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PRODUCTION OF MICROSHOOT USING THE TEMPORARY IMMERSION SYSTEM (TIS) IN POTATO CULTIVARS

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SUMMARY

The Temporary Immersion System (TIS) Bioreactor's performance in cultivation for several commercial potato cultivars became the prevailing study's review focus. The experiment in a split-plot design had two factors. The first factor comprised the type of culture system (conventional and TIS Bioreactor) treatment used as main plots. The second factor was the four potato cultivars (Atlantic Malang, Dayang Sumbi, Granola L., and Maglia) used as subplots. The TIS Bioreactor culture has the highest average in all studied variables compared with conventional cultures at the multiplication stage. In the TIS Bioreactor, potato cultivar Dayang Sumbi excelled in the number of axillary branching and number of primary roots. At the same time, genotype Granola L. surpassed the height of the plantlets of other cultivars. Meanwhile, the cultivar Atlantic Malang shone in the number of nodes, with Maglia in the number of leaves. Using the TIS Bioreactor produces microshoots with more nodes and can be beneficial as a propagation organ. The Dayang Sumbi cultivar was superior to all other potato cultivars.

Keywords: Potato (*Solanum tuberosum* L.), cultivars, conventional *in vitro*, TIS Bioreactor, microshoots, axillary shoots, number of nodes

Key findings: The Temporary Immersion System (TIS) Bioreactor can be advantageous as an alternative to the cultivation system to maximize the productivity of plantlets and microtubers of several potato cultivars, which is beneficial. The success of increasing the axillary branching and the number of internodes through the TIS system will encourage an increase in the production of microshoots as one of the primary seed sources.

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INTRODUCTION

The potato (*Solanum tuberosum* L.) is one of the most valuable root vegetable crops and ranks fourth as a food staple, after corn, rice, and wheat. Its global production is approximately 300 million tons yearly, increasing steadily (Jennings *et al.*, 2020). It grows in a temperate climate for use in the food and starch industries (Kurek *et al.*, 2016). Potatoes are the third most popular plant serving as humans' staple food worldwide (Alamar *et al.*, 2017; Chitchumroonchokchai *et al.*, 2017).

The limited availability of certified and quality potato seed stock, accounting for 10% of the nation's total demand for certified potato seed, is the prime cause of the country's low potato yield (Hidayat, 2011). The Indonesia Vegetable Crops Research Institute in West Java, Indonesia, has successfully released several high-yielding potato cultivars. However, until now, cultivar Granola, with the early-maturation category, still dominates 90% of the total domestic potato planting area.

In vitro technology is an alternative method for producing certified pathogen-free seeds in the potato seed industry. In Indonesia, the seed sources come from the G0 (generation zero) plantlets and tubers. The multiplication of high-quality potato seeds is a one-generation flow pattern, with vegetative propagation using tubers or cuttings as seeds. The propagation pattern could begin with the breeder seeds' (BS) production via in vitro initiation to produce micro cuttings or microtubers. Then, the generated breeder seeds attain planting to produce foundation seed (FS/G0). The foundation seeds sustain replanting to create seed stock (SS/G1), which again proceeds to sow for further development of the spores (ES/G2) (Directorate of Horticultural Seeds, 2014).

In vitro micropropagation, potato typically serves many uses for new cultivars and breeding lines, germplasm storage, transportation, and production of minitubers that are simple to store, transport, distribute, and produce disease-free crops in a short

period throughout the year (Karjadi, 2016). In the in vitro propagation of potatoes, the meristem tissue can be a convenient source of propagules in the form of an apical shoot, nodal cutting, and microtuber, with one or two primordial leaves (Xhulaj and Gixhari, 2018).

Successful micropropagation of potatoes has been using various types of explants, such as tuber buds (Xhulaj and Gixhari, 2018), lateral buds (Hajare *et al.*, 2021; Yancheva and Kondakova, 2016), and many other different plant parts (De-Morais *et al.*, 2018). Therefore, it is now an effective way of multiplying new and existing cultivars under disease-free conditions. These micropropagated potato plants' production can be via nodal cuttings, utilized directly to produce microtubers. According to past studies on various plant species, the explants' growth incurs influences from diverse factors, including genotype makeup, media composition, and growth nature under laboratory conditions (Naresh *et al.*, 2011).

