talenta PUBLISHER

InJAR
Indonesian Journal of Agricultural Research
Journal homepage: https://injar.usu.ac.id

Optimization medium at the propagation and formation stage of micro tubers some varieties of potato

Melvi Wahyu Angraini Nasution[1], Luthfi Aziz Mahmud Siregar[1*], Mohammad Basyuni[2]
[1]*Department of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia*
[2]*Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Medan, Indonesia*
[*]Corresponding author: luthfi1@usu.ac.id

ARTICLE NFO

Article history:
Received 12-06-2024
Revised 08-11-2024
Accepted 21-03-2025
Available online 27-03-2025

E-ISSN: 2615-5842
P-ISSN: 2622-7681

How to cite:
M. W. A. Nasution, L. A. M. Siregar, and M. Basyuni "Optimization medium at the propagation and formation stage of micro tubers some varieties of potato", *Indonesian J. Agric. Res*, vol. 8, no. 1, pp. 9-16, Mar. 2025.

http://doi.org/10.32734/injar.v8i1.16798

ABSTRACT

Potato cultivation in Indonesia is inadequate and experiences fluctuations. One of the obstacles is the limited availability of land for seed propagation and the high cost of in vitro media. The aim of this research is to increase the efficiency of MS media (Murashige and Skoog) for cultivating several potato varieties to form micro tubers in vitro. The research was conducted at the Biotechnology Laboratory, Faculty of Agriculture, USU Medan from June to November 2023. The research was carried out by planting node cuttings from four potato cultivars (Granola, Dayang Sumbi, Atlantic Malang, and Maglia) on MS medium with optimal concentration (1/2 MS, 3/4 MS, and 1 MS). This research uses two different stages: the propagation stage, namely shoot micropropagation, and the micro tuber creation stage. The results showed that the optimization of 3/4 MS media concentration showed quite good growth compared to 1/2 MS media concentration for growing potato explants in terms of the number of segments, number of leaves and number of primary roots. Furthermore, the optimization of media concentration and varieties did not show any significant influence or difference in the formation of micro tubers based on statistical tests.
Keywords: in vitro, micro tubers, ms medium, potato varieties, propagation

1. Introduction

Demand for potatoes continues to increase every year. Indonesia imported potatoes in 2022 was 1,87 million kilograms and in 2023 was 7,16 million kilograms [1]. The lack of quality and adequate potato seeds is a major obstacle to potato production in Indonesia because it is unable to meet the demand of potato farmers. Based on data from [2], national potato production shows 1,42 million tons in 2022 and 1,25 million tons in 2023. Seeds are very important for the success of potato cultivation in Indonesia. Apart from that, the limited availability of potato seeds in Indonesia is partly due to limited land allocation for seed cultivation [3].

The quality of the potato seeds currently in circulation is worse because the seeds from the previous harvest were susceptible to disease attacks, causing a decline in potato production. Tissue culture is a method used to produce potato seeds that are large, uniform in size, and free of disease [4]. The in vitro approach is a step in the process of producing G0 plant material for potato seed production. The technique used to produce superior potato seeds is a one-generation flow propagation pattern [5]. Murashige and Skoog (MS) media are widely used as planlet growing media because of their high nitrate, potassium and ammonium content, which are

important for promoting plant growth. However, excessive amounts of media may not be optimal for adequate growth and development of explants and plantlets in vitro.

The use of 3/4 MS media on Mentha spicata plants resulted in increased growth [6]. Tetsumura et. al. [7] found that reducing MS media content in blueberry plants led to increased shoot and root growth. Purwanto et. al. [8] proved that a mixture of 1/4 MS media and 150 ml of coconut water was sufficient to develop potato explants. The administration of the growth regulator (ZPT) meta-Topolin (mT) at 2 ppm in MS media variants with strengths of 1, 3/4 and 1/2 had a significant effect on the growth of potato shoots and roots [9]. In addition, the use of culture media is one of the costs associated with in vitro propagation techniques. Achieving the ideal modified media concentration levels in vitro culture is essential to effectively produce planting material using in vitro procedures [10]. Based on this background, it is necessary to carry out in vitro research on several potato varieties to determine the optimal effect of reducing the nutrient concentration of MS media in terms of growth and development at the propagation and micro tuber formation stages.

