states that the usual sucrose added to the media is 30 g / l. Elaleem [10] received the highest number of tubers in Amera and Diamant cultivars on MS medium containing 80 g / l of sucrose. MS medium with 30 g / l sucrose produced shoots without micro tubers.

Regulating substances that have an effect on the in vitro induction of potato micro tubers are cytokinins, gibberellins and retardants. Suhadi et al. [11] reported several synthetic retardants commonly used in horticultural cultivation, namely Daminozide (Alar and B-nine), Chloromequat (cystocele), Ancymindol (A-Rest), Maleic hydrazine and Paklobutrazol. The use of paklobutrazol has an effect on the weight variable of sweet potato, while the use of jasmonic acid for the variable percentage of plantlets forms micro potato [12]. According to Sakya et al. [13] utilize coumarin will accelerate the entry of plants into the reproductive phase because the energy to carry out this growth is accumulated for tuber formation and at the right concentration will produce the largest number of tubers.

The response of coumarins in plants can act as stimulants or inhibitors of plant growth, depending on the concentration [14]. The most significant effect of coumarin on root growth [15], affects respiration, photosynthesis, metabolism, and the activity of various enzymes [16].

For the induction of micro tubers, it is necessary to add growth regulators BAP and Coumarin. The BAP concentration commonly used for tuber induction is 5 mg / l medium [17]. Environmental factors also affect plant physiological processes. Temperature affects plant metabolic processes, growth regulator activity and micro tuber induction processes. Negative effect on the induction of potato micro tubers Advantages of using micro tubers. Additionally, it has been reported that microtuber derived plants have higher yield compared to seed tuber derived plants [18]. Further histological study of microtuber revealed that microtuber periderm is made up of multiple layers of meristematic phellogen cells which confer microtuber disc as a potential explant for multiple shoot regeneration [19].

According to Anisa [20], the higher tuber weight per plant was obtained from the treatment of 100 ppm coumarin, this is thought to be due to the role of growth inhibitors that flow photosynthate to the tubers as energy, thereby increasing tuber formation. Sari et al. [21] stated that giving BT.4.1 rhizobacteria with 100 mg / l coumarin could increase the leaf area of potato plants. It was further reported that the combination of auxins, cytokinins, and retardants gave the best results in inducing the Kufru Jyoti potato micro tuber variety [22]. The best nutritional medium is 2.5 mg / L BAP + 0.5 mg / L NAA + 30 g / L sucrose + 1.0 ppm methyl jasmonate) according to different ratios of sucrose and methyl jasmonate. Used in nutritional media [23].

Incubation room temperature is a limitation in the induction of potato micro tubers. High incubation temperatures inhibit the formation of potato tubers [24], because potato plants require low temperatures to be able to grow well and produce optimally [25].

Temperature regulation and application of growth regulators will affect the formation of potato micro tubers. However, if the settings are not correct, it will affect or even reduce tuber induction. Therefore, it is necessary to research the use of this technology to get a lot of micro tubers. The purpose of this study was to obtain the best coumarin concentration and temperature for micro tuber induction.

RESEARCH METHODS

This research was conducted from September 2019 to January 2020. The research location was at the Tissue Culture Laboratory, Faculty of Agriculture, Andalas University. Padang

The materials used in this study were Atlantic variety potato plantlets and the chemical constituents of MS (Murashige and Skoog) media, distilled water, 70% alcohol, 96% alcohol, sprits, sucrose, coumarin, detergent, 1 mol / L NaOH, 1 mol HCl. / L, in order (bacto agar)

This experiment was carried out with a 2-factor factorial experimental method with 4 replications in a completely randomized design (CRD). The first factor is the concentration of coumarin consisting of 5 levels, namely: 0, 25, 50, 75 and 100 mg / L of coumarin. The second factor is the temperature of the incubation room which consists of 3 levels, namely: 20, 25 and 30 ° C. The data were analyzed by using the F test followed by Tukey's test at the 5% level.

All rooms involved with activities such as the culture room and media stock room were cleaned using 70% alcohol. For laminar air flow cabinets sterilized using UV light and 70% alcohol. The cleaned culture bottles were then sterilized by autoclaving at a pressure of 15 Psi (pounds per square inch) at a temperature of 121 0C and maintained for 30 minutes. Other planting tools such as petri dishes, tissue, tweezers, clear plastic, and scissors are sterilized using an autoclave.