Mass propagation of potato seeds can proceed using a bioreactor system approach, such as the Temporary Immersion System (TIS). The TIS is an alternative culture system for enhancing the multiplication of buds and microtubers. The temporary immersion system is a widely used micropropagation enhancer of numerous crop plants (Othmani *et al.*, 2017). Its successful application for large-scale propagation has existed in various crop plants (Kunakhonnuruk *et al.*, 2019). It is an option for enhancing biological performance, facilitating semi-automated micropropagation, and decreasing production costs (Xhulaj and Gixhari, 2018). The successful TIS Bioreactor application has aided in cultivating various potential crops and conserving spheres. In addition, TIS has proven to provide opportunities for different crop plants' mass propagation in a relatively short time at a low cost.

Micro shoots, used as seeds, can also be a stimulant to produce microtubers through the TIS. The TIS has the potential to produce several G0 tubers equivalent to the yield of prime plantlet seeds, with large tubers and

high biomass (Gautam *et al.*, 2021). Hence, the objective of the presented study was to evaluate the potential of micro shoot multiplication of several existing superior cultivars using the Temporary Immersion System approach.

MATERIALS AND METHODS

The present research ran from September 2020 to March 2021 at the Tissue Culture Laboratory PT. Hijau Surya Biotechindo, West Kisaran, Asahan Regency, North Sumatra, Indonesia.

The materials and tools

The plant material used were four potato cultivars (Atlantic Malang, Dayang Sumbi, Granola L., and Maglia) obtained from the Tissue Culture Laboratory of the Indonesian Vegetable Crops Research Institute, West Java, Indonesia. The potato micro shoot culture's maintenance ensued in subculturing on an MS medium (Murashige and Skoog, 1962) containing no growth regulators. In this case, the middle and upper node segments resulted in a better explanatory height, wider leaf size, and sturdier rooting pattern (Rai *et al.*, 2012). The culture media material used is a stock solution of MS basic media, a widely utilized specific planting medium with a high content of nitrates, potassium, and ammonium.

The culture preparation tools included analytical scales, rulers, gas stoves, glass stirrers, hollow term, autoclaves, measuring cups, volume pipettes, and pH meters. The tools used in the explants' planting process included a Laminar Air Flow Cabinet (LAFB), petri dishes, Bunsen, test tube sized 2.4 cm × 14.6 cm, a glass culture bottle with a capacity of 250 ml, medium vessel (4000 ml) and culture vessel (5600 ml) TIS (SETIS™ platform), tweezers, scalpel handles, sprayers, scalpel knives No. 11, and matches. However, at the incubation stage, the tools used included bioreactor pumps, four fluorescent lamp pieces (20 watts), air conditioners, culture racks, black fabrics, and label paper.

Experimental design

The existing research employed a split-plot design with two factors. The first factor comprised two types of culture systems, TIS and the conventional semi-solid culture system, used as main plots. The second factor as subplots were the explants from four potato cultivars, Atlantic Malang, Dayang Sumbi, Granola L., and Maglia. Overall, the study consisted of eight treatment combinations with three repeats; thus, the total experimental units were 24.

Sterilization

The equipments included test tubes, planting tools, glass culture bottles with a capacity of 250 ml, and sterilized TIS Bioreactor vessels. Before entering the sterilization stage, culture bottles and planting tools, such as tweezers, scalpel handles, and Petri dishes, sustained thorough washing with soap under running water. Specifically, TIS vessels, made of transparent polycarbonate, have two compartments: the lower compartment for culture media with a volume of 4000 ml; In contrast, the upper partition accommodates potato explants with a volume of 5600 ml. This TIS Bioreactor includes equipment that can receive sterilization using an autoclave, with the filter and pipe sections of the TIS Bioreactor wrapped in aluminum foil before the sterilization process. Sterilization using an autoclave occurred at 121 °C for 60 min at a pressure of 17.5 psi.

