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# MICROPROPAGATION OF POTATO (Solanum tuberosum L.) cv. GRANOLA IN LIQUID MEDIUM USING AERATION SYSTEM FOR G0 SEED PRODUCTION

# Perbanyakan *in vitro* tanaman kentang (*Solanum tuberosum* L.) cv. Granola pada media cair menggunakan sistem aerasi untuk produksi benih G0

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#### ABSTRACT

Disease-free potato seeds of high quality can be obtained via in vitro culture. The use of liquid medium during in vitro cultures might boost the number of plantlets produced, however, the problem of hyperhydricity in plantlets was often encountered. This study aimed to investigate effects of different sucrose concentrations and application of aeration system on micropropagation of potato cv. Granola using liquid medium. Aseptic nodal explants with 3-4 nodes from established in vitro cultures were subjected to MS liquid medium with a factorial treatment of three sucrose concentrations (0, 7.5, 15 g. L<sup>-1</sup>) and two culture (with and without aeration). The results showed that MS medium with 7.5 g L<sup>-1</sup> sucrose was the best medium to produce the highest number of shoots and nodes. Furthermore, it was found that application of aeration system in MS liquid culture decreased plantlet hyperhydricity and increased the number of shoots, number nodes, plantlet height, as well as improved plantlet morphology and vigor. Application of the aeration system in liquid medium produced 200-230 new potato plants per bioreactor in the acclimatization stage and an average of 2773.5 G0 mini tubers.

Keywords: Coconut water, micro tuber, multiplication, potato seed, sucrose

#### ABSTRAK

Benih kentang bebas penyakit berkualitas tinggi dapat diperoleh dari kultur *in vitro*. Penggunaan media cair selama kultur *in vitro* dapat meningkatkan jumlah planlet yang dihasilkan, namun masalah hiperhidrisitas plantlet masih sering dijumpai. Penelitian ini bertujuan mengetahui pengaruh perbedaan konsentrasi sukrosa dan penerapan sistem aerasi terhadap perbanyakan kentang cv. Granola menggunakan media cair. Eksplan dengan 3-4 nodus dari kultur *in vitro* ditumbuhkan pada media cair MS dengan perlakuan faktorial, faktor pertama: tiga tingkat konsentrasi sukrosa (0, 7,5, 15 g. L<sup>-1</sup>) dan faktor kedua: dua sistem kultur (dengan dan tanpa aplikasi aerasi). Hasil menunjukkan bahwa media MS dengan 7,5 g. L-1 sukrosa merupakan media terbaik untuk menghasilkan jumlah tunas dan nodus terbanyak. Selanjutnya ditemukan bahwa penerapan sistem aerasi pada kultur cair MS menurunkan hiperhidrisitas plantlet dan meningkatkan jumlah tunas, jumlah buku, tinggi plantlet, serta memperbaiki morfologi dan vigor plantlet. Penerapan sistem aerasi pada media cair menghasilkan 200-230 tanaman kentang baru per bioreaktor dalam tahap aklimatisasi dan rata-rata 2773,5 G0 umbi mini.

Kata Kunci: Air kelapa, benih kentang, multiplikasi, sucrose, umbi mikro

#### INTRODUCTION

Potatoes are one of the horticultural crops that are used as the main source of non-rice carbohydrate food in Indonesia. There are many types of potato based on the morphology of the leaves (Oves et al. 2021). Compared to other cultivars, potato cv. Granola is more popular and preferred to be consumed by Indonesian people, which then encouraging many farmers to cultivate Granola. In addition, the potential yield of Granola is higher than other potato cultivar such as Atlantic. Granola potato yield reaches 30-35 tons/ha with a relatively short planting period (80 days), whereas the cultivar Atlantic has an average yield of only 8-20 tons/ha with a longer planting period, i.e. around 100 days (BPTP 2014).

