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Liquid culture for efficient in vitro propagation of potato (*Solanum tuberosum* L.) using bioreactor system

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Abstract: The bioreactor is one method of in vitro culture for plant propagation. Some plants that have used this method in propagation are date palms, ornamental plants, and medicinal plants, but potato seed production has not yet been carried out. In vitro culture activities usually use solid or semi-solid media, namely culture media with the addition of agar. In a bioreactor system, the media used does not use agar so it is called liquid media. Propagation methods using tissue culture generally use a non-bioreactor culture system which causes production prices to increase. Therefore, it is interesting to use a bioreactor system in potato seed production. The research aimed to compare the best combination of bioreactor system treatment and non-bioreactor culture system with the addition of IBA for the growth and development of Amudra potato seed plantlets. The experimental design used a Complete Randomized Design (CRD) with 6 treatments, and 4 repetitions. The treatments in this study were non-bioreactor without IBA (control), non-bioreactor + IBA 1 mg L^{-1}, non-bioreactor + IBA 2 mg L^{-1}, bioreactor without IBA (control), bioreactor + IBA 1 mg L^{-1}, and bioreactor + IBA 2 mg L^{-1}. Bioreactor treatment (control) is better than other treatments. This can be seen from the number of shoots 7.30, stem diameter of 2.83 mm^3, leaf area of 1.42 cm^2, number of leaves of 9.60, and total chlorophyll content of 0.32 mg L^{-1}. The results indicate that bioreactor culture with a liquid medium could be used for mass micropropagation of *S. tuberosum* L.

Keywords: Plant growth regulator; Seeds production; Shoot regeneration.
Abbreviation: IBA_Indole Butyric Acid.

Introduction

Potato (*Solanum tuberosum* L.) is a plant that is used as a source of carbohydrates and has the potential for food diversification so potato development in Indonesia has a priority place. The nutritional value contained in potato tubers includes carbohydrates 18.05 g, protein 2.01 g, 9.00 mg of calcium, 20 mg of vitamin C (Haverkort et al., 2023), vitamin B1 0.09 mg, and vitamin B2 0.03 mg (Dereje & Chibuzo, 2021). This causes a high demand for potatoes in society. This is not by potato production results which are only 1.83 kg/m^2, while the potential production of potato plants is 2.5-3.0 kg/m^2 (Erlangga, 2023). Continuously increasing demand must be balanced with high and stable production.

National potato production is currently 1.314.650.000 kg with a planting area of 6.822.300 m^2, while potato crop productivity can reach 2.5 kg/m^2 (Rosdiana et al., 2023). This low production is because farmers still use potato seeds from the next generation which are produced independently using conventional methods. It is essential to use certified seeds because this will guarantee the quality of the seeds (Halterman et al., 2011). However, there are still many farmers who use advanced-generation seeds. This condition is caused by the high price of quality potato seeds, while the selling price of consumption potatoes is relatively lower. Therefore, it is necessary to have a method of propagating potato seeds with low commercial prices for farmers. One way is to use seed propagation using tissue culture techniques.

To produce potato seeds in plantlet form, in vitro culture techniques can be used. This technique can produce potato plantlets in large quantities in a relatively short time. The general media used for explant growth is MS (Murashige and

Skoog) media with a complex nutrient composition. The cultivation media material used is MS base media stock solution, a widely used specific planting medium with high nitrate, potassium, and ammonium content for the growth of potato explants (Siregar et al., 2023). Currently, a bioreactor system has been developed using in vitro culture techniques. In the bioreactor system, liquid MS media is used, whereas in the conventional system, semi-solid media is used (added agar). The purpose of using liquid media is to make it easier for the explants to absorb nutrients, whereas the liquid media is obtained from basic MS media without the addition of solidifying agents (Da Silva et al., 2020).

Propagation methods using tissue culture generally use semisolid media which is usually called agar (Ohnuma et al., 2020). However, the price is quite expensive, so there needs to be the right solution to reduce production costs. Nowadays there is a new technology that is used to accelerate plantlet growth but can reduce production costs, namely the Bioreactor system (María et al., 2022). Several types of bioreactors have been studied (Ramírez-Mosqueda & Iglesias-Andreu, 2016). Of the various types of bioreactors, the Air-Lift system is the easiest system to find (Afreen, 2006). This type is the most economical because it can be modified simply. The systematics or workings of the Air-Lift require air pressure as the main non-mechanical driving factor which functions as a supplier of oxygen (aeration), stirring (agitation), and circulation. Research using a bioreactor has also been carried out on *Bletilla striata* (Zhang et al., 2018), *Fragaria ananassa* (Camargo et al., 2019), *Vanilla planifolia* (Ramírez-Mosqueda & Bello-Bello, 2021), *Anthurium andreanum* (Carvalho et al., 2019), dan *Eucalyptus grandis* (Souza et al., 2020).

