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Temporary immersion system: a system to improve the quality of potato seeds (*Solanum tuberosum* L.) cultivar 'Granola Lembang'

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Abstract: High-quality, virus-free, and vigorous potato plantlets are crucial for producing subsequent generations of potato seeds. This study aimed to optimize potato seed development using two distinct propagation systems: a temporary immersion system (TIS) employing liquid media and a conventional system utilizing semi-solid media. A factorial, completely randomized design was employed, with the propagation system (TIS vs. conventional) and sucrose concentration (30, 40, 50, 60 g L^{-1}) as factors. Results indicated superior potato seed growth in the TIS compared to the conventional system. Specifically, plantlets grown in TIS with 30 g L^{-1} sucrose exhibited a greater height (13.43 cm) than those in the conventional system with 30 g L^{-1} sucrose (8.23 cm). Similar trends were observed for leaf number and root length, with TIS + 30 g L^{-1} sucrose yielding 14.55 leaves and a root length of 15.00 cm, compared to 8.55 leaves and 6.76 cm in the conventional system, respectively. The total chlorophyll content in the TIS + 30 g L^{-1} sucrose treatment was 1.45 mg L^{-1}, while the conventional system + 30 g L^{-1} sucrose treatment yielded 0.98 mg L^{-1}. Virus testing confirmed the absence of the four major potato viruses (PVX, PVY, PVS, and PLRV) in all treatments. These findings suggest that mass propagation of high-quality, virus-free potato seeds can be effectively achieved using TIS with the addition of 30 g L^{-1} sucrose.

Keywords: micropropagation, plantlet, *S. tuberosum*, TIS.
Abbreviation: PLRV_Potato Leaf Roll Virus; PVS_Potato Virus S; PVX_Potato Virus X; PVY_Potato Virus Y; TIS_Temporary Immersion System.

Introduction

Potato plants can be propagated using several types of seed, which are distinguished by their method of production. These include true potato seed (TPS), plantlets, and microtubers. True potato seeds are the result of potato flower pollination. The seeds resulting from this sexual reproduction can produce genetically diverse phenotypes, potentially reducing disease transmission (Rasool, 2023). Potato plantlets are grown in vitro in sterile and controlled environments, with their quality dependent on the nutrient medium and environmental conditions (Hou et al., 2022). These plantlets, sometimes referred to as Breeder Seed (BS), are produced in the laboratory through in vitro culture. Microtubers are small tubers also produced through in vitro potato culture. They can be planted directly in the field and produced seasonally (Sivakumar et al., 2024). The production of virus- and disease-free seed potatoes with high yield potential is crucial. For potatoes, high-quality (virus- and disease-free) seed is essential and can be obtained through both conventional and modern in vitro culture techniques, including bioreactors. Seed potatoes produced through meristem culture in vitro are considered high-quality due to their virus-free status (Boubaker et al., 2023). Potato productivity is influenced by biotic and abiotic factors, as well as seed quality. Temporary Immersion Bioreactor (TIB) technology offers an alternative approach to obtaining quality seed potatoes (Tapia et al., 2018). Potato seed growth in bioreactor systems has been shown to be superior to conventional methods (Husen et al., 2024). A bioreactor provides a controlled environment for plant propagation through culture with liquid media and a fluid/air inflow and outflow system (Murthy et al., 2023). This system allows for direct contact between plant explants and the nutrient medium (Regueira et al., 2018). However, continuous direct contact between potato explants and the nutrient medium in bioreactor systems can increase hyperhydricity (Husen et al., 2024). Hyperhydricity is a physiological, biochemical, and anatomical disorder that can occur in plant explants grown in vitro (Zunazri et al., 2024).

Preventive measures are necessary to mitigate hyperhydricity, as it can significantly reduce plant yields in in vitro culture systems. One such measure involves temporarily immersing explants in the in vitro culture system to avoid constant contact with the nutrient medium.

The Temporary Immersion System (TIS) integrates several components into a single unit designed for in vitro plant propagation. Compared to conventional systems utilizing semi-solid media, TIS offers advantages such as enhanced efficiency, reduced labor requirements, and accelerated plantlet growth rates (Pożoga et al., 2024). TIS facilitates periodic (temporary) direct contact between plant explants and nutrients, typically for a few minutes at a time (Daurov et al., 2024). This system's improved efficiency stems from enhanced oxygen availability, more accessible nutrient uptake for explants leading to increased biomass and reduced propagation time (Gianguzzi and Sottile, 2024), and the potential for producing disease- and pest-free plants (Mancilla-Álvarez et al., 2021). The TIS approach is suitable for large-scale production due to its capacity to generate uniform plantlets in substantial quantities and within a relatively short timeframe (Tarraf et al., 2024).

Several studies have substantiated the efficiency of TIS in plant propagation. For example, TIS enhances shoot proliferation during banana propagation (Bello-Bello et al., 2019). *Gerbera jamesonii* "Shy Pink" plantlets grown in TIS exhibited a greater height (88.87 mm) compared to those in conventional semi-solid media (71.74 mm) (Lim et al., 2024). *Castanea sativa* x *C. crenata* grown in liquid media supplemented with 1% sucrose showed an increase in the number of shoots, leaf width, and leaf length (Gago et al., 2022). Sucrose serves as a critical carbon source in in vitro plant culture, as plants in this environment are typically not autotrophic and, therefore, require carbohydrates as a primary carbon source (Gago et al., 2021). *Viola ucriana* plants grown in TIS exhibited a significant increase in the number of shoots (7) compared to the conventional system (2.45), with similar trends observed in shoot length (24.13 mm in TIS vs. 11.65 mm in the conventional system) (Capaci et al., 2024). Building upon these findings, this study aims to analyze the optimal culture system and carbon source (sucrose) for the rapid mass production of potato seeds (plantlets).

Results and Discussion

Chlorophyll content

The total chlorophyll content in TIS was greater than in the conventional system; the TIS + 30 g L^{-1} treatment yielded a value of 1.45 mg L^{-1}, compared to 0.98 mg L^{-1} in the conventional system (Table 1). Furthermore, within TIS, the addition of sucrose at a concentration of 30 g L^{-1} resulted in higher chlorophyll content compared to a higher concentration of 60 g L^{-1}. Chlorophyll content of *Eucalyptus saligna* in liquid medium supplemented with 30 g L^{-1} sucrose (3.6 mg L^{-1}) was higher than with 45 g L^{-1} (2.1 mg L^{-1}), a trend also observed in semi-solid media (Reisdörfer-Schorr et al., 2023). In the conventional system treatment (semi-solid media), no significant difference in chlorophyll content was observed despite varying sucrose concentrations. This is likely due to limited carbon source availability for plantlets in semi-solid media (conventional system), unlike plantlets grown in liquid media (TIS).

Visually, potato seeds in TIS appeared healthier, exhibiting deep green leaves and stems compared to plantlets in conventional systems (Figure 1d). This is likely attributed to the ease with which plantlets utilize carbon and nutrient sources in the liquid medium (TIS). In TIS, oxygen transport within the vessel is efficient, mitigating oxygen limitations and promoting growth (Aka Kaçar et al., 2020). Consistent with these findings, previous research has shown that Amudra potato plantlets grown in a bioreactor (liquid media) had a total chlorophyll content of 0.32 mg L^{-1}, while in semi-solid media, the total chlorophyll content was 0.24 mg L^{-1} (Husen et al., 2024). The deep green leaves indicative of healthy potato seeds, as opposed to yellowish leaves, likely