2. Method

This research was conducted at the Biotechnology Laboratory, Faculty of Agriculture, Universitas Sumatera Utara, Medan. This research was conducted from June to November 2023.

2.1. Materials and tools

In vitro research requires a high level of material sterility and a good method can grow healthily. Criteria for healthy plantlets include normal morphology, free from microbial contamination, bright plant color, well-developed roots, environmental stress resistance, and genetic stability [11].

The material that will be used for this research is node cuttings from potato plantlets of Granola, Dayang Sumbi, Atlantic Malang, and Maglia varieties aged four weeks after planting, obtained from the plant collection of the Olifira Tissue Culture Laboratory on Jln. Karya Wisata (Johor) Medan, Murashige and Skoog (MS) media stock solution, granulated sugar, BAP, rubber bands, heat-resistant plastic, solidifying agar, distilled water, alcohol, sodium hypochlorite, NaOH, HCl, filter paper, tissue, matches, and labels.

The tools that will be used for this research are Laminar Air Flow Cabinet (LAFC), autoclave, analytical balance, hot plate, magnetic stirrer, pH meter, culture tube, test tube, culture rack, measuring cup, measuring flask, beaker glass, measuring pipette, rubber suction bulb, micro pipette, stirring spoon, petri dish, dissection tools (scalpel, blade, bayonet tweezers, dental tweezers, and scissors), bunsen lamp, handsprayer, chlorophyll meter, refrigerator, camera, and stationery.

2.2. Research stages

The research stages are as follows:

2.2.1. Sterilization of tools

The petri dish and culture bottle were soaked in sodium hypochlorite solution for 1 hour. After that, the instrument is cleaned with soapy water and scrubbed gently using a sponge or scrub brush. Then rinse with clean water until the remaining soap is gone. Next, the instruments were dried on a storage rack and sterilized using a pressurized autoclave at a pressure of 1 atmosphere and a temperature of 121 ºC for a duration of 60 minutes.

2.2.2. Preparation of stock solution

The ingredients used are solutions of macro nutrients, micro nutrients and vitamins, weighed using analytical scales according to the concentration of each treatment (1/2 MS, 3/4 MS and 1 MS). The ingredients are put into a glass beaker and one liter of distilled water is added. After that, each stock solution treatment was homogenized with a magnetic stirrer and put into solution storage bottles. Then the bottles are labeled with the name of the stock solution treatment and the date of manufacture. After that, the stock solution was stored in the refrigerator.

2.2.3. Media creation

a. Shoot micropropagation stage

The media used for micropropagation were Murashige and Skoog (MS) solid media, with each treatment enriched with 30 g/ liter of granulated sugar. After that, it is homogenized using a magnetic stirrer. Next, the acidity of the media solution was measured using a pH meter with the optimum pH for potato growth, pH 5.8

(using 1 N NaOH and 1 N HCl to raise and lower the pH). After that, the treatment media was added to solidify 6.5 gr/ liter and homogenized using a magnetic stirrer and cooked to boil using a hot plate. Then, the media was transferred into each treatment bottle as much as 25 ml for each treatment bottle (bottle volume 330 ml) and closed using heat-resistant plastic. Next, the media was put into an autoclave to be sterilized at a pressure of 1 atm and a temperature of 121 ºC for 15 minutes. The media was stored in the culture room at 20 ºC and incubated for 7 days.

b. Micro tuber formation stage

The media used for micro-tuber induction is the same treatment media as the propagation stage media, but after the plantlets are 3 weeks old, solid MS media is added with micro-tuber formation media. In the formation of micro tubers, 80 gr/liter of sucrose and 5 mg/liter of BAP were added to the media according to the research [12]. After all the media compositions are mixed, then add distilled water to the limit of one liter. Next, the acidity of the media was measured at pH 5.8. Then the bottles were covered with heat-resistant plastic and sterilized in an autoclave at 121 ºC, pressure 1 atm, for 15 minutes. Next, the culture bottles containing three week old plantlets after subculture were added with 20 ml of tuber media per treatment bottle.