The use of PGR (Plant Growth Regulator) can be seen from its function in detail. Several types of PGR include the auxin group, namely IBA (indole-3-butyric Acid), IAA (indole-3-acetic acid), NAA (Naphthalene Acetic Acid), and 2,4-D (dichlorophenoxy-acetic acid). IBA is a synthetic auxin that has more stable properties compared to other types of auxin such as IAA, IBA is more appropriate for stimulating root activity because it works longer and is more stable (Mohapatra & Batra, 2017). According to research results by Hajare et al., (2021) Giving an IBA concentration of 1.0 mg L^{-1} with the addition of IAA 0.5 mg L^{-1} can produce several roots and shoots in potato plantlets. Based on this analysis, this research was carried out to obtain the best combination of bioreactor system treatment and non-bioreactor culture system for the growth and development of Amudra potatoes.

Results and Discussion

Developing vigorous plantlets

The combined treatment of the bioreactor system with 1 mg L^{-1} IBA showed that the plantlets experienced vitrification/hyperhyricity, this was indicated by the morphology of the plantlets being clear, glassy, and transparent (Figure 1b). According to Alves et al., (2021), plantlet hyperhydricity is often found in cultures using liquid media due to excess water in plant cells. In contrast to plantlets grown in a combination of non-bioreactor culture systems with IBA 1 mg L^{-1}, plantlets have more optimal root growth and normal plantlet growth does not experience hyperhydricity (Belachew et al., 2020).

Plantlets with a combination of a bioreactor system with 2 mg L^{-1} IBA experienced etiolation that caused plantlet growth to be stunted, one of the things that influenced this was the lighting factor. Liquid medium is very suitable for culture with shoot explants (Ramírez-Mosqueda & Bello-Bello, 2021), This is because explants which often float on the top surface of the liquid medium can experience adequate gas diffusion so that the part of the shoot that protrudes above the media can grow more quickly compared to using a non-bioreactor culture system. This research has previously been carried out (Carrión & Tapia, 2019) in several varieties of potato plants by soaking for a certain time.

Combining a bioreactor system with 2 mg L^{-1} of IBA aged up to 28 d after inoculation (DAI) caused etiolating. This can happen due to the lack of light received by the plantlets (Zapata-Arias et al., 2014). According to Caibin & Huachun (2017), Low light intensity in potato plantlet inoculation can stimulate the formation of auxin which is a compound that stimulates cell growth and makes plantlets long and slender. The morphology of plantlets in the combination of bioreactor treatment with IBA 0 mg L^{-1} and IBA 2 mg L^{-1} had taller and stronger stems compared to the non-bioreactor culture system. This is thought to be because, in the bioreactor system, there is quite good gas exchange, so it can influence plantlet growth. In this way, oxygen transportation runs well, thereby reducing oxygen limitations, so that it can stimulate growth (Aka Kaçar et al., 2020).

Plantlets aged 21 DAI experienced very significant growth compared to 14 DAI and 7 DAI. Plantlets in the combination treatment of the bioreactor system with 0 mg L^{-1} IBA began to stand upright and have sturdy stems. This also happened to plantlets treated with a combination of a bioreactor system with IBA 2 mg L^{-1}. However, plantlets in this treatment experienced etiolation and contamination at 28 DAI. Bacterial and fungal contaminants in the bioreactor system grew faster than plantlets treated with the non-bioreactor culture system. This happens because the agitation process accelerates the spread of mold throughout the bottle area (Karyanti et al., 2018b). To prevent microorganism contamination, several equipments such as scissors, tweezers, silicone tubing 9.6 mm, and Schott bottles that are in direct contact with the explants are soaked in 10% Clorox solution for 24 hours. Because the explants used are guaranteed to be sterile, what needs to be considered to prevent contamination is the equipment used during the experiment. Clorox (sodium hypochlorite) can be used to sterilize culture equipment and plant explants because it contains sodium hypochlorite which is effective in suppressing the growth of bacteria and fungi (Bonetta et al., 2021). The environment or room where the experiment is carried out is always maintained, where 70% alcohol is sprayed thoroughly every 3 days in the experiment room. One sterility chemical that is quite effective and safe is alcohol (Al Shikh & Milosevic, 2020).

Number of shoots

Plantlets grown in bioreactors had better shoot growth compared to plantlets grown in non-bioreactor systems (Table 1). The average number of shoots appearing on each plantlet was 5.00. The bioreactor system treatment without the addition of IBA (control) was significantly different