The decline in potato production was mostly caused by crop failure due to viral infection (Charkowski et al. 2020), which could be caused by the use of tuber seeds from the previous harvest (Aighewi et al. 2015). This problem can be prevented by using tissue culture-derived virus-free seeds through meristem culture (Mohapatra and combination Batra 2017). The of chemotherapy thermotherapy and techniques can help eliminate potato culture viruses (Bamberg et al. 2016). Virus-free potatoes can be produced by combining meristem culture techniques with virus-free testing techniques using the enzyme-linked immunosorbent assay (ELISA) or molecular method (Gong et al. 2019, Zhang et al. 2019). Other advantages of potato seed production through tissue culture, are that this method enables the production of healthy and superior quality true-to-type seeds, a large number of seeds in a shorter time, and without the need for a large production area (Sugihono and Hasbianto 2014, Forbes et al. 2020). The addition of 6benzylamino purine (BAP) in culture media can help maximize shoot propagation and support seed production (Cheng et al. 2020, Hajare et al. 2021). The use of tissue culture methods for potato production has been successfully carried out previously in a largescale production and the seeds were capable of growing on soils (Kim and Mei 2012, Dessoky et al. 2016, Singh et al. 2019).

Farmers, however, are more interested in using the seeds from secondary tubers because they are cheaper than those from tissue culture, hence lower production costs. Efforts to reduced cost of potato seed production through tissue culture technique can be done by eliminating gelling agent, reducing sucrose concentration and adding natural source of growth regulator such as coconut water to substitute BAP in the medium.

Sucrose concentrations of 5 g. L<sup>-1</sup> had been shown to stimulate explant growth in potato cultivar Granola (Rai et al. 2015). High concentration of sucrose up to 8% stimulated the formation of micro tubers (Hossain et al. 2017, Salem and Hassanein plant 2017). Natural growth-regulating substances such as cytokinin, auxin, gibberellin, and abscisic acid were found in coconut water (Rao and Najam 2016). The coconut for addition of water the replacement of plant growth regulator formation buds stimulated the of by triggering cell division and effectively increased the multiplication of shoots in Atlantic potato (Karyanti et al. 2018); induced formation of adventitious the shoots of Corvlus avellana L. (Prando et al. 2014). and increased height and number of shoots in Tribulus terrestris L. (Akhiriana et al. 2019).

Potato plantlets could be produced using liquid medium. The use of liquid medium in potato culture increased the number of nodes and produce plantlets with more vigor. Potato plantlets easily absorbed nutrients in liquid medium rather than in solid medium (Qureshi et al. 2014). However, the use of this culture system caused inhibition exchange. resulting of air in low multiplication rates and plant survival rates. Another alternative culture system is to provide an air supply to the medium. The conditions in the aerated system might stimulate cell metabolism so that growth performance becomes more activated and faster. Liquid culture is also used in the production of micro tubers by immersion system (de L Tapia et al. 2018), or with an aeration system (Mamiya et al. 2020). A simple and inexpensive container bioreactor can help produce potato seeds in large quantities and be more economical. Thus,

this study aimed to obtain the optimum culture system and sucrose concentration, for in vitro propagation of potato using liquid media with a simple bioreactor container. The finding of this research can be used to support the government's program to provide disease-free potato seeds.

# MATERIALS AND METHODS

## **Location and Time**

This research was conducted at the Plant Micropropagation Laboratory, Center for Biotechnology, National Research and Innovation Agency, Science and Technology Park, South Tangerang City, Banten, Indonesia. The research was carried out around 2019 January-December.

# Materials

The plant materials used in this study were established potato cv. Granola cultures from the fifth subculture from the plant tissue culture laboratory collection. Aseptic nodal explants with 3-4 nodes were subjected to MS (Murashige and Skoog 1962) basal liquid medium consisted of macro and micro salts, and (in mg. L<sup>-1</sup>) 0.1 thiamine-HCl, 0.5 pyridoxine-HCl, 0.5 nicotinic acid, 2 glycine, 100 myo-inositol, and enriched with 15% coconut water and sucrose at various concentrations. The pH of all media were adjusted to 5.8 prior to being autoclaved for 15 minutes at 121°C.