The medium used was MS0 media as a plantlet growth medium in solid form. The degree of acidity is measured in the range of 5.8 - 6. If the pH is acid-base, 0.1 N KOH is added and 0.1 N HCl is added if it is alkaline. Then, the media was added with bacto agar as a compactor for 8 grams / liter and stirred using a magnetic stirrer hotplate until the medium boiled [7,9,26].

After boiling, MS 0 media was put into a culture bottle with a volume of 20 ml / bottle. Then, the bottle is closed with plastic and the neck of the bottle is tied with a rubber band, then the media is sterilized using an autoclave with a pressure of 15 Psi at a temperature of 121 0C for 15 minutes after

that it is transferred to an incubation room with a temperature of 20° C. The media is incubated for about one week [4,9,26].

The planting material used was the nodes of potato explants of Atlantic varieties cultured on MS 0 media. The plantlets were cut with scissors and tweezers which had been sterilized in a laminar air flow cabinet into cuttings consisting of two nodes.

The explants were planted in a Laminar Air Flow Cabinet (L AFC) which had been irradiated using UV light. All planting equipment needed to plant the explants are sprayed with 70% alcohol before entering the L AFC box. Planting tools such as tweezers, scissors, soaked in 96% alcohol then burned using a Bunsen.

The explant consisted of two nodes placed in a culture bottle containing the media, 1 culture bottle containing 2 explants. The bottles filled with explants were then closed using glass plastic and tied with a rubber band on the neck of the bottle and then covered with plastic wrap. The explants were incubated in a sterile room with controlled light and temperature. The explants that have been planted in the media are stored in a storage rack with a temperature of 20 °C.

The seasoning medium used consisted of liquid MS medium with a concentration of coumarin according to each treatment, namely: 0, 50 and 100 mg / l plus 5 ppm BAP. The addition of the ground media is carried out in the Laminar Air Flow Cabinet (L AFC). Sterilization of the ground media by autoclaving with a pressure of 15 Psi for 15 minutes. The addition of the seeding medium was carried out when the plantlets were 6 weeks after planting. You do this by adding 20 ml / bottle of seasoning media to the bottle containing the plantlets. After that the culture bottles were placed on the culture racks according to the placement plan and the storage conditions treated.

At a temperature treatment of 20° C the culture bottles were incubated at 20° C with total darkness, the culture racks were covered with black plastic that was not translucent in an air-conditioned room, while for the 25° C treatment the culture bottles that had been given the ground

media were incubated at 25° C in total darkness, the culture shelf is covered with black plastic which is opaque in a room without air conditioning. At a temperature of 30 ° C, the culture bottles that have been given the ground media are incubated in total darkness, to create an incubation temperature of 30 ° C using a temperature control device then one culture rack is coated with opaque black plastic, 1 thermostat is installed on the rack and 3 incandescent lamps 100 watts to generate heat. Then installed a thermometer on the rack to see that the temperature remains constant at 30 °C. Finally, a timer is attached to the mains socket and is set to keep the lights on for 8 hours during the day. The plants were incubated for 10 weeks after adding the ground media until harvesting.

Maintenance includes maintaining cleanliness, separating explants or media contaminated with fungi or bacteria by removing them from the culture shelf. The bottles containing explants are neatly arranged to make it easier to observe. Maintenance is done by spraying 70% alcohol around the culture racks where the culture bottles are placed. Spraying is done 2 times a day.

Harvesting of the micro tubers was carried out at the 10th week after the addition of the ground media, which was done by removing the plantlets and micro tubers. Then the micro tubers were observed.

RESULTS AND DISCUSSION

Time of Tuber Emergence

There was an interaction between coumarin concentration and incubation room temperature on the induction of potato micro tubers (Table 1). The fastest tuber induction occurred at 100 g / l of coumarin and an incubation room temperature of 30 ° C for 13.28 days and the longest was 0 g / l of coumarin at 30 ° C, namely 47.53 days after the addition of the ground medium.