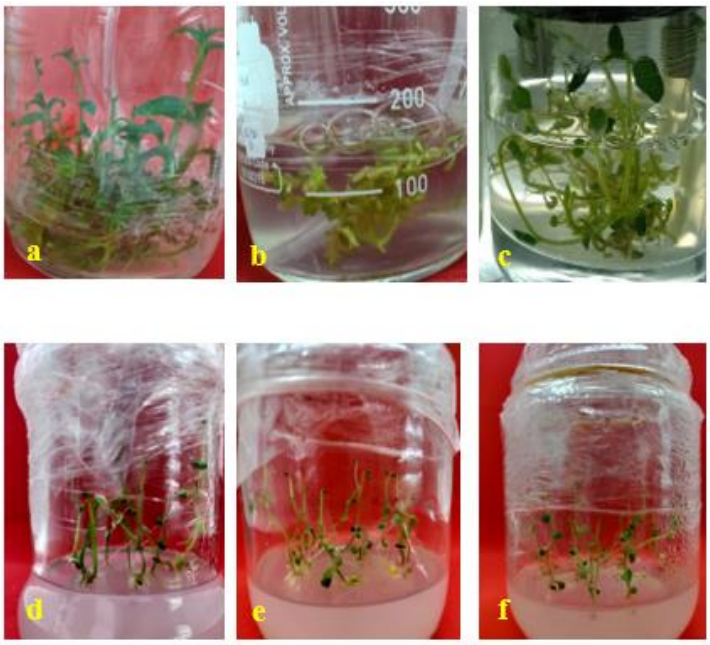

Fig 1. Morphology of plantlets grown using bioreactor at 21 DAI; (a) No IBA (Control); (b) IBA 1 mg L^{-1}; (c) IBA 2 mg L^{-1}; and non-bioreactor system (d) No IBA (Control); (e) IBA 1 mg L^{-1}: (f) IBA 2 mg L^{-1}.

Table 1. Number of potato plantlet shoots at 28 DAI.

Treatments	Number of Shoots			
	7 DAI	14 DAI	21 DAI	28 DAI
Non-Bioreactor No IBA	1.05 d	1.98 b	2.55 d	3.93 ab
Bioreactor No IBA	1.25 ab	5.00 d	5.90 c	7.30 c
Non-Bioreactor + IBA 1 mg L^{-1}	1.20 a	1.58 ab	3.13 a	4.53 b
Bioreactor + IBA 1 mg L^{-1}	2.00 c	3.00 c	3.00 a	3.00 a
Non-Bioreactor + IBA 2 mg L^{-1}	1.43 ab	1.28 a	3.20 ab	4.15 b
Bioreactor + IBA 2 mg L^{-1}	2.00 c	3.00 c	3.00 a	3.00 a

Numbers followed by the same letter are not significantly different from the DMRT test at the $p>0.05$ level. Days after inoculation (DAI)

Table 2. Leaf area plantlet at 28 DAI.

Treatments	Average leaf area (cm^2)
Non-Bioreactor No IBA	0.27 a
Bioreactor No IBA	1.42 cd
Non-Bioreactor + IBA 1 mg L^{-1}	0.28 a
Bioreactor + IBA 1 mg L^{-1}	0.95 b
Non-Bioreactor + IBA 2 mg L^{-1}	0.21 a
Bioreactor + IBA 2 mg L^{-1}	1.13 c

Numbers followed by the same letter are not significantly different from the DMRT test at the $p>0.05$ level. Days after inoculation (DAI)

compared to all existing treatments. At the end of the observation, the bioreactor system without IBA (control) had the highest number of shoots, namely 7.30 (Table 1). This is following the research results of Siregar et al., (2023), the use of TIS Bioreactor is very effective compared to conventional systems in increasing the number of shoots of potato plantlets of Atlantic Malang, Dayang Sumbi, Granola Lembang, and Maglia varieties.

In a bioreactor system, plantlets have a greater opportunity to absorb carbon gas because they have a good aeration system. In this case, it is possible that the plantlets only need endogenous hormones for their growth and the addition of exogenous hormones inhibits growth. The growth of lateral branches in most plantlets is probably due to the use of large containers and sufficient gas supply (Mancilla-Álvarez, Eucario, Juan Antonio Pérez-Sato, Rosalía Núñez-Pastrana, José L. Spinoso-Castillo, 2021). In contrast to plantlets that grew in the non-bioreactor system (control) and with the addition of IBA 1 mg L^{-1} and 2 mg L^{-1}, shoot growth was quite different from that in the bioreactor system. Potato plantlets probably only need indigenous hormones, the addition of exogenous hormones (IBA) causes the growth of potato plantlets to be suboptimal. The growth of potato plantlets of Atlantic Malang, Dayang Sumbi, Granola Lembang, and Maglia varieties was very good in the bioreactor even though there was no addition of growth regulators (Siregar et al., 2023).

One of the problems that often occur in bioreactor systems is vitrification/hyperhydricity, which is a condition where plantlets have excess water in their cells (Kim et al., 2020). In this case, the plantlets in the bioreactor with the addition of L^{-1} or 2 mg L^{-1} of IBA experienced abnormal growth, the