Sterilization also proceeded in the Laminar Air Flow Cabinet (LAFB) as the cultivator's work environment by spraying 70% alcohol on the table surface and LAFB glass walls, then drying using tissue or cotton. Turning on the UV for two hours on the LAFB before use can ensure the complete eradication of bacteria and fungi that cause contamination.

Preparation of the culture media

The medium used in the shoot multiplication stage was a simple medium of Murashige and Skoog (MS) using 30 g/l sucrose. The setting of the media pH was at 5.8 before sterilization.

For the treatment in conventional culture, the medium gained an additional 7 g/l agar. Furthermore, heating the media solution until homogeneous for distribution to 250 ml media bottles amounted to 50 ml, covering the culture bottles with aluminum foil, then tightly tied with a rubber band. The media underwent autoclaving at 121 °C for 30 min with a pressure of 17.5 psi. The autoclaved media's storing and incubating continued in the culture room at 22 °C ± 2 °C for seven days before use.

Shoot multiplication

The explants used were nodal segments of the in vitro cultured plants of the four potato cultivars under study. Maintaining them in test tubes contained 10 ml of gelled MS medium + 30 g/l sucrose + 7 g/l agar (Difco-Bacto), and then incubated in the culture room at 22 °C ± 2 °C with a light intensity of 3000 lux sourced fluorescent lamps. For a conventional culture system, seven nodal segments (1.5–2.0 cm), with a single node each, attained culturing on a 250 ml culture vessel containing 50 ml MS medium with 7 g/l agar. For the TIS, 60 nodal segments acquired refining on a 4000 ml media vessel containing 2000 ml of liquid culture media.

The cultures' incubation in the culture room had continuous light provided from fluorescent tubes with a light intensity of 3000 lux, at 22 °C ± 2 °C temperature. For TIS cultures, the liquid medium immersion achieved regulation with a frequency of eight times/day for 2 min/3 h. This level of immersion was considerably good for the process of shoot multiplication (Perez-Alonso *et al.*, 2007). After three weeks of subculture, data recording commenced on the variables, i.e., number of nodes, axillary branching, primary roots, and leaves and plant height.

Statistical analysis

The evaluation of data obtained used a two-way analysis of variance (ANOVA). The treatments with a significant effect incur further comparison with the Duncan Multiple Range Test (DMRT) at a 5% level.

RESULTS

The culture systems significantly affect the number of axillary shoots in the four potato cultivars at three weeks after culturing. The TIS Bioreactor culture produced an average number of axillary shoots of 1.68 pieces, while in the conventional, the average was only 0.85. The potato cultivars did not show significant differences in amount of axillary buds. However, the interaction between the culture system and the cultivars revealed substantial differences, especially for Dayang Sumbi, Granola L, and Maglia, with the highest number of axillary shoots using the TIS Bioreactor. In the cultivar Atlantic Malang, the TIS Bioreactor and conventional culture systems showed nonsignificant differences (Table 1). The emergence of apical shoots was first visible in Granola L., with the latest to emerge, the cultivar Maglia. However, the apical shoots of all cultivars surfaced within the first week of culturing in the culture medium. Most plantlets produced lateral branches after two weeks of culture using the conventional system, whereas plantlets in TIS horizontal stems came out only after three weeks of culture (Figure 1).

The culture systems significantly affected the average number of potato nodes produced after three weeks of culture. The TIS Bioreactor culture provided the average number of nodes at 9.14, compared with the conventional (7.39). However, the potato cultivars had a nonsignificant impact on the average number of nodes per plant (Table 2). The interaction between the culture systems and the potato cultivars notably affected the average node number. The treatment of different culture systems also influenced the morphology of the internode distances for all the cultivars. Atlantic Malang, Granola L., and Maglia showed more nodes using the TIS Bioreactor than the conventional culture system; however, no difference was apparent for Dayang Sumbi. In general, plantlets in the TIS Bioreactor culture treatment had farther distances between nodes, i.e., one to 3.5 cm, and in conventional culture, the distance was around 0.5 to 1.5 cm (Figure 2).

Table 1. The number of axillary shoots (#) of four potato cultivars using the TIS and conventional culture system three weeks after culture.