2.2.4. Explant planting

UV Laminar Air Flow Cabinet (LAFC) is turned on for one hour, then turned off. Next, the planting area is sprayed with 70% alcohol and wiped with tissue. The bunsen lamp is turned on to sterilize the planting tools. The planting tools are put into a glass bottle containing 96% alcohol and burned over a bunsen. Next, on a petri dish, potato explants from four week old plantlets are cut to a length of ± 1.5 cm with one leaf per node. The explants are then planted in culture bottles contains 25 ml of treatment media (three explants/ bottle volume 330 ml), then closed and sealed with heat-resistant plastic. After being sealed, the explants were arranged on shelves randomly based on the predetermined treatment and stored in the incubation room for four weeks at a temperature of 22 ºC.

2.2.5. Plantlet subculture

After the plantlets were four weeks old, they were subcultured using the same method as before. The explants are planted in culture bottles contains 27 ml of treatment media (three explants/bottle volume 330 ml). Next, the bottles were arranged randomly on the culture rack and incubated at room temperature 22 ºC for three weeks.

2.2.6. Micro bulb induction

The plantlets used came from 108 bottles of healthy culture left over from the subculture results. The culture bottle containing three week old plantlets was added with 20 ml of tuber media. After that, the culture bottle containing the plantlets was incubated in a rack covered with black cloth (dark conditions) which was placed in an incubation room at a temperature of 20 ºC for six weeks for the formation of micro tubers.

2.3. Analysis method

The research used a completely randomized design analysis with 2 factors. Factor I is the potato plant varieties: Granola (V1), Dayang Sumbi (V2), Atlantic Malang (V3), and Maglia (V4). Factor II is MS concentration with 3 levels: 1/2 MS (M1), 3/4 MS (M2), and 1 MS (M3). The parameters observed were the number of nodes, number of leaves, number of lateral branches, number of primary roots, tuber formation time, number of tubers, tuber diameter, and fresh weight of tubers. This research is pure research experimental. The data obtained were analyzed using Analysis of Variance (ANOVA). If the treatment has a real effect, then continue with the Duncan Multiple Range Test (DMRT) at the 5% level with the help of Statistical Analysis System (SAS) software version 9.4.

3. Results and Discussion

3.1. Effect of MS medium optimization on the propagation stage of several potato plant varieties in vitro

The number of nodes and leaves is a determining indicator of the success of the propagation stage using micro shoot cuttings (nodes). Primordial leaves will grow and nodes will automatically form where the leaves sit. These two organs cannot be separated from the propagation stage, especially shoot micropropagation in potato plants because the planting material used as explants is a node organ with one leaf. The success of this propagation stage is marked by the growth of explants that start from one node with one leaf and are able to grow into plantlets that have many nodes and many leaves which can be used again as explants for the next propagation stage.

Indonesian Journal of Agricultural Research Vol. 08, No. 01 (2025) 9 – 16

Table 1. Recapitulation of average propagation stage parameters at 4 weeks after planting

Varieties	Concentration			Average
	½ MS	¾ MS	1 MS	
	Number of nodes (fruits)			
Granola	4.67	5.11	5.72	5.17
Dayang Sumbi	4.33	4.89	5.44	4.89
Atlantic Malang	5.17	5.89	6.00	5.69
Maglia	4.83	5.44	5.78	5.35
Average	4.75 [b]	5.33 [a]	5.74 [a]	
	Number of leaves (pieces)			
Granola	6.83	7.33	9.83	8.00 [b]
Dayang Sumbi	6.00	6.89	8.78	7.22 [b]
Atlantic Malang	7.83	8.67	10.00	8.83 [a]
Maglia	7.94	10.22	8.33	8.83 [a]
Average	7.15 [b]	8.28 [a]	9.24 [a]	
	Number of lateral branches (fruit)			
Granola	0.71	0.78	0.88	0.79
Dayang Sumbi	0.78	0.83	1.05	0.89
Atlantic Malang	0.71	0.71	0.78	0.73
Maglia	0.80	0.71	0.78	0.76
Average	0.75	0.76	0.87	
	Number of primary roots (fruit)			
Granola	3.50	4.78	5.83	4.70
Dayang Sumbi	5.11	5.22	5.33	5.22
Atlantic Malang	5.61	5.11	6.22	5.65
Maglia	4.67	5.22	5.33	5.07
Average	4.72 [b]	5.08 [a]	5.68 [a]	

Note: Numbers followed by the same letter in the same row and column are not significantly different based on DMRT 5%.