The experiment was conducted in a completely randomized design with three replications. Treatments were applied in a 3 × 2 factorial arrangement, consisted of three levels of sucrose concentrations (0, 7.5, 15 g.  $L^{-1}$ ) and two culture systems, namely with and without application of aeration. Each experimental unit consisted of 30 explants per culture vessel. Thus, as many as 540 explants were used in this experiment. Culture vessels of 2,000 mL, each of which containing 200 mL of liquid medium were used in this experiment. All cultures were maintained in a culture room with illuminance from cool-white fluorescent liaht of approximately 1500 lux, with a photoperiod of 16 hours of light and 8 hours of darkness per day and at temperature of  $25 - 26 \pm 2^{\circ}C$ .

## **Observation and statistical analysis**

Observations were made weekly, and in the 4<sup>th</sup> week after subculturing, the plantlets were removed from the culture container. Then quantitative measurements were carried out on the number of shoots produced per explant, the number of nodes produced per plantlet, the multiplication rate, and the length of the plantlets. The data were statistically analyzed with a 5% significance level using the two-way analysis of variance. Further tests were carried out using the Duncan Multiple Range Test at a 5% significance level. Data analysis was performed using the XL Stat statistical program.

# **Production using bioreactors**

A bioreactor is prepared from a bottle with a volume of 2,000 mL, equipped with a 0.022 µm hose and filter. Each bioreactor bottle is filled with the same basal MS liquid medium with 7.5 g. L<sup>-1</sup> sucrose and enriched with 15% coconut water, at a volume of 200 mL/Liter. The explants were planted aseptically, twenty explants were planted per bottle. Each explant consisted of three nodes. The bioreactor was grown in a culture room at 26 ± 2°C, with a light intensity of approximately 1500 lux, with a photoperiod at 16 hours of light and 8 hours of darkness per day. Incubation was carried out for 4 weeks, and then the plantlets could be acclimatized.

### Acclimatization

Acclimatization was carried out in the District of Pangalengan, Bandung Regency, West Java Province. The acclimatization media consisted of rice husks charcoal and sterile cocopeat (1:1). The plantlets were cut into pieces of two nodes and soaked in a fungicide solution at of 2 g. L<sup>-1</sup> for 2 minutes. The plantlet cuttings were planted carefully and were grown in a plastic hood for 2 weeks. Cutting maintenance was carried out for 4 weeks by watering and fertilizing. The potato seeds were ready to be planted after 6 weeks at *ex-vitro* condition and can be used as a source of plant materials to produce G0 potato tubers.

#### Production of mini tubers (G0)

The production of G0 tubers was carried out in a greenhouse. The planting media consisted of sterile cocopeat and sterile rice husks charcoal. A planting rack of 6 meters  $\times$  1 meter in size was prepared. The potato seeds were planted with a spacing of 10 cm  $\times$  10 cm. Watering is done in the morning and evening by misting. In addition, fertilization is done regularly once a week. G0 potato tubers were harvested after the plants were 4 months old.

#### **RESULTS AND DISCUSSION**

#### **Plantlet morphology**

The morphological observations of potato culture without aeration in the third week of culture showed hyperhydricity in many shoots. Hyperhydricity is а morphological and physiological disorder found in plants cultured in vitro with transparent, glassy, or translucency appearance of the stems leaves and short internodes (Figure 1).

The abnormalities mentioned above were common for *in vitro* plantlets (Yaseen et al. 2013). Hyperhydrated plantlets are easily damaged because they contain a lot of water. These abnormal plantlets often fail to be acclimatized. This result was different from that of the aerated cultures, in which the plantlets grew more vigorously and normally. The occurrence of plantlet hyperhydricity *in vitro* can be reduced by applying aeration to the environment and culture (Bhatia and Sharma 2015).