Cultural system	Potato Cultivars				Means (#)
	Atlantic Malang	Dayang Sumbi	Granola L.	Maglia	
TIS Bioreactor	1.83 ab	1.89 a	1.39 a-e	1.61 a-c	1.68 a
Conventional	1.56 a-d	0.33 fg	0.53 fg	0.98 d-f	0.85 b
Means (#)	1.70	1.11	0.96	1.30	

Values followed by the same letter in a column or row show no significant difference at the 0.05 level with Duncan's Multiple Range Test.

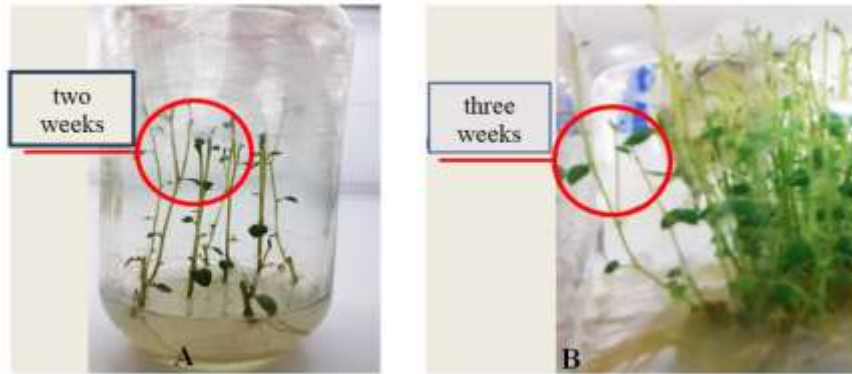


Figure 1. The emergence of lateral shoots in conventional culture (A) and TIS Bioreactor culture (B).

Table 2. The number of nodes (#) in four potato cultivars using the TIS and conventional culture system three weeks after culture.

Cultural System	Potato Cultivars				Means (#)
	Atlantic Malang	Dayang Sumbi	Granola L.	Maglia	
TIS Bioreactor	9.61 a	8.44 bc	9.00 a	9.50 a	9.14 a
Conventional	7.01 c	8.67 ab	6.78 c	7.11 bc	7.39 b
Means (#)	8.31	8.56	7.89	8.31	

Values followed by the same letter in a column or row show no significant difference at the 0.05 level with Duncan's Multiple Range Test.

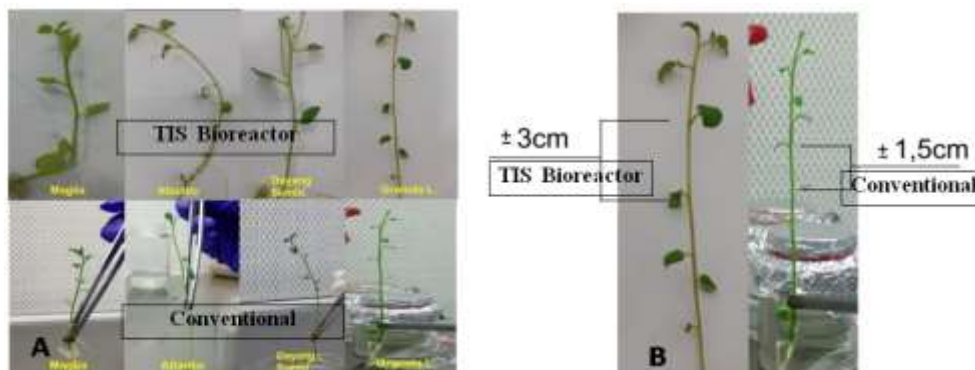


Figure 2. Morphology of the plantlets of four potato cultivars (A), and morphological differences in distance between plantlets' nodes (internodus) aged three weeks after planting in TIS Bioreactor and conventional culture systems (B).

Table 3. Plant height (cm) of four potato cultivars using TIS and conventional culture system three weeks after culture.