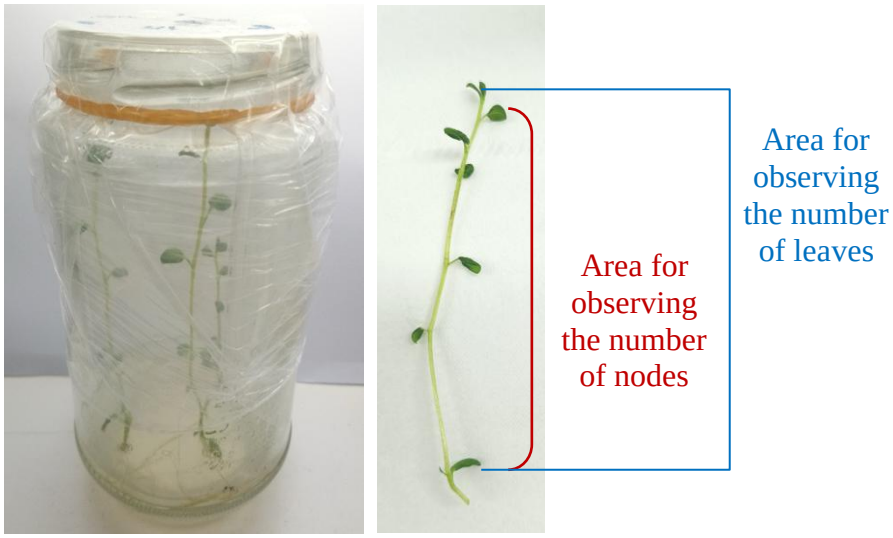

Figure 1. Observation of the number of nodes and leaves on planlets aged 4 weeks after planting

In Table 1, the results of observing potato plantlets at 4 weeks after planting, the parameters for the number of nodes, number of leaves, and number of primary roots were obtained from an optimization concentration of 3/4 MS and the parameters for the number of lateral branches obtained from an optimization concentration of 1/2 MS were not significantly different from the concentration of 1 MS. This proves that for potato plants the use of 3/4 MS and 1/2 MS concentrations is optimal and is able to produce the number of nodes, number of leaves, number of lateral branches and number of primary roots as the concentration of 1 MS. This is because explants have endogenous ZPT which can encourage plant tissue towards organ formation. The literature [13] stated that the natural hormones produced in a plant are said to be sufficient to support its development. As a

result, the addition of nutrients or hormones from external sources does not result in a marked increase in plant growth.

It is also estimated that the capacity of plantlet variants to thrive under the right conditions will result in nearly identical growth in each variety parameter. This is in line with research by [14] who found that environmental factors have a direct effect on plantlet physiology and have the ability to increase the rate of hormonal activity in plant tissue. The incubation environment for potato planlets must be aseptic, at a temperature of 22 ^{o}C, and with 1.000 lux lighting for 16 hours/ day. Furthermore, literature [12] reported that Granola was superior in terms of plantlet height and number of tubers, while Dayang Sumbi was superior in terms of number of shoots, primary roots, diameter, wet weight and tuber formation time. Although Maglia has more leaves than Atlantic Malang today, Atlantic Malang has more nodes and a greater doubling rate.

The interaction between MS concentration treatments and various potato plant varieties did not differ significantly for all parameters. This is thought to be because each potato variety is quite adaptive to the MS media content so that differences in concentration have no significant effect on its growth (the response of each potato variety is almost the same in each MS concentration treatment). This is in accordance with the literature [14] which states that MS media is designed to meet the nutritional needs of most plants at certain concentrations. In some varieties, certain concentrations have met optimal growth needs. Changes in concentration can cause excess or deficiency of nutrients that have no positive or negative effect on plant growth.