In general, at a temperature of 20 °C, the emergence time of tubers is faster than at 25 and 30 °C, this is due to the lower the incubation room temperature, the faster the induction of tubing, where the potato plant is a plant that requires a

low temperature to be able to produce well. The results of research by Otrshy et al. [27] showed that there was a decrease in the percentage of tuber crops from 88% to 64%, the number of tubers per plant from 0.88 to 0.66, and tuber size from 2.2 mm to 1.6 mm when the incubation room temperature was increased from 17 ° C to 25 ° C. Furthermore, Suharjo et al. [28] stated that high temperatures inhibit the growth and induction of potato micro tubers.

Based on the results of this study, the use of coumarin has been able to eliminate the negative effects of high temperatures. Coumarin which is a phenolic compound is able to inhibit gibberellin biosynthesis, with the increasing concentration of coumarin given, the faster the appearance of tubers.

Plantlets began to experience initiation in the second week after the addition of tuber media (WAT) which was marked by swelling of the stolon and accumulation of starch, then an increase in tuber size, including volume and tuber weight (Fig. 1). Micro tuber formation begins with tuber initiation and accumulation of starch resulting in an increase in tuber volume and weight.

The formation of micro potato tubers at a high temperature of 30 ° C with a coumarin concentration of 100 mg / L gave the tuber emergence time faster than the coumarin concentration of 50 mg / L and without coumarin administration. It is thought that the higher the coumarin concentration given at high temperatures will be able to speed up the appearance of the

Table 1. Time of tuber emergence at various coumarin concentrations at incubation room temperature

Coumarin concentration (mg/L)	Incubation Room Temperature (°C)		
	20	25	30
days.....		
0	19.44 a B	22.25 a B	47.51 a
25	18.97 ab A	21.53 ab A	20.34 b A
50	18.14 abc A	18.34 bc A	15.29 c B
75	16.12 bc A	16.67 c A	18.74 b A
100	15.46 c AB	16.34 c A	13.26 c B

Note: Means with different letters, in the same row and column differ significantly ($p \leq 0.05$, according to Tuckey's test).



Fig. 1. Micro tuber

tubers. Rykaczewska [29] inform that at high temperatures there is an increase in the rate of respiration and endogenous gibberellin in plants, thus inhibiting tuber formation. High temperatures induce plant development, make plant stems thin, leaves small, long stolon, increase the number of internodes and inhibit tuber formation.

Increasing the concentration of coumarin given can inhibit plant vegetative growth. The inhibition by giving coumarin causes the action of the gibberellin hormone in plants to be inhibited, thus accelerating the plant to enter generative growth. This is because the energy used during the formation of nodes and branches is accumulated for tuber formation, so the time needed to form tubers is relatively faster [30]. In vitro rooting can occur because the growing environment conditions and the composition of the media used are able to encourage tuber initiation, especially when viewed from the high speed of tuber growth. Wattimena [26] report that tuber emergence time can be influenced by several factors, namely, the source of explants, media composition, growth regulators, low culture room temperature (18 - 20 ° C), dark conditions during induction of tubing and high sugar concentrations.

Number of Tubers per Plantlet

There was no interaction between coumarin and incubation room temperature on the number of potato tubers in vitro. In Table 2 indicate that an increase in coumarin concentration increases the

number of micro tubers formed. The highest number of micro tubers was found in the coumarin administration of 100 mg / l and the lowest was obtained at 0 mg / l of coumarin application. The application of several coumarin concentrations had a significant effect on the number of potato micro tubers produced per plantlet. The application of coumarin concentrations of 0 mg / l, 25 mg / l, 50 mg / l and 75 mg / l showed a relatively similar effect on the number of micro tubers and was significantly different from the coumarin concentration of 100 mg / l. The highest number of micro tubers was produced at a coumarin concentration of 100 mg / l, namely 1.67 tubers, compared to a concentration of 0 mg / L, namely 1.12 tubers and a concentration of 50 mg / l, namely 1.33 tubers.