Cultural System	Potato Cultivars				Means (cm)
	Atlantic Malang	Dayang Sumbi	Granola L.	Maglia	
TIS Bioreactor	12.64 a-c	13.39 ab	13.83 a	11.09 a-d	12.74 a
Conventional	7.59 e-h	8.27 de	8.25 d-f	6.46 e-h	7.64 b
Means (cm)	10.11	10.83	11.04	8.78	

Values followed by the same letter in a column or row show no significant difference at the 0.05 level with Duncan's Multiple Range Test.

Table 4. The number of primary roots (#) of four potato cultivars using the TIS and conventional culture system three weeks after culture.

Cultural System	Potato Cultivars				Means (#)
	Atlantic Malang	Dayang Sumbi	Granola L.	Maglia	
TIS Bioreactor	5.55 b-d	9.28 a	7.11 a-c	7.39 ab	7.33 a
Conventional	4.68 d	5.44 b-d	4.88 cd	5.11 b-d	5.03 b
Means (#)	5.12 b-d	7.36 a	6.00 a-c	6.25 ab	

Values followed by the same letter in a column or row show no significant difference at the 0.05 level with Duncan's Multiple Range Test.

On average, the culture systems meaningfully impacted plant height after three weeks. In the TIS, the average plant height reached 12.74 cm, while the plantlets' height in conventional culture reached 7.64 cm. However, the potato cultivars showed a nonsignificant difference in the plant height in each culture system (Table 3). The interaction of the two culture systems and potato cultivars presented significant differences in plant height. The potato cultivars Atlantic Malang, Dayang Sumbi, and Granola placed with TIS Bioreactor had extensive differences from the same cultivars when put in the conventional culture. Meanwhile, the potato cultivar Maglia with TIS Bioreactor culture displayed significant differences. Conversely, Atlantic Malang and Maglia cultivars placed in standard culture did not significantly differ from the two other cultivars, Dayang Sumbi and Granola, in the same culture.

The culture systems drastically affected the number of primary plantlet roots within three weeks of culture. In the TIS Bioreactor culture, the average number of primary tubers was 7.33, but in the conventional, the average was 5.03. The potato cultivars Dayang Sumbi and Atlantic Malang showed significant

disparities, whereas others did not show differences for the said trait (Table 4). The interaction between the culture systems and the cultivars also significantly influenced the number of primary roots. The cultivar Dayang Sumbi with TIS Bioreactor remarkably differed from all the conventionally cultured cultivars and with Atlantic Malang in the TIS Bioreactor. However, potato cultivars showed nonsignificant differences with conventional culture.

Morphologically, differences in the primary roots of potato cultivars' plantlets also occurred when cultured with TIS Bioreactor and conventionally. In TIS Bioreactor cultures, the plantlets' root morphology looks thicker than the plantlets' roots derived from conventional cultures. The primary root colors were similar in all treatments; however, greenish-beige dominated (Figure 3). In addition to primary and secondary roots, adventitious ones were 1–5 cm on the aerial part of the plantlets' node in all the treatments. For standard cultures, adventitious roots emerge early, two weeks after planting. In TIS Bioreactors, the adventitious roots surfaced from the plantlets three weeks after planting (Figure 4).

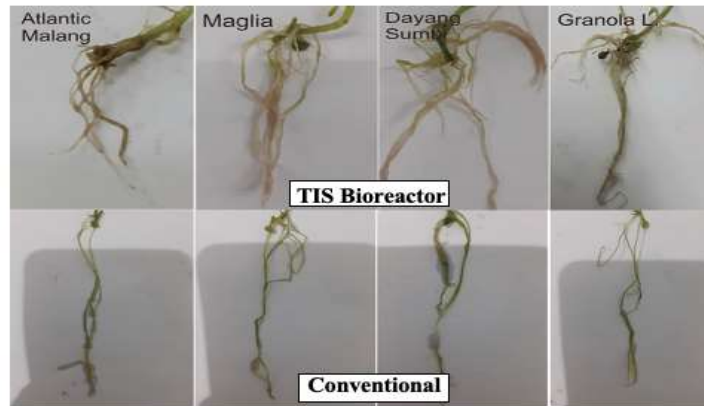


Figure 3. Primary root morphology of the plantlets of four potato cultivars in the TIS Bioreactor and conventional culture systems at three weeks after culturing.