Apart from plantlet morphology, leaf morphology was also observed. In this study, two types of potato leaves were found, namely fully round leaves (Figure 2A) and compound leaves (Figure 2B). The leaf shapes of potato plantlets could be used as an indicator of the physiological state of the culture. The physiological state of the culture would be related to the criteria for plantlets that could be continued as a source of propagule stocks in seed production at the ex vitro stage. The rounded leaf shape indicated that the plantlet was at juvenile stage, whereas the compound leaf shape suggested the plantlet were mature or had aging. experienced The plantlets recommended as a source of propagule stocks for the ex vitro stage were those with rounded leaves. According to a previous

study (Karjadi 2014), the propagation of potato plants by stem cuttings (*ex vitro*) would be improved if the initial source of stocks were harvested from young (juvenile) plantlets. Based on the results of the culture observations in the liquid medium, rounded leaves were produced at 2-4 weeks of culture. After that, the plantlets would age and produced compound leaves.

The morphology of plantlets in the culture system with and without aeration had significant differences (Figure 3). In aerated culture, plantlets were significantly more vigorous (Figure 3A) compared to those in



Figure 1. Morphology of potato plantlets with hyperhydricity



**Figure 2.** Morphology of juvenile leaves (A) and mature leaves (B. The horizontal line below the sample image shows the 1 cm scale



Figure 3. Plantlet morphology at 4 weeks of culture in the aerated system (A) and non-aerated system (B). The white horizontal lines show the 1-cm scale

the non-aerated system, in which the plantlets looked vitrified (Figure 3B). The use of cultures without aeration in a sealed container potentially also increased the hyperhydricity because it increased humidity (Hassankhah et al. 2014).

previous study А reported the comparison of the use of solid and liquid media on the growth of three cultivars of potato plantlets from Kenya. The results showed that the use of liquid media gave more roots, and more leaves per plantlet segment compared to those in solid media. Root formation of plantlets in liquid media was thought to have originated from the ease with which the roots penetrated the liquid medium compared to the solid media. Thus, faster plantlet growth occurred in liquid media (Mbiyu et al. 2012).

More adventitious root formation was found in the plantlets grown in the nonaerated system (Figure 3B) than in the aerated system (Figure 3A). Adventitious roots often formed in plants under stressed because the environment does not support their growth, such as when the plants are submerged in water. When the plants are submerged in water, air diffuses into the plantlets at slower rate than when the plants are not immersed in water. The lower oxygen availability in the plant tissue would stimulate the formation of adventitious roots (Steffens and Rasmussen 2016).

#### Number of nodes

Explants were subcultured in liquid media using the nodal section. All parts of the upper, middle and lower nodes give the same growth response (Rai et al. 2012). Explants subjected to the liquid medium were nodal segments from established All parts of the nodes used as cultures. explants, namely the top, middle and lower parts, showed similar growth responses. The use of different culture systems as treatments in this study resulted in significant differences in the number of nodes However. produced. different sucrose concentrations and interactions between the two factors had no significant effect. The average number of nodes in the aerated system (711.56) was higher and significantly different than that without aeration (286.89) (Table 1).

In the culture system without aeration,

**Table 1.** The number of nodes in potato shoot multiplication in two culture systems with different sugar concentrations at four weeks of culture

Treatment	Aeration (S1)	Non-Aeration (S2)	Average
G1 (0 g L <sup>-1</sup> )	693	214	453.5
G2 (7.5 g L <sup>-1</sup> )	844	308	576
G3 (15 g L <sup>-1</sup> )	597.67	338.67	468.17
Average	711.56ª	286.89 <sup>b</sup>	

Note: Numbers followed by the same letter in the same column and row show no significant difference at the 5% level based on the Duncan's Multiple Range Test (DMRT)