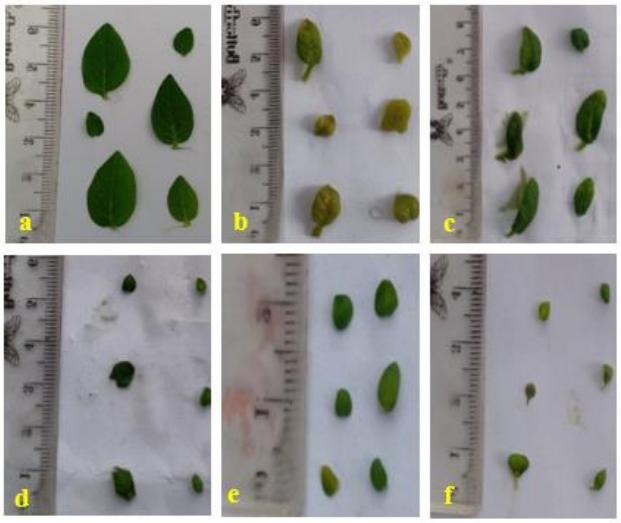

Fig 2. Leaf area observed using bioreactor at 28 DAI; (a) No IBA (Control); (b) IBA 1 mg L^{-1}; (c) IBA 2 mg L^{-1}; and non-bioreactor system (d) No IBA (Control); (e) IBA 1 mg L^{-1}; (f) IBA 2 mg L^{-1}.

Table 3. Number of potato plantlet leaves.

Treatments	Number of leaves			
	7 DAI	14 DAI	21 DAI	28 DAI
Non-Bioreactor No IBA	3.60 a	5.68 bc	7.63 cd	8.63 ab
Bioreactor No IBA	4.25 ab	7.00 d	7.20 c	9.60 d
Non-Bioreactor + IBA 1 mg L^{-1}	4.30 bc	4.23 a	5.55 a	8.50 ab
Bioreactor + IBA 1 mg L^{-1}	4.00 a	6.00 c	6.00 b	6.70 a
Non-Bioreactor + IBA 2 mg L^{-1}	4.30 bc	5.70 b	6.73 b	9.03 bc
Bioreactor + IBA 2 mg L^{-1}	4.75 d	5.30 ab	8.20 d	9.00 b

Numbers followed by the same letter are not significantly different from the DMRT test at the $p>0.05$ level. Days after inoculation (DAI).

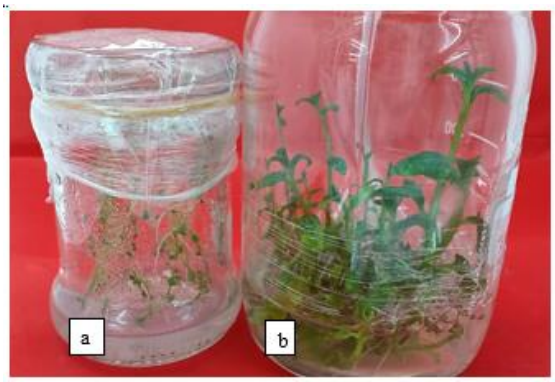

Fig 3. Number of leaves at 28 DAI observation; (a) non-bioreactor no IBA; (b) bioreactor no IBA.

Table 4. Chlorophyll a, b, and total chlorophyll at 28 DAI.

Treatment	Chlorophyll (mg L^{-1})		
	a	b	total
Non-Bioreactor No IBA	0.12 a	0.12 b	0.24 b
Bioreactor No IBA	0.14 d	0.18 d	0.32 d
Non-Bioreactor + IBA 1 mg L^{-1}	0.12 bc	0.13 c	0.25 c
Bioreactor + IBA 1 mg L^{-1}	0.09 a	0.05 a	0.14 a
Non-Bioreactor + IBA 2 mg L^{-1}	0.11 b	0.10 b	0.21 b
Bioreactor + IBA 2 mg L^{-1}	0.10 ab	0.12 b	0.22 b

Numbers followed by the same letter are not significantly different from the DMRT test at the $p>0.05$ level. Days after inoculation (DAI).

plantlets experienced vitrification and etiolation so the growth of new shoots was not optimal. This may be because the plantlets do not need additional exogenous hormones (IBA). After all, the environment in the bioreactor is sufficient for the growth of potato plantlets. The greater concentration of IBA results in reduced growth of banana plants in vitro (Khatun et al., 2017). The growth of plantlets in the bioreactor without the addition of IBA looks better, this can be seen from the plantlets which have green, large stems, lots of leaves, and are vigorous (Figure 1a). Previously it was also reported that plantlets in the TIS Bioreactor had a larger stem morphology compared to plantlets grown in a

conventional (non-bioreactor) system (Nurul-Afza et al., 2023).

Leaf area

The results of the variance analysis showed that the highest average leaf area was found in the bioreactor treatment with a combination of IBA 0 mg L^{-1} (1.422), shown in Table 2. Figure 2 shows significant differences in leaf area in all treatments. The leaf area in the bioreactor + IBA 0 mg L^{-1} treatment had a wide, sturdy shape, and fresh green color with larger leaf veins compared to other treatments. The leaf area in the 1 mg L^{-1} bioreactor + IBA system treatment was yellow with a curled leaf shape, this occurred because the plantlets experienced fertilization so the leaves did not grow optimally. This also happened in the non-bioreactor + IBA 1 mg L^{-1} culture system treatment, some leaves were found to be slightly yellow in color. Leaf area indicates an optimal photosynthesis process. The photosynthesis process will take place more optimally in plants with large leaf sizes and a large number of leaves (Pertamawati, 2012).