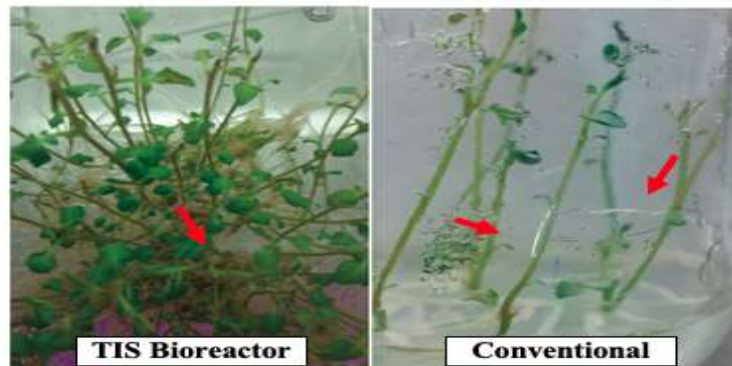


Figure 4. The appearance of adventitious roots in the plantlets at third week after planting in the TIS Bioreactor culture and the plantlets in conventional culture.

The culture systems notably affected the number of plantlet leaves in three weeks. With the TIS Bioreactor, the average number of produced leaves was 8.86 compared with conventional culture (6.39). However, the potato cultivars had nonsignificant impacts on plantlet leaves. The interaction between the culture systems and potato cultivars significantly affected the increase in plantlet leaves. The four cultivars cultured in the TIS Bioreactor did not show any relevant differences with each other. For conventional culture, Dayang Sumbi significantly differed from the cultivar Malang Atlantic (Table 5).

Regarding leaf size, plantlets in TIS Bioreactor culture have broader leaves than conventional ones. However, double or

compound leaves appeared early on the plantlets three weeks after planting. The most dominant double leaves were evident in the cultivar Dayang Sumbi. In standard culture, double leaves generally appear in the plantlets six weeks after planting. Several yellow-looking leaves were also apparent in TIS Bioreactor cultures (Figure 5).

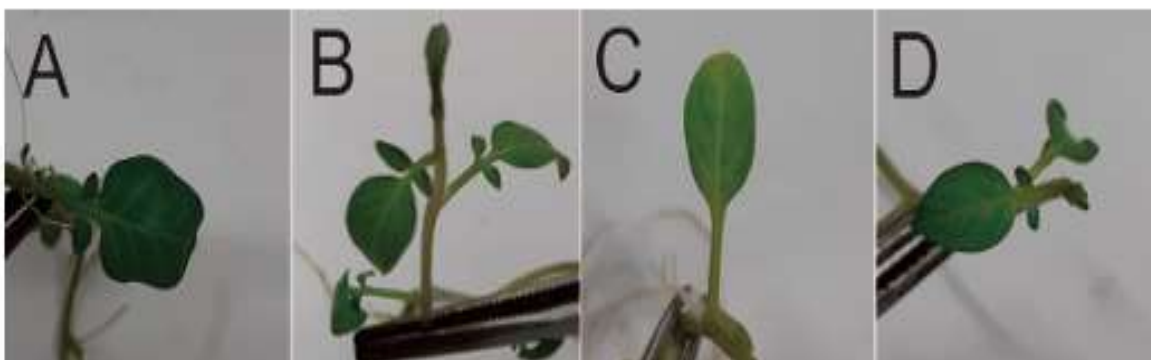
DISCUSSION

Results indicated the vegetative growth of explants of the four potato cultivars differed when cultured using the conventional and Temporary Immersion System (TIS). An increase in axillary branching appeared in TIS

Table 5. The number of leaves (#) of four potato cultivars using the TIS and conventional culture system three weeks after culture.

Cultural System	Potato Cultivars				Means (#)
	Atlantic Malang	Dayang Sumbi	Granola L.	Maglia	
TIS Bioreactor	9.11 a	8.28 a	8.78 a	9.28 a	8.86 a
Conventional	5.79 c	7.67 ab	5.87 bc	6.22 bc	6.39 b
Means (#)	7.45	7.97	7.32	7.75	

Values followed by the same letter in a column or row show no significant difference at the 0.05 level with Duncan's Multiple Range Test.