Table 2. Potato node multiplication	rates in two in	vitro culture systems
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Treatments		Number of Nodes	Multiplication Rates (times)
System	Sucrose Concentrations (gL <sup>-1</sup> )	(after 4 weeks of culture)	
Aeration	0	693	6.93
	7.5	844	8.44
	15	598	5.98
Non-Aeration	0	214	5.35
	7.5	308	7.7
	15	339	8.45



Figure 4. Internode distance of potato plantlets in aerated system with 7.5 g L<sup>-1</sup> sucrose (A), and non-aerated system with 15 g L<sup>-1</sup> sucrose (B)

explant growth was slower than in the aerated system. The growth inhibition of explant in the non-aerated system was probably due to the presence of ethylene gas. The gas was one of the products of the explant metabolism which accumulated or was trapped in the culture environment due to the absence of an air exchange facility in the culture system (Cournac et al. 1991).

The multiplication rate of nodes is important for the production of plantlets as a source of propagule stock. Increased yield of plantlets through aerated systems can support G0 seed production and reduce production costs. The multiplication rate was obtained by dividing the number of nodes in the fourth week of culture with the number of initial explants used. The mean potato node multiplication rate in each treatment at four weeks of culture was 5-8.5 times more than the initial node planted (Table 2). The optimum treatments that increased the multiplication rate by 8.44 and 8.45 times were the aerated system supplemented with sucrose  $(7.5 \text{ g.L}^{-1})$  and the non-aerated system with the addition of sucrose (15 g. L<sup>-</sup> <sup>1</sup>), respectively.

The use of the aerated system reduced the amount of sucrose supplemented in the medium. It appeared that the plantlets used the sucrose more effectively. These results indicated that the culture media not only needs nutrients and vitamins but also carbohydrates, which were obtained from the sucrose added. Sucrose substitutes the carbon source usually obtained by plants from the air in the form of  $CO_2$ .

Although the two treatment combinations could achieve the optimum multiplication rate, the morphology formed between the two treatment combinations was significantly different. In the aerated system, sucrose addition at a 7.5 g. L<sup>-1</sup> concentration level produced an internode distance of about 3 cm, which was approximately twice as long as that of the non-aerated system with 15 g. L<sup>-1</sup> sucrose, which was 1.5 cm (Figure 4).

The longer distance between nodes was desired because it would enable more seeds to be produced (Rai et al. 2012) since it facilitated the micro-cutting techniques. Micro-cuttings at a distance further away from the growing point would ease the acclimatization as it would prevent the axillary from being buried or covered by the acclimatization media, thus inhibiting the growth of lateral shoots from nodal explants.

#### Number of shoots

The shoot formation from nodal explants was significantly influenced by the use of different culture systems and sucrose concentrations. However, there was no significant interaction between the two factors (Table 3). The average number of lateral shoots produced by the aerated system (72.22 shoots) was significantly different compared to those without aeration (30.78 shoots) (Table 3). Addition of sucrose at 7.5 g. L<sup>-1</sup> produced the highest number of shoot buds (68.63). The number of shoots at 7.5 g. L<sup>-1</sup> sucrose was significantly different from that of the control treatment without

 Table 3. Number of potato shoots in two culture systems with different sucrose concentrations at four weeks of culture

Treatment	Aeration (S1)	Non-Aeration (S2)	Average
G1 (0 g L <sup>-1</sup> )	55.33 <sup>b</sup>	17 <sup>c</sup>	36.17 <sup>b</sup>
G2 (7.5 g L <sup>-1</sup> )	101.67 <sup>a</sup>	36 <sup>bc</sup>	68.83 <sup>a</sup>
G3 (15 g L <sup>-1</sup> )	59.67 <sup>b</sup>	39.33 <sup>bc</sup>	49.5 <sup>ab</sup>
Average	72.22ª	30.78 <sup>b</sup>	

Note: Numbers followed by the same letter in the same column and row show no significant difference at the 5% level based on the Duncan's Multiple Range Test (DMRT)

sucrose (36.17 shoots). However, the figure was not significantly different from that of medium with 15 g. L<sup>-1</sup> sucrose (49.5 shoots) (Table 3).