3.2. Effect of optimizing the use of MS medium on the micro tuber formation stage of several potato plant varieties in vitro

The appearance of potato micro tubers in plantlet culture which has been added with tuber formation medium with appropriate criteria for acclimatization is an indicator of the success of the potato micro tuber formation stage. In Table 2, based on observations that have been made, the response of MS concentration, the response of the four varieties of potato plants, and the interaction between the two at the *in vitro micro-tuber formation stage* with the parameters of tuber formation time, number of tubers, tuber diameter, and fresh weight of tubers for 6 MSI showed that the results were not significantly different for all of these parameter treatments. In this case, a concentration of 1/2 MS is able to produce tubers due to the addition of a growing medium in the form of 80 gr/liter sucrose which can regulate the growth rate and metabolism of plant tissue. According to the literature [15], this research supports the assumption that the addition of 80 gr/liter sucrose is the most optimal method to produce rapid microtuber development. Sucrose has the ability to inhibit gibberellin, a hormone involved in the process of plant development. Suppression of growth results in the accumulation of assimilate in the stems and leaves, thereby triggering tuber development [16].

Reducing the MS concentration was able to provide a growth effect that was almost the same as the normal concentration, presumably due to the addition of a growing medium in the form of BAP with a concentration of 5 mg/liter [12] which could regulate the growth rate and metabolism of plant tissue in general, thereby influencing the diameter and fresh weight of micro tubers. This is in accordance with the literature of [17] who stated that the presence of exogenous cytokinin hormones also plays a role in cell division and enlargement activities which directly influence the tuber formation process. Good photosynthate production is also a factor in producing tuber diameter and weight. This is in accordance with the literature of [18] which states that plants that are able to produce more leaves will influence photosynthate production. High photosynthate will be beneficial for plants to be able to increase the diameter and weight of larger tubers.

The appearance of potato micro tubers in plantlet culture which has been added with tuber formation medium with appropriate criteria for acclimatization is an indicator of the success of the potato micro tuber formation stage. Results with a micro tuber diameter of 4.86 mm turned out to be less desirable because they were considered to limit the success rate of transplantation in the field. This is in accordance with the literature of [19] who stated that a diameter of potato micro tubers > 6 mm is more desirable because with this size the micro tubers can be planted directly without going through an acclimatization process. Furthermore, the literature [20] added that micro-tubers with small diameters only store minimal amounts of nutrients, which results in slow plant growth and generally have a relatively short life time in the field.

Indonesian Journal of Agricultural Research Vol. 08, No. 01 (2025) 9 – 16

Table 2. Recapitulation of the average parameters of the micro tuber formation stage, aged 6 weeks after induction in the tuber medium

Varieties	Concentration			Average
	½ MS	¾ MS	1 MS	
	Tuber formation time (days)			
Granola	22.88	23.00	21.17	22.35
Dayang Sumbi	21.17	21.25	18.83	20.42
Atlantic Malang	24.42	23.75	21.00	23.06
Maglia	20.50	19.77	19.67	19.98
Average	22.24	21.94	20.17	
	Number of tubers (fruit)			
Granola	1.75	4.00	1.72	2.49
Dayang Sumbi	2.22	3.00	2.67	2.63
Atlantic Malang	1.42	3.50	1.00	1.97
Maglia	2.50	1.43	1.17	1.70
Average	1.97	2.98	1.64	
	Bulb diameter (mm)			
Granola	3.68	3.28	2.92	3.29
Dayang Sumbi	4.19	4.40	3.94	4.17
Atlantic Malang	3.24	4.50	2.57	3.44
Maglia	3.26	4.86	2.80	3.64
Average	3.59	4.26	3.06	
	Tuber fresh weight (gr)			
Granola	0.01	0.04	0.03	0.03
Dayang Sumbi	0.05	0.09	0.07	0.07
Atlantic Malang	0.04	0.10	0.02	0.05
Maglia	0.04	0.11	0.08	0.07
Average	0.03	0.09	0.05	

Note: Numbers followed by the same letter in the same row and column are not significantly different based on DMRT 5%.