**Figure 5.** Multiple and compound leaf morphology (A) and (B); leaves with yellowing symptoms (C) and (D) on potato plantlets using with TIS Bioreactor system at three weeks after culturing.

for each potato cultivar compared with the regular culture system. The identical phenomenon occurs when propagating caper shoots (*Capparis spinosa* L.) with TIS. Bioreactors exhibited significant differences in the shoots' number and length, and a higher growth rate was evident compared with a conventional solid medium culture system (Gianguzzi *et al.*, 2019). The study showed that axillary branching emerged in all treatments during the first week of incubation, even though the explants grew on media without adding exogenous growth regulators. It indicates that endogenous phytohormones in explants can naturally stimulate the growth and development of axillary meristems (Yagiz *et al.*, 2020; Haq *et al.*, 2021).

The appearance of lateral branches in most plantlets has strong suspicions of an effect of the influence of the size of the culture container (Aragon *et al.*, 2014). In this case, the earlier emergence of lateral branches in conventional culture might be due to adapting to the conditions of the culture container.

Unlike the case with plantlets in the TIS Bioreactor culture, even with a fast metabolic rate, the size of the culture container can balance, which was taller and broader and equipped with an aeration system, with the plantlets not experiencing stress from lack of oxygen; thus, the initial growth of plantlets focused more on the apical shoots. Earlier works have proven that plantlets in TIS Bioreactor culture have higher stem morphology than plantlets in conventional culture systems (Nurul-Afza *et al.*, 2023).

Nodal explants culture in TIS Bioreactor increased the number of produced nodes. Nodes have a vital role in the growth of axillary shoots as the forerunner to forming buds, stolon, and microtubers, depending on the composition of the media and the growth environment period (Husna *et al.*, 2014). The culture systems not only affect the number of nodes, but also impact the morphology of all the potato cultivars' plantlet internodes. In general, plantlets in the TIS Bioreactor culture had longer internodes than plantlets in

conventional culture systems. The distance between nodes was also one of the determining factors for success in the acclimatization stage using micro cuttings. Cutting plantlets with too close spacing between nodes will bury the nodes in the acclimatization media; hence, the growth of lateral shoots becomes stunted. Therefore, plantlets resulting from multiplication with TIS Bioreactor culture with farther distances between nodes proved more profitable (Karjadi, 2016).

In addition to the influence of the culture systems, the genetic factors were also noteworthy, which affected the cultivar's morphological and physiological characteristics. It is a fact that the cultivar Dayang Sumbi has an average number of nodes almost the same as the cultivar Granola L., as one of its parents. The same thing also happened to the cultivar Maglia, with an average number of nodes comparable to one of its parents, the cultivar Atlantic Malang. On the multiplication rate parameter, the initial number of explants at the time of planting was according to the inoculum density, where too high inoculum density will cause phenotypic malformations and reduce the quality of plant growth. However, the low inoculum density causes sub-utilization of culture space (Perez-Alonso *et al.*, 2007).

Observations related to the multiplication rate demonstrate that explants from four potato cultivars grown in the TIS Bioreactor culture system responded better than those in conventional culture systems, proving that the culture system can significantly affect the number of nodes. The differences were visible when the explants entered 2–3 weeks after culturing. The four potato cultivars in TIS Bioreactor culture showed a faster multiplication rate than in conventional culture. It suggests that the physical properties of different culture media also influence the multiplication process, which is closely related to the capacity of plant tissues to absorb nutrients from the culture medium (Mirzabe *et al.*, 2022).

In the TIS Bioreactor culture, equipped with an aeration system, temporary immersion can provide the plantlets with a better growth

environment. The culture media in liquid form can affect all parts of the plant during provisional immersion for the maximum absorption of nutrients to occur without any signs of hyperhydricity (Ahmadian *et al.*, 2017). In addition, maintaining the homogeneity of the culture media continued by the presence of an aeration system; thus, it significantly enhances the quality of metabolism in plant tissues. Generally, a fast multiplication rate will correlate with other growth parameters' increase, such as the number of shoots, height, roots, and leaves in plantlets.