In the bioreactor system, excellent gas exchange occurs and increased nutrient absorption by the explants (Martínez-Estrada et al., 2019). Plants grown through in vitro culture absorb nutrients not only through the roots but also through the leaf organs (Hwang et al., 2022). In fact, in this system the entire explant epidermal tissue will come into contact with nutrients, thereby increasing the media's greater nutrient absorption (Kim et al., 2020).

Number of leaves

There was a significant difference in the leaf number parameters of the combined treatment of the bioreactor culture system + 0 mg L^{-1} IBA for all treatments (Table 3). This indicates that giving IBA to the bioreactor culture system has a real influence on the number of leaves that grow. The number of leaves during the incubation period is influenced by active cell division so leaf development is also faster. This is thought to be due to the speed of aeration and the number of bubbles entering the culture environment which accelerates respiration (Camargo et al., 2019). According to Karyanti et al. (2018a), Leaves are organs needed by plantlets for photosynthesis and other metabolic processes, so the leaves that are formed determine the success of plantlets when they are acclimatized. The results of visual observations (Figure 3), plantlets in the bioreactor without IBA (control) had a greater number of leaves were thicker, and had wider leaf morphological characteristics compared to the non-bioreactor culture system treatment.

Chlorophyll content

The lowest total chlorophyll was found in the combination of bioreactor culture system treatment with IBA 1 mg L^{-1} (Table 4), this was because the plantlets underwent vitrification which caused the low green color of the leaves contained in the plantlets (Robert et al., 2006). Chlorophyll a and b are influenced by the type of pigment that functions actively as a reaction center or photosystem, namely photosystem I (absorbs light with a wavelength of 680 nm) and photosystem II (absorbs light with a wavelength of 700 nm). Photosystem II produces energy from electrons released for photophosphorylation which then produces ATP, which is the unit of energy exchange in cells. At the same time, light ionizes photosystem I releasing electrons which ultimately reduce NADP to NADPH. The ATP and NADPH produced in photosynthesis trigger various plantlet biochemical processes. This biochemical process can stimulate plantlet growth.

Photosynthesis in plantlets will produce biochemical processes that will provide benefits, including increasing the rate of multiplication. An increase in chlorophyll concentration was detected in the media without the addition of IBA to the bioreactor system. Increased chlorophyll concentrations cultivated in ventilated containers compared to airtight ones have been reported in *Dianthus caryophyllus* (Thi et al., 2019), *Anthurium andreanum* L., (Martínez-Estrada et al., 2019) and *Colocasia esculenta* L. Schott (Mancilla-Álvarez, Eucario, Juan Antonio Pérez-Sato, Rosalía Núñez-Pastrana, José L. Spinoso-Castillo, 2021).

Number of nodes

There is a difference in the number of potato plantlet nodes growing in a bioreactor system and a non-bioreactor (conventional) system. It can be seen that in the non-bioreactor system, almost all treatments showed a higher number of nodes compared to plantlets grown in the bioreactor system (Table 5). The highest value for the number of nodes was found in the non-bioreactor system treatment with the addition of IBA 1 mg L^{-1} (8.65), this could occur due to the action of the IBA hormone. The addition of a growth regulator in the form of IBA can affect the number of nodes of potato plantlets of the Kufri Bahar variety (Kumari, 2023). However, the length of plantlets in non-bioreactor systems is not much longer than plantlets in bioreactor systems, this is because plantlets in non-bioreactor systems have shorter distances between nodes (Figure 5). This will certainly affect the acclimatization stage of potato plantlets later.

Acclimatization of potato plantlets is intended to obtain potato seeds in tuber form which is carried out in a screen house. One of the determining factors for the success of the acclimatization stage is the distance between nodes. Using plantlets that have short node spacing for the acclimatization stage can affect the growth of acclimatized plantlets, with close spacing between nodes can bury the nodes in the acclimatization medium so that the growth of lateral shoots will be stunted (Siregar et al., 2023). Production of potato plantlets with a longer distance between nodes will be very profitable for the production of potato seeds in tuber form (Karjadi, 2016).

Plantlet height

When observed, the height of plantlets between those grown in non-bioreactor and bioreactor systems appeared to be significantly different. The bioreactor system treatment without IBA (control) had a higher plantlet height (9.13 cm) compared to the non-bioreactor system treatment without IBA (7.17 cm) (Table 6). Plantlets grown in a bioreactor system with the addition of 2 mg L^{-1} IBA had the highest mean value (10.93 cm) compared to other treatments. This can happen because the plantlets experience etiolation. One of the causes of etiolation is the intensity of light received and the high production of the hormone auxin at the tip or shoot (Cavallaro et al., 2022). In this case, it can occur because the concentration of the IBA hormone given is quite large, namely 2 mg L^{-1}, so it can cause etiolation in potato plantlets (Figure 1c).