Figure 2. Fresh weight of micro tubers in harvested potato plantlets

Regarding the results of the fresh weight of micro tubers of 0.11 gr, the literature [17] states that to be used as good propagule seeds, micro tubers must meet certain criteria, namely > 5 mm in diameter with fresh or wet weight tubers > 0.10 gr/ tuber. However, the literature [21] states that micro tubers with a wet weight of > 0.25 gr are considered to have a shorter dormancy period than micro tubers with a wet weight of < 0.25 gr, so they are more recommended as seeds to produce tubers (G0) is better. In this case, the increase in the fresh weight of micro tubers is influenced by the size of the tuber cells, so that the greater the number and diameter of the tubers, the greater the wet weight of the tubers [22]. Therefore, based on these facts, the number, diameter, and wet weight of micro tubers are often indicators of success in the microtuberization process [23].

4. Conclusion

The results showed that the optimization of 3/4 MS media concentration showed quite good growth compared to 1/2 MS media concentration for growing potato explants in terms of the number of segments, number of leaves and number of primary roots. Furthermore, optimization of media concentration and varieties did not show any significant influence or difference in the formation of micro tubers based on statistical tests.

The recommendation for this research is that further testing should be carried out using MS medium concentration optimization treatments and different varieties up to the acclimatization stage in order to obtain better concentration and variety optimization in the propagation stage and micro tubers formation stage of potato plants.

References

[1] Ministry of Agriculture of the Republic of Indonesia. (2020). *Creative Food Agribusiness.* [Online]. Available: https://cybex.pertanian.go.id/mobile/artikel/90156 (accessed March, 25, 2023).

[2] Central Statistics Agency Indonesia. (2023). *BPS – Statistic Indonesia. National Vegetable Crop Production*. [Online]. Available: https://www.bps.go.id/id/statistics-table/2/NjEjMg==/produksi-tanaman-sayuran.html (accessed July 11, 2023).

[3] Hamdani, J. S. Sumadi, Kusumiyati, and H. Ruwaidah, "Growth and yield of G0 cultivar medium potato seeds on various planting media compositions and watering intervals in medium plains," *Journal of Cultivation*, vol. 19, no. 3, pp. 1237 - 1238, 2020.

[4] E. Sulistiani and S. Yani. *Production of Plant Seeds Using Tissue Culture Techniques.* Seameo Biotrop, Bandung, 2012.

[5] Directorate of Horticultural Seeds. (2014, February, 06). *Technical Propagation and Certification of Potato Seeds* (*Solanum tuberosum* L.). Ministry of Agriculture. [Online]. Available: www.hortikultura.pertanian.go.id (accessed March 25, 2023).

[6] D. Fadel, S. Kintzios, A. S. Economou, G. Moschopoulou, and H.I.A. Constantinidou, "Effect of different strengths of medium on organogenesis, phenolic accumulation and antioxidant activity of spearmint (*Mentha spicata* L.)," *The Open Horticulture Journal*, vol. 3, no. 1, pp. 31 - 35, 2010.

[7] T. Tetsumura, et al., "Evaluation of basal media for micropropagation of four highbush blueberry cultivars," *Sci Hort Journal*, vol. 4, no. 72, pp. 119, 2008.

[8] P. Purwanto, A. S. D. Purwantono, and S. Mardin, "Modification of MS media and treatment with the addition of coconut water to grow potato plant explants," *Agrin Journal of Agricultural Research*, vol. 11, no. 1, pp. 36 - 42, 2007.

[9] T. Setiawati, A. Zahra, R. Budiono, and M. Nurzaman, "In vitro propagation of potato plants var. granola with the addition of meta-topoline in murashige and skoog modified media," *Journal of Metamorphosis*, vol. 5, no. 1, pp. 44 – 40, 2018.

[10] Firmawati and A. D. Kerti, "*Modification of MS media by adding a concentration of ZPT Benzyl Adenine (BA) on the growth of potato plantlets (Solanum tuberosum L.) in vitro",* Thesis - University of Muhammadiyah Malang, Malang, 2018. [Online]. Available: https://eprints.umm.ac.id/41506/ (accessed March 23, 2023).