This is thought to occur because of the effect of coumarin which can inhibit the action of gibberellin so that it can induce tuber formation, so the amount produced is more, while the 0 mg / l coumarin treatment is more focused on vegetative growth of plants which causes the resulting tubers to be low. The same thing was also reported by Kianhmer et al. [31]; Hasni et al. [30]) that increasing the concentration of coumarin can increase the number of micro tubers formed. This is also in accordance with Warnita [17] that the number of tubers is influenced by the composition of the media used. Retardants are able to stimulate seeding by inhibiting gibberellin biosynthesis. Inhibition of growth results in the accumulation of assimilates on the stems and leaves, thus inducing tuber formation.

Table 2. Number of tuber per plantlet at various coumarin concentrations at incubation room temperature

Coumarin concentrations (mg/L)	Incubation Room Temperature (°C)			Average
	20	25	30	
0	1.22	1.00	1.50	1.12 b
25	1.28	1.55	1.29	1.37 b
50	1.37	1.34	1.28	1.33 b
75	1.20	1.50	1.46	1.39 ab
100	1.77	1.50	1.75	1.67 a
Rata - rata	1.37	1.38	1.46	

Note: Means with different letters, in the same column differ significantly ($p \leq 0.05$, according to Tuckey's test).

The delay in tuber formation at high temperatures will also affect the number of tubers formed. However, the addition of coumarin can reduce the negative effects of high temperature. Kianmher et al. [31] found that the number and size of tubers continued to increase with increasing concentrations of coumarin in the growing medium.

Diameter of Tuber

There was no interaction between the administration of several coumarin concentrations and the incubation room temperature on the diameter of the micro tubers. The application of several coumarin concentrations and the incubation room temperature also did not affect the diameter of the micro tubers (Table 3).

In this study, coumarin application was thought to be able to inhibit gibberellin biosynthesis, so that the metabolic yield could be allocated for tuber formation. Coumarin as an inhibitor functions to stimulate the rooting by inhibiting the performance of gibberellin so that the accumulation of assimilates on the stems and

leaves and encourages tuber formation. Hasni et al. [29] stated that the coumarin concentration of 25 mg / l had a significant effect on increasing the percentage of plantlets that produced the largest tubers and the diameter of micro tubers. Kusumaningrum [32] states that tuber initiation can be formed if environmental conditions are adequate, such as the concentration of growth regulators, temperature or incubation room temperature and irradiation. The size of the tubers is also influenced by the distance between the tubers and the growing medium. Tubers that are close to the media and even submerged in the media generally have a larger size than the tubers that are located far above the media

Here it can be seen that the tendency is that at lower temperatures can produce a larger tuber diameter compared to higher temperatures. The negative effects of temperature can be suppressed by application of retardants such as coumarin. The size of the micro tubers is an indication of the success of his performance in the field. The larger the tuber size the better it looks in the field [33]. The diameter of tubers and tubers and haulm can be seen in Fig. 2

Table 3. Diameter of tuber at various coumarin concentrations at incubation room temperature

Coumarin Concentrations (mg/L)	Incubation Room Temperature (°C)		
	20	25	30
0	0.48	0.35	0.46
25	0.52	0.44	0.55
50	0.58	0.53	0.55
75	0.60	0.46	0.43
100	0.46	0.45	0.45

Note: not significantly different at F test 5 %

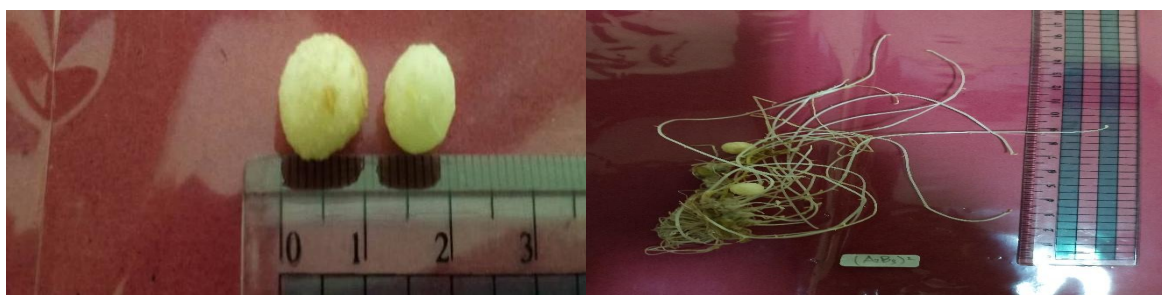


Fig. 2. Diameter of tuber and tuber and haulm (50mg/l coumarin + temperature 30°C)

Fresh Weight of Tuber per Plantlet

Treatment of several coumarin concentrations an incubation room temperature does not have any effect toward fresh weight of tuber. Also there is no interaction between the coumarin concentration and the incubation room temperature toward fresh weight of tuber (Table 4).