The bioreactor system treatment without IBA was not significantly different from the bioreactor system treatment with the addition of 2 mg L^{-1} IBA on plant height. The

Table 5. Number of nodes plantlet.

Treatment	Number of nodes			
	7 DAI	14 DAI	21 DAI	28 DAI
Non-Bioreactor No IBA	4.23 ab	5.50 b	5.90 b	7.10 ab
Bioreactor No IBA	4.00 a	4.10 a	5.80 ab	7.50 bc
Non-Bioreactor + IBA 1 mg L^{-1}	4.55 c	6.33 d	7.48 d	8.65 d
Bioreactor + IBA 1 mg L^{-1}	4.00 a	5.10 ab	5.10 a	5.70 a
Non-Bioreactor + IBA 2 mg L^{-1}	5.03 cd	5.83 b	6.83 c	7.93 c
Bioreactor + IBA 2 mg L^{-1}	4.00 a	5.90 c	6.80 c	7.00 b

Numbers followed by the same letter are not significantly different from the DMRT test at the $p>0.05$ level. Days after inoculation (DAI).

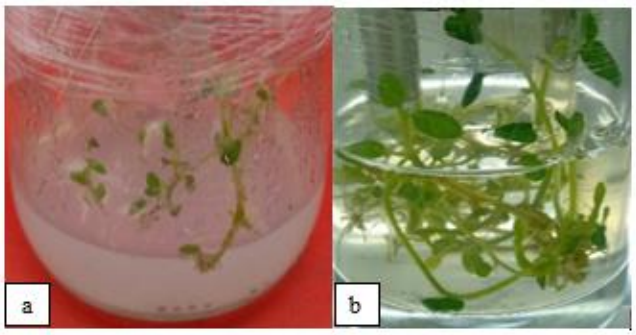

Fig 4. Morphology of number of nodes at 21 DAI observation; (a) non-bioreactor system + IBA 2 mg L^{-1}: (b) bioreactor system + IBA 2 mg L^{-1}.

Table 6. Plantlet high at 7 DAI, 14 DAI, 21 DAI, and 28 DAI.

Treatment	Plantlet High (cm)			
	7 DAI	14 DAI	21 DAI	28 DAI
Non-Bioreactor No IBA	3.20 bc	4.61 bc	5.70 ab	7.17 b
Bioreactor No IBA	2.23 a	3.23 a	6.98 c	9.13 c
Non-Bioreactor + IBA 1 mg L^{-1}	3.35 c	5.28 c	6.27 b	7.18 b
Bioreactor + IBA 1 mg L^{-1}	2.85 ab	4.50 b	4.60 a	4.87 a
Non-Bioreactor + IBA 2 mg L^{-1}	3.67 c	4.74 bc	6.19 b	7.39 b
Bioreactor + IBA 2 mg L^{-1}	2.30 a	1.15 cd	7.10 d	10.93 c

Numbers followed by the same letter are not significantly different from the DMRT test at the $p>0.05$ level. Days after inoculation (DAI).

bioreactor system treatment without IBA was significantly different from the non-bioreactor system treatment without IBA regarding plant height with values of 9.13 cm and 7.17 cm respectively (Table 6). This situation can occur most likely because the bioreactor system uses liquid media so it is easier for plantlets to use it. Culture media in liquid form can influence explant growth because there is maximum nutrient absorption (Ahmadian et al., 2017). Siregar et al., (2023) added that the aeration system in the TIS Bioreactor will increase metabolic processes in plant tissue.

Node distance

Node distance analysis in the variance table has significant differences between culture systems with bioreactors and non-bioreactor culture systems (Table 7). Plantlets with small stems and not optimal height will produce closely spaced nodes (Septiani, 2019). Meanwhile, plantlets with long node spacing, but with stems that are not dark green in color and have a weak twisting shape indicate etiolation of the stem (Makowski et al., 2023).

Stem diameter

The best stem diameter was found in the bioreactor + IBA 1 mg L^{-1} treatment (Table 8). The stem diameter is influenced by the aeration supply provided through the aerator as a carbon source (Aka Kaçar et al., 2020), Apart from that, in the bioreactor container, the homogeneity of nutrients in the media is also maintained by movement. Air circulation and homogeneity of nutrients in the media are important for plant growth and development to help improve the quality of metabolic processes in plant tissue (Karyanti et al., 2018b). The combination treatment of 1 mg L^{-1} IBA with a bioreactor system had a large stem diameter but plantlets could not grow normally. This is thought to occur due to an imbalance between the endogenous and exogenous auxin hormones (Hoque, 2010).

The plantlets are submerged with 1 mg L^{-1} MS IBA media which aims to initiate root emergence, however, the exogenous auxin which is continuously soaked causes an imbalance with the plantlet's endogenous auxin. According to Yasmin et al., (2011), The existing imbalance of exogenous and endogenous hormones affects the absorption that occurs throughout the surface of the stem. The absorption process in plant cells is influenced by the permeability of the cell membrane and the difference in water potential between inside and outside the cell. Absorption by plant cells will cause turgor pressure in the cells which will ultimately result in cell enlargement and swelling on the plantlet surface.