Observational evidence indicates that the culture systems and the interactions between the culture system and the potato cultivars influence the increase in plantlet height. Compared with plantlets in the conventional culture, TIS Bioreactor culture has an increased plant height. It indicates a rapid rise in plant tissue metabolism due to nutrient intake and homogeneity of cultural media maintained in the TIS Bioreactor culture. In addition to fundamental differences related to aeration and temporary immersion systems, variances in the size of experimental containers also affect the height of the plantlets. The larger the culture container used, the more the explants will grow, and the smaller the culture container, the more limited the growth of the potato's explants (Septiani, 2019). However, the shoots and seedlings grown in the TIS culture grew larger. The broad and transparent culture container in the TIS Bioreactor also benefitted in maximizing light penetration for plantlet growth; hence, plantlets can experience typical growth with upright and sturdier stem conditions and do not experience the etiolating conditions.

Regarding plant material, investigations showed that plant conditions *in vitro* can also represent the general picture of plants *ex vitro*. Factually, the cultivar Granola L., under *in vitro* conditions, has the maximum stem height compared with other cultivars. It aligns with the morphological characteristics of the cultivar Granola L., which indeed has a higher stem size (60–70 cm) in *ex vitro* conditions. The cultivar Maglia has the same genetic characteristics as one of its parents,

the Atlantic Malang, with a stem height of only 50 cm (Vegetable Crops Research Institute, 2019). Based on the observations, it established that the culture system and potato cultivars, as well as the interaction between them, impact the increase of primary plantlet roots. The primary roots appeared in the first week of incubation in all treatments. It showed that endogenous auxin in explants spontaneously stimulates organogenic events, leading to root formation (Setiawati *et al.*, 2018).

The phenomenon related to the earlier emergence of primary roots in explants of the cultivar Granola L. and the primary roots' lengthiest appearance in the cultivar Maglia can refer to the genetic explanation of a correlation between the time of root emergence and the emergence of apical shoots. In this case, root formation will occur rapidly in apical shoots that fully grow because the process of root formation itself has stimulation from auxin, which is synthesized in the apical and shoot meristem (Raspor *et al.*, 2020). The difference in the morphological appearance of the primary plantlet roots in the TIS Bioreactor culture, which looks thicker than the primary plantlet roots in conventional culture, might be due to the availability of adequate oxygen and the homogeneity of the culture media, which the aeration system has maintained. Liquid culture equipped with a slow and continuous aeration system can produce elongation, thickening, and twice the number of roots compared with root growth in stationary culture media with agar media (Georgiev *et al.*, 2014).

Following the observations on other parameters, the TIS Bioreactor culture, equipped with an aeration system and temporary immersion, also positively affects the increase in the number of leaves with larger sizes than plantlet leaves in the conventional culture. It implies that the TIS Bioreactor culture can favorably affect the activation of metabolic substances in plant tissues, impacting plant growth rates, including the parameter of an increasing number of leaves (Karyanti *et al.*, 2018). However, the high metabolism of plant tissue in the TIS Bioreactor culture results in symptoms of

premature aging, which can be visible in the morphology of the plantlet leaves. These symptoms manifested by early multiple and compound leaves' appearance when the plantlets were three weeks after culturing. In addition, several leaves appear to turn yellow due to chlorophyll overhaul, in which the transfer of starch accumulation transpired to younger leaves (Husna *et al.*, 2014). The most dominant multiple leaves were apparent in the cultivar Dayang Sumbi plantlets. In the conventional culture, numerous leaves generally appeared when the plantlets were at the age of six weeks. Based on observations and data analysis results regarding the number of nodes, shoot length, and root formation, it is possible to conclude that the TIS Bioreactor can safely benefit in the micro shoot multiplication of the potato cultivars.

CONCLUSIONS

The use of TIS Bioreactor culture showed better conditions for the growth and development of shoots and micro plantlets for the four potato cultivars (Atlantik Malang, Dayang Sumbi, Granola L, and Maglia) compared with the use of culture technique in the solid medium.

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