It is assumed that the concentration of coumarin given has been able to suppress the performance of gibberellins so that almost the same tuber weight is formed. According to Warnita [17] fresh weight of tuber is also related to the number and size of tubers. A large number of tubers and a large diameter of tuber, will give the high fresh weight of tuber.

The temperature of the incubation room also had almost the same effect on the fresh weight of the tubers, meaning that the temperature of 20-30 ° C was still suitable for tuber formation. At 30 ° C, there is a tendency for fresh weight of tuber to be higher than at 20 ° C and 25 ° C. In contrast to the research of Sumarni et al. [34] which states that

tuber production is influenced by the rate and duration of tuber filling. The filling rate of the tubers decreases with increasing temperature. High temperatures inhibit the translocation of assimilates to tubers, so that tuber formation is inhibited.

High temperature induces the development of plants with thin stems, small leaves, long stolon, an increase in the number of internodes, inhibition of tuber formation and a decrease in the ratio of fresh weight of tuber to total fresh weight [29]. Micro potato tubers at several concentrations of coumarin at 30 ° C can be seen in Fig. 3.

The effect of high temperature stress also depends on the phase of plant growth, the earlier the plant is exposed to high temperatures, the greater the negative effect on tuber growth and yield [29]. In contrast to Suharjo et al. [24] high incubation temperatures inhibit the formation of potato tubers, because potato plants require low temperatures to grow well and produce optimally [25].

Table 4. Fresh weight of tuber per plantlet at various coumarin concentrations at incubation room temperature

Coumarin concentration (mg/L)	Incubation Room Temperature (°C)		
	20	25	30
0	0.138	0.127	0.165
25	0.192	0.134	0.205
50	0.194	0.177	0.217
75	0.211	0.124	0.217
100	0.171	0.146	0.188

Note: not significantly different at F test 5 %

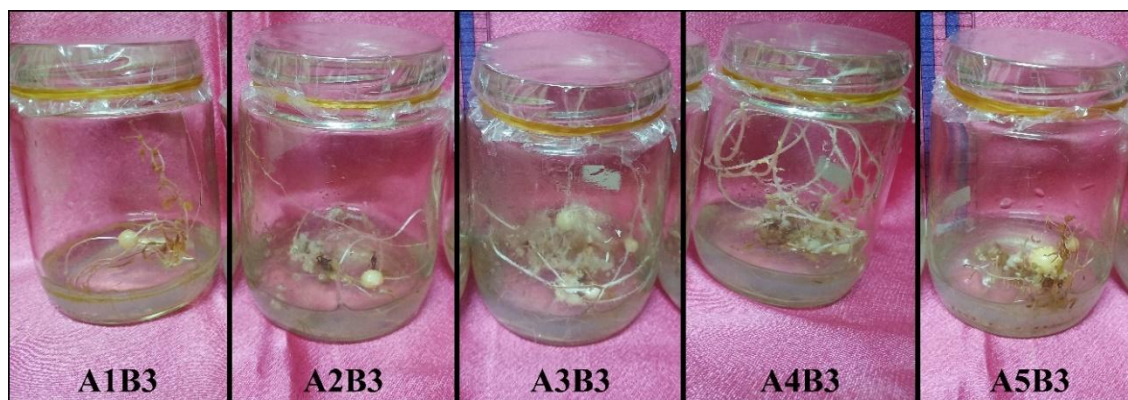


Fig. 3. Potato micro tubers at some coumarin concentrations at 30 ° C

Low fresh weight of tuber is associated with a low number of tubers and a small diameter of tuber. The more the number of tubers and the larger the diameter of tuber, the weight of the micro tubers produced will be higher [35]. The fresh weight of tuber was very much affected by the number and the size of tuber. Suharjo et al. [36] report that the best growing media to induce tuber formation, improve tuber size, and increase tuber weight in vitro was 5 g l⁻¹ BAP combined with 7.5 mg l⁻¹ paclobutrazol.