[11] Romodhon, S. "*The effect of various planting media on acclimatization orchid (Dendrobium sp.),"* Repository – Universits Medan Area, Medan, 2017. [Online]. Available: https://repositori.uma.ac.id/jspui/handle/123456789/9178 (accessed October, 03, 2024).

[12] L. A. M. Siregar, I. Safni, S. Andriani, and S. P. Hernosa, "Production of microshoots using the temporary immersion system in potato cultivars," *Sabrao Journal of Breeding and Genetics*, vol. 55, no. 6, pp. 2227 – 2238, 2023.

[13] U. Siron, N. Noertjahyani, Y. Taryana, and R. Romiyadi, "Effect of concentration of growth regulators naphthalene acetic acid and benzyl amino purine on the growth of dendrobium spetabile orchid protocorms on cultivars in vitro," *Paspalum Agricultural Scientific Journal*, vol. 7, no. 1, pp. 16 – 23, 2019.

[14] Y. S. Hidayat, D. Efendi, and Sulassih, "Morphological characteristics of several genotypes of potato (*Solanum tuberosum* L.) cultivated in Indonesia," *Comm. Horticulturae J.*, vol. 2, no. 1, pp. 28 - 34, 2018.

[15] A. U. Husna, L. A. M. Siregar, and Y. Husni, "Growth and development of potato nodes (*Solanum tuberosum* L.) due to modification of sucrose concentration and addition of 2-isopentenyladenine in vitro," *Online Journal of Agroecotechnology*, vol. 2, no. 3, pp. 997 – 1003, 2014.

[16] S. Sonnewald and U. Sonnewald, "Regulation of potato tuber sprouting," *Planta*, vol. 239, no. 1, pp. 27 – 38, 2014.

[17] M. Ulfa, Elimasni, Sofyan, and M. Zaidun, "Production of micro tubers in potato plants (*Solanum tuberosum* L.) granola variety with the addition of young coconut water," 2017. [Online]. Available: http://repositori.usu.ac.id/handle/123456789/6166 (accessed March 25, 2023).

[18] D. Z. Zelelew, L. Sewa, T. K. Tesfai, and M. G. Biniam, "Effect of postassium levels on growth and productivity of potato varieties," *American Journal of Plant Science*, vol. 7, no. 12, pp. 1629 – 1638, 2016.

[19] A. N. Perez, E. Jimenez, D. Feria, A. Capote, R. Barbon, E. Quiala, and M. Chavez, "Effect of inoculum density and immersion time on production of potato microtubers in temporary immertion systems and field studies," *Articulo Cientifico*, no. 3, 2007.

[20] L. Turnip, *"The Effect of Tuber Weight and Time of Cytokinin Soaking Treatment on Breaking Dormancy of Potato Seeds (Solanum tuberosum* L.*),*" Thesis - University of North Sumatra, Faculty of Agriculture, Master of Agrotechnology Program, Medan, 2019. [Online]. Available: https://repositori.usu.ac.id/handle/123456789/26651 (accessed April 01, 2023).

[21] A. Aslan and J. Iqbal, "Combined effect of cytokinin and sucrose on in vitro tuberization parameters of two cultivars diamant and red norland of potato (*Solanum tuberosum* L.)," *Sir. J. Bot*, vol. 42, no. 2, pp. 1093 – 1102, 2010.

[22] R. Sembiring, M. Hayati, and E. Kesumawati, "Potato tuber (*Solanum tuberossum* L.) formation due to the application of different concentrations of coconut water in vitro," *Earth Environ Sci*, vol. 7, no. 11, pp. 1755-1315, 2020.

[23] C. Lubis, *"Production of Micro Tubers of Potato Plants (Solanum tuberossum* L.*) Granola Variety by Planting Young Corn Extract,"* Thesis - University of North Sumatra, Faculty of Sains, Master of Biology Study Program, Medan, 2018. [Online]. Available: https://repositori.usu.ac.id/handle/123456789/6168 (accessed March 25, 2023).