Based on research and morphological analysis results, it can be seen that the treatment of the bioreactor without IBA (control) shows the best treatment. This treatment shows that the plantlets have strong vigor, which is characterized by an upright and thick stem diameter, the plantlets have

Table 7. Node distance 7 DAI, 14 DAI, 21 DAI, and 28 DAI.

Treatment	Node distance (cm)			
	7 DAI	14 DAI	21 DAI	28 DAI
Non-Bioreactor No IBA	0.63 b	1.24 d	1.46 ab	1.71 bc
Bioreactor No IBA	0.36 a	0.72 a	1.50 bc	1.87 c
Non-Bioreactor + IBA 1 mg L^{-1}	0.79 bc	1.07 bc	1.47 ab	1.76 bc
Bioreactor + IBA 1 mg L^{-1}	0.50 a	0.79 ab	0.85 a	0.99 a
Non-Bioreactor + IBA 2 mg L^{-1}	0.88 d	0.99 ab	1.15 a	1.42 ab
Bioreactor + IBA 2 mg L^{-1}	0.62 b	1.15 cd	1.60 d	1.90 d

Numbers followed by the same letter are not significantly different from the DMRT test at the $p>0.05$ level. Days after inoculation (DAI).

Table 8. Diameter stem of the potato plantlet at 28 DAI

Treatment	The diameter of potato plantlet (mm^3)
Non-Bioreactor No IBA	0.41 a
Bioreactor No IBA	2.83 c
Non-Bioreactor + IBA 1 mg L^{-1}	0.64 a
Bioreactor + IBA 1 mg L^{-1}	2.94 cd
Non-Bioreactor + IBA 2 mg L^{-1}	0.85 a
Bioreactor + IBA 2 mg L^{-1}	2.10 b

Numbers followed by the same letter are not significantly different from the DMRT test at the $p>0.05$ level. Days after inoculation (DAI)

wide leaves, the plantlets have a high number of shoots, and there are no signs of vitrified plantlets. This is thought to be because the entire surface of the explant is in direct contact with nutrients. The use of liquid media provides better growth than semisolid media, this is because the explants have much greater direct contact with the nutrient media (Ramírez-Mosqueda & Bello-Bello, 2021).

Material and Methods

In vitro culture

This research was carried out using in vitro culture, namely growing potato plant explants in sterile bottles and vessels with MS basic media. Using in vitro culture, plants can be produced in large quantities in a relatively short time. In this research, two culture systems were used, namely conventional in vitro culture and a bioreactor system. The difference is the use of liquid and semisolid media, and in the bioreactor system, there is regulation of the oxygen supply in the growth container.

Plant material

The planting material used was potato plantlets of the Amudra F4 variety which were grown in MS media without growth regulators for one month (Figure 6).Amudra potato variety produced by the Tissue Culture Laboratory of the Indonesian Vegetable Crops Research Institute, West Java, Indonesia newly named to The Agricultural Instruments Standardization Agency of Vegetable Plants. The Amudra variety was obtained by crossing the Shepody variety with Ritex. Shepody variety introduced from Canada, the progeny of a cross between Bake-King x F58050. The Ritex variety was obtained by crossing the IP 81001-1 with MF-1.

The plantlets were then cut along four nodes per sample (Figure 7). The purpose of the plantlets used along the 4 nodes is so that they are not completely submerged in the liquid media of the bioreactor. The experimental design used a completely randomized design (CRD) with 6 treatments, 4 repetitions, and 10 samples per treatment. The treatments in this study were non-bioreactor without IBA (control), non-

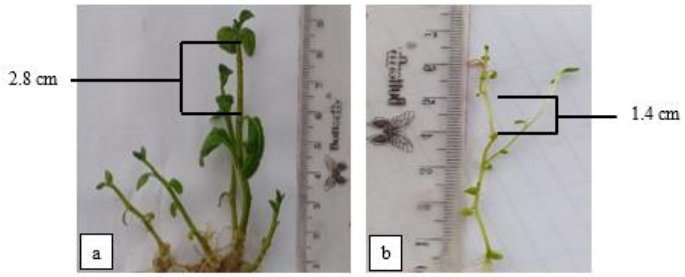

Fig 5. Distance between nodes in plantlets using (a) bioreactor with no IBA[1] and (b) non-bioreactor culture system treatment with

Fig 6. Initial seeds of potato

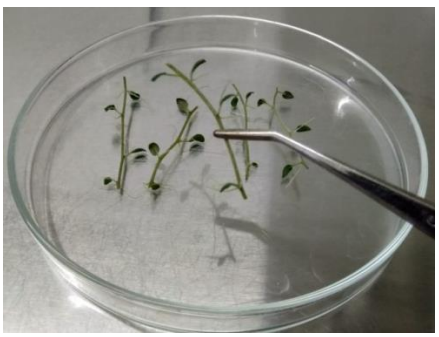

Fig 7. Potato explants consisting of 4 nodes.