Fresh Weight of Shoot

There was no interaction between the application of several coumarin concentrations and incubation room temperature on the fresh weight of shoot. The application of several coumarin concentrations and the incubation room temperature also had no effect on the fresh weight of shoot (Table 5).

Increasing the temperature of the incubation room from 20 - 30 ° C has not had an effect on the increase in fresh tuber weight. In general, potatoes are better roasted at lower temperatures. Sumarni et al. [34] which states that tuber production is influenced by the rate and duration of tuber filling. The filling rate of the tubers decreases with increasing temperature. High temperatures inhibit the translocation of assimilates to tubers, so that tuber formation is inhibited.

The application of coumarin from 0-100 mg / l had no effect on the fresh weight of potato shoots. In accordance with the function of coumarin is to inhibit the biosynthesis of gibberellins, the growth of shoots will be suppressed so that the shoot weight becomes low and assimilates are directed to the tubers. This is also related to tuber diameter

and tuber weight. This is in line with Weaver's [37] statement that coumarin as a retardant can inhibit physiological and biochemical processes in the plant body. Coumarin as a retardant is able to stimulate rooting by inhibiting the biosynthesis of gibberellins which plays a role in plant growth. Inhibition of plant growth results in the accumulation of assimilates on stems and leaves so as to induce tuber formation [17].

Fresh Weight of Root

There was no interaction between applications several coumarin concentrations and incubation room temperature to fresh weight of root. The application of several coumarin concentrations had an effect on fresh weight of root but the incubation room temperature also did not affect fresh weight of root (Table 6).

While the incubation room temperature had no effect on fresh weight of root, although the incubation room temperature increased gave almost the same effect. This is probably because the roots are in a medium that is always wet and can also absorb nutrients from the media that can support root growth.

Table 6 show that the higher the coumarin concentration result the higher the fresh weight of the roots, although between 50, 75 and 100 mg / l were not significantly different. Increasing the concentration of coumarin suppressed fresh weight of shoot and conversely increased fresh weight of root. Good root growth will be able to absorb the nutrients available in the media. Roots that grow well will optimize nutrient transportation from the roots to the top of the plant, so that it will indirectly increase the fresh weight of the roots.

Table 5. Fresh weight of shoot at various coumarin concentrations at incubation room temperature

Coumarin concentration (mg/L)	Incubation Room Temperature (°C)		
	20	25	30
0	0.40	0.48	0.71
25	0.61	0.55	0.66
50	0.68	0.58	0.67
75	0.69	0.54	0.79
100	0.61	0.65	0.66
KK = 20.31 %			

Note: not significantly different at F test 5 %

Table 6. Fresh weight root at various coumarin concentrations at incubation room temperature

Coumarin concentration (mg/L)	Incubation Room Temperature (°C)			
	20	25	30	Average
0	0.065	0.030	0.146	0.080 b
25	0.048	0.054	0.096	0.066 b
50	0.148	0.084	0.153	0.128 a
75	0.103	0.202	0.184	0.163 a
100	0.174	0.161	0.177	0.171 a
Average	0.108	0.106	0.151	

Note: Means with different letters, in the same column differ significantly ($p \leq 0.05$, according to Tuckey's test).

Long and short of root will effect nutrient absorption and root growth. The roots are long and plunged into important to reach water and nutrients are much deeper on the media [38]. Good nutrient uptake will effect root growth and have a positive correlation with root weight fresh.