The presence of sucrose as a carbon source in the in vitro growing media was used by explants to form fully-developed Sucrose can increase starch organs. accumulation in plastids in the formation of shoots and roots in vitro culture (Saji and Sujatha 1998). The number of shoots produced by the 7.5 and 15 g. L<sup>-1</sup> sucrose treatments was not significantly different (Table 3). Both treatments contained a significant amount of sucrose relative to the control (0 g. L<sup>-1</sup> sucrose). The high sucrose concentration in the culture media helped the explant tissue obtain sufficient nutrients, supporting faster multiplication of the plant cells (Ni'mah et al. 2012).

The non-aerated system with 15 g. L<sup>-1</sup> sucrose might have provide the optimum conditions to increase the number of shoots. In contrast, the aerated system's optimum sucrose concentration was 7.5 g. L<sup>-1</sup>. This showed that the presence of aeration in the culture system could change the character of the medium so that it would also affect the growth of plantlets. The use of an aerated system lowered the sucrose concentration needed for optimal results (Mohamed and Alsadan 2010). This would be beneficial because it would reduce the production cost of potato plantlets. In another study, high  $CO_2$  concentration (1.000 µmol. mol<sup>-1</sup>) without sucrose addition in the culture medium was shown to be better for the growth and development of internode numbers, tissue moisture content, leaf length, leaf width, and total chlorophyll content compared to other treatments (Park et al. 2018). The resulting proliferation did not require the addition of synthetic growth regulators, so this medium was is cheaper. The use of organic hormones such as coconut water can help increase the number and diameter of potato shoots. These results are in accordance with the research reported by Sembiring et al. (2021). Other studies related to potato propagation require the addition of BAP hormones and vitamins (Salem and Hassanein 2017, Kazemiani et al. 2018). Optimal media with the addition of 4 mg. L<sup>-1</sup> BAP, mg. L<sup>-1</sup> IAA and 1 mg. L<sup>-1</sup> GA<sub>3</sub> can induce micro tubers (Borna et al. 2019). The use of plant growth regulators increases production costs and will have an impact on seed costs.

# **Plantlet length**

Results of the experiment showed that the use of aeration in the culture system significantly increased plantlet height. Meanwhile, the different sucrose concentrations and the interaction between the two factors did not have a significant effect on the height increase of potato plantlets.

The use of the aerated system resulted in significantly higher plantlet lengths (14.35 cm) compared to the non-aerated system (9.47 cm) (Table 4). The use of different concentrations of sucrose did not give significantly different results on the plantlet length parameters (Table 4). In the nonaerated treatment, the increase in sucrose concentration triggered the increase in plant length, but this was not the case with those treated with aeration.

These results indicated that the use of an aerated system did not require a high concentration of sucrose in comparison with the non-aerated system. The use of aeration and an appropriate concentration of sucrose could increase the plantlet height and produce more vigor morphology on the

**Table 4.** Plantlet lengths on potato shoot multiplication in two culture systems with different sucrose concentrations at four weeks of culture

Treatment	Aeration (S1)	Non-Aeration (S2)	Average
G1 (0 g/l)	14.3ª	9.53 <sup>b</sup>	11.93ª
G2 (7.5 g/l)	14.49 <sup>a</sup>	8.62 <sup>b</sup>	11.55ª
G3 (15 g/l)	14.27 <sup>a</sup>	10.27 <sup>b</sup>	12.24 <sup>a</sup>
Average	14.35 <sup>a</sup>	9.47 <sup>b</sup>	

Note: Numbers followed by the same letter in the same column and row show no significant difference at the 5% level based on Duncan's Multiple Range Test (DMRT)



Figure 5 Production of potato plantlets using an aeration system



Figure 6. Production of mini tuber potatoes, a. Potato seeds, b. Potato tuber production in Green House, c, mini tubers

plantlets compared to those without aeration.