bioreactor + IBA 1 mg L^{-1}, non-bioreactor + IBA 2 mg L^{-1}, bioreactor without IBA (control), bioreactor + IBA 1 mg L^{-1}, and bioreactor + IBA 2 mg L^{-1}. Materials needed during the research includeplant growt regulator , sterile distilled water, 96 % alcohol, 70 % alcohol, 1 N NaOH, 1 N HCl, 10 % Clorox, plastic wrap, tissue, aluminum foil, filter paper, dish soap, the label paper, and liquid MS media. Media for plant

culture is media that contains several macro, micronutrients and several types of vitamins that are useful for explant growth (Murashige & Skoog, 1962).

Experimental site

The research was carried out at the Biotechnology Laboratory, Muhammadiyah University of Malang. The experiment lasted for 5 months, carried out in July-November 2022. The plantlet incubation room was conditioned at a temperature of 18° C at night, and 20° C during the day. Photoperiodism is 16 hours of light, 8 hours of darkness, humidity of 60%, and light intensity of 5000 lux.

Bioreactor preparation

The tools used were a 500 ml Schott bottle (Duran®, Germany), silicone tubing 9.6 mm, and a syringe filter 0.2 µm (Whatman, Germany) which was modified to become a simple bioreactor tool (Figure 8). The transfer of nutrient media is assisted by a pump with a pressure of 0.30 MPa. Gas exchange in the explant bottle can occur by perforating the bottle cap and inserting silicon tubing, and a syringe filter to maintain the quality of the gas (microorganisms) entering the explant bottle. In in vitro culture, bioreactors are used to propagate plants in large quantities and in a relatively short time. A bioreactor is a device consisting of a vessel containing liquid media with airflow control in it to supply oxygen for the explant. In this research, what is said to be a Non-Bioractor is a conventional culture system. Conventional culture usually uses a tightly closed glass bottle, adds agar to the medium (semi-solid media), and does not regulate the timing of nutrition or oxygen supply.

Tool sterilization

The tools that have been washed are then soaked in 2 L of 10% Clorox solution for 24 h. Next, the tool is rinsed again using running water. The bottle is drained in a cool place by turning it upside down. After the tools are dry, put them in PP (Polypropylene) plastic and then close them tightly with a rubber band. The equipment was sterilized for 40 min at 121° C (Kryukov et al., 2022).

Media and sterilization

The basic media used are Murashige and Skoog (MS) with a liquid texture (Sigma-Aldrich, United States). MS media was made by taking the available stock solution and measuring pH 5.8. MS media is put into a Scott bottle and then closed tightly so that there are no air spaces. The bottle is then placed in an iron basket. MS media was sterilized in an autoclave at 121°C for 15 min (Hwang et al., 2022). Once cool, the media is removed from the autoclave and ready for use.

Explant inoculation

The explants used in this research came from plantlets produced by the Tissue Culture Laboratory of the Indonesian Vegetable Crops Research Institute, West Java, Indonesia.

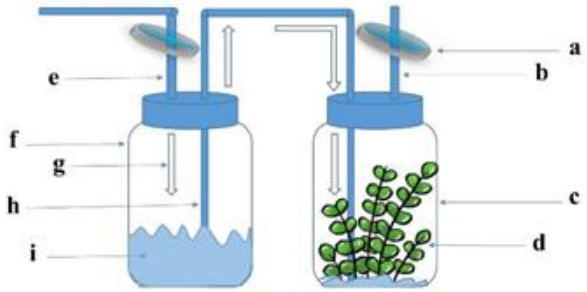

Fig 8. Bioreactor schematic. (a) Syringe filter; Silicone tubing; (b) Bottle for growing explant; (c) Explant/plantlets; (d) Silicone tubing (connecting air compresor and bioreactor media bottle); (e) Bioreactor media bottle; (f) Flow of incoming air pressure (g) the air compressor; (h) pipe; (i) MS liquid media.

The plantlets are then multiplied by cutting the plantlets along 4 nodes. Next, the pieces were put into a bioreactor container containing liquid MS media. For non-bioreactors, explant pieces are grown in semi-solid media in culture bottles. The prepared plantlets were then planted in liquid MS + IBA media in a Scott bottle as a vessel. The number of samples in one treatment was 10 samples. The plantlet is placed on a petri dish in the LAF, then the plantlet is cut along four nodes using a scalpel blade. The explant is inserted into the vessel using tweezers to keep the explant sterile. Observations were carried out for 4 weeks.

Statistical analysis

This research data is presented in the form of an average table which is then analyzed using a normality test using the Kolmogorov-Smirnov test and a homogeneity test using the Levene's test. Analysis of variance used two-way ANOVA. If the treatment shows a significantly different effect on the observation results, then further analysis of the Duncan Multiple Range Test (DMRT) is carried out at a significance level of 5 %. Data processing was carried out using SPSS version 29.

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