CONCLUSION

1. There is an interaction between coumarin concentration of 100 ppm and incubation room temperature of 30 ° C to the time of potato micro tuber emergence, namely 13.26 DAT.
2. The application of coumarin with several concentrations has an effect on time of tuber emergence, number of tuber, diameter of tuber and fresh weight of root.
3. The temperature of the incubation room affects the time of micro tubers emergence of the potato

ACKNOWLEDGEMENTS

Thanks to Mr. Dean of the Faculty of Agriculture, University of Andalas, which has funded research through correspond BOPTN Fund Agreement No. 01 / PL / SPK / PNP / Faperta-Unand / 2019, Head of UPPM Faculty of Agriculture, and all those who have helped for the implementation of this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. BPS. Statistics of Seasonal Vegetable and Fruits Plants Indonesia. Jakarta: BPS Indonesia; 2019.
2. Warnita. Study of seeding patterns of several potato genotypes in the field and in vitro in the effort to supply seeds. Andalas University Postgraduate Dissertation, Padang. 2006;185.
3. Hidayat MI, Sudjuko S. The technique of rapid propagation of potato seed tubers. Indonesian Vegetable Crops Research Institute. Bandung. 2016;28.
4. Saha S, Ahmed M, Islam MM, Reme RN, Ali MR. Effect of different levels of sucrose on microtuberization and different substrates on minituber production from potato meristem culture. IOSR Journal of Agriculture and Veterinary Science. 2013;4(6):58-62.
5. Saputra A. Effect of giving several coumarin concentrations on potato (*Solanum tuberosum* L.) rooting in vitro. Thesis Faculty of Agriculture; 2009.
6. Bodani A, Chauhan JS. In vitro sterilization protocol for micropropagation of *Solanum tuberosum* cv. Kufri Himalini. Academia Arena. 2010;2(4):24-27.
7. Murashige T. Plant propagation through tissue cultures. Ann. Rev. Plant Physiol. 1974;25:135-166.
8. Gamborg OL, Shyluk JP. Nutrition, media and characteristics of plant cell and tissue culture, In Thorpe T.A. (Ed.) Plant Tissue Culture Methods Application in Agriculture. Academic Press Inc., New York. 1981;21-41.

9. Pierik RLM. In vitro culture of higher plants. Martinus Nijhoff Publishers, Dordrecht, The Netherlands. 1987;344.
10. Elaleem KGA, Modawi RS, Khalafalla MM. Micro tuber Induction of two Potato (*Solanum tuberosum* L.) Varieties namely, Almera and Diamant. International Research Journal of Biological Sciences. 2015;4(3):84-89.
11. Suhadi I, Nurhidayati BA. Sharon. The effectiveness of synthetic retardants on the growth and longevity of sunflowers (*Helianthus annuus* L.). Journal of Agrifor. 2017;XIV(2):219-228.
12. Amalia IA, Nuraini Sumadi S, Mubarak E, Suminar. Formation of potato micro potato (*Solanum tuberosum* L.) on various media compositions in vitro. Cultivation Journal. 2017;16(3):389 – 393.
13. Sakya TA, Yunus A, Samanhudin U, Baroroh. The effect of coumarin and aspirin on induction of potato microtuber. Agrosiences. 2003;5(1):19-28.
14. Al-Wakeel SM, Gabr MM, Abu-El-Soud WM, Pious AM. Coumarin and salicylic acid activate resistance to *Macrophomina phaseolina* in *Helianthus annuus*. Acta Agronomica Hungarica. 2013;61(1):23–35.
15. Ahrabi F, Enteshari S, Moradshahi A. Allelopathic potential of para-hydroxybenzoic acid and coumarin on canola: Talaieh cultivar. Journal of Medicinal Plants Research. 2010;5:5104–5109.
16. Zhou X, Song H, Wang J. Effects of coumarin on net nitrate uptake and nitrogen metabolism in roots of alfalfa (*Medicago sativa*). Allelopathy Journal. 2013;31:377.
17. Warnita. Modification of potato rooting media with growth retardant substances. Straw Journal. 2008;1(1):50-52.
18. Hossain MS, Hossain MM, Haque MM, Haque MM, Sarkar MD. Varietal evaluation of potato microtuber and plantlet in seed tuber production. International Journal of Agronomy. 2017;Article ID 7520297. DOI: 10.1155 / 2017/7520297
19. Borna RS, Hoque MI, Sarker RH. 2019. In vitro microtuber induction and regeneration of plantlets from microtuber discs of cultivated potato (*Solanum tuberosum* L.). Plant Tissue Cult. & Biotech. 2019; 29(1):63-72, (June)
20. Anisa, F. Effect of chitosan and coumarin on growth and yield of potato (*Solanum tuberosum* L.) G2 Cultivar Granola. Agri Journal SCI. 2014;1(4):Hal 100-110.
21. Sari, H.P., W. Warnita, and Indra Dwipa. 2019. Rhizobacteria and Coumarin Applications on Growth and Tuber Formation of Potato (*Solanum tuberosum* L.). J. Agron. Indonesia, 47 (2): 188-195
22. Dhaka M, Nailwal TK. High efficiency macropropagation of potato (*Solanum tuberosum* L.) cv. Kufru Jyoti in Kumaun Hills. J. Plant Breeding and Crop Sci. 2015; 7 (7):203-210.
23. Vural GE, Ozsan T, Gozen V, Onus AN. In vitro Micro Tuber Formation in Potato (*Solanum tuberosum* L.): is there any Relation between Methyl Jasmonate, Sugars, and Explants?. International Journal of Biotech Trends and Technology (IJBT). 2018;8(1):1-8.
24. Suharjo UKJ, Fahrurrozi, Sudjatmiko S. 2008. Encouraging the formation of micro potato tubers at high temperatures with the application of paclobutrazol, coumarin, CCC, and ancymidol. Proceedings of the National Potato Week Seminar. Lembang, Bandung, 22-23 August; 2008.
25. Stark KL, Love SM. Potato production system. California: Mcgraw Hill; 2003.
26. Wattimena GA. Development of quality potato propagules from superior potato cultivars to support increased potato production in Indonesia. Scientific Oration of Permanent Professor of Horticulture, Faculty of Agriculture, IPB, Bogor; 2000.
27. Otrshy M, Nazarian F, Struik PC. Effects of temperature fluctuation during in vitro phase on in vitro microtuber production in different cultivars of potato (*Solanum tuberosum* L.). Plant Cell Tiss Organ Cult. 2009;98(2):213-218.
28. Suharjo UKJBG, Murcitra T, Pamekas, Haryuni. In vitro induction of micro potato tubers at high temperature with multiple tuber promoter. Biogenesis. 2017;5(1):61 - 69.