#### Potato plantlet production

In order to prove the results of the studies that have been carried out, the next step is to test the production of potato granola plantlets. Potato plantlet production is carried out using a bioreactor container with an aeration system. Optimum use of culture media from the results of studies that have been carried out using modified MS basal media with the addition of 150 mL. L<sup>-1</sup> coconut water and 7.5 g. L<sup>-1</sup> sucrose, the culture media was able to produce optimal shoot growth. Potato explants propagated in the bioreactor were virus-free and certified cultures. Potato plantlet production only takes about 25 days and planlets were ready

to be acclimatized (Figure 5). Every single container of the bioreactor-produced new plantlets that were ready to be acclimatized. This innovation can help speed up the production of potato plantlets in large quantities, more economically with easy application.

One container of the bioreactor could produce about 200 - 230 potato seeds in the acclimatization stage. The application of planting techniques in liquid culture media is an important factor in the success of the growth and development of explants to produce vigorous plantlets. Potato plantlets grown with an aeration system have stronger stems, so that the percentage of mortality in the acclimatization stage is very low. Potato plants produced in vitro using axillary shoot

Table 5. Average number of mini tubers from the 1st, 2nd and 3rd plantings

Container	1 <sup>st</sup> plantings	2 <sup>nd</sup> plantings	3 <sup>rd</sup> plantings
	Number of Tubers	Number of Tubers	Number of Tubers
Container 1	2506	2701	3004
Container 2	3040	2452	2450
Container 3	2445	3022	2841
Container 4	2602	3155	2655
Container 5	3102	2876	2451
Average	2739	2841.2	2740.2

proliferation techniques, microtubes, or somatic embryogenesis was not found to have phenotypic differences (Sharma et al. 2007).

# Mini tuber production

Production of disease-free potato plantlets using a bioreactor system and mini tuber production is one of the best propagation alternatives. Mini tubers can be produced in large quantities, and are easy to store, and have a longer shelf life (Hossain et al. 2017). Plantlets produced by the aeration system have a survival rate of about 98% at the acclimatization stage. It produces uniform, disease-free potato seeds in large quantities. The production of G0 tubers was carried out under controlled environmental conditions and growing media (Figure 6). The greenhouse was maintained in environmental conditions to prevent contamination or the spread of disease. Potato seeds were planted carefully. Maintenance was carried out for four months until mini tubers (G0) were produced. One container of bioreactor could produce tubers (G0) of around 2773.5 (Table 5). Virus-free potato seeds produced using tissue culture has broad prospects to support the availability of quality seeds in various countries (Singh et al. 2019). The mini tuber production method can also be done aeroponically. According to Tierno et al. 2014 the production of mini tubers with an aeroponics system can increase by 60 - 80% from the commonly used system and the tuber size is more uniform. According to Caliskan et al. 2021, the use of an aeroponics system in the production of potato mini tubers could produce about 787 – 1168 tubers.m<sup>-2</sup>, an increase of 2 - 4 times compared to conventional planting. Mini tuber production was influenced bv environmental conditions, both temperature and humidity, that affect stress (Oraby et al. 2015, Kuncoro et al. 2021). The combination of in vitro potato propagation using a bioreactor could be combined with an aeroponics system to increase the production of mini tubers and be more economical.

# CONCLUSION

Plantlet production of potato cv. Granola with vigorous morphology,

increased number of nodes, and plantlet length was optimally achieved in a liquid culture of MS medium with the aerated system and sucrose concentration of 7.5 g. L<sup>-1</sup>. This liquid culture method would enable the production of potato plantlets at a lower cost than that using a solid culture medium. Each bioreactor aerated 844 nodes and produced 200-230 new seeds, and produced an average of 2773.5 mini tubers.

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