29. Rykaczewska K. The impact of high temperature during growing season on potato cultivars with different responses to environmental stresses. *American Journal of Plant Sciences*. 2013;4:2386-93.
30. Hasni VUA, Barus FET, Sitepu RCB. Hutabarat. Response adding to the coumarin in to micro tuber production of potato plantlets (*Solanum tuberosum* L.) varieties of granola. *Online Journal of Agroecotechnology*: 2014;2(4):1552-1562.
31. Kianmehr B, Parsa M, Otrushy M, Mohallati MN, Moradi K. Effect of plant growth regulators during in vitro phase of potato microtuber production *Journal of Agricultural Technology*. 2012;8(5):1745-59
32. Kusumaningrum IS. Evaluation of in vitro growth and micro tuber production of several potato clones (*Solanum tuberosum* L.) from crosses of Atlantic and Granola cultivars [thesis]. Bogor Agricultural University. Bogor; 2007.
33. Kawakami J, Iwama K. Effect of potato size on the growth and yield performance of field grown plants. *Plant Prod. Sci*. 2012; 15 (2):144-148.
34. Sumarni E, Suhardiyanto H, Saptomo SK. Root zone cooling in potato seed production using an aeroponic system. *J. Agron. Indonesia*. 2013;41:154-159.
35. Color. Effect of growth media and photoperiod on potato microtuberization. *Journal of Agrosia Deed*. 2007;10(2):167-171.
36. Suharjo UKJ, Hasanudin H, Pamekas T, Pujiwati H, Vanturini A. Promoting tuber formation in vitro with benzyl amino purine and paclobutrazol at different concentrations. *Agrosia Deed*. 2019;22(1): 29-35.
37. Weaver. *Plant Growth Substances in Agriculture*. Mc Millan Publishing Co Inc. New York; 1972.
38. Warnita, Akhir N, Vina. Growth response of two varieties chrysanthemum (*Chrysanthemum* sp.) on some media composition. *International Journal on Advanced Science, Engineering and Information Technology*. 2017;7(3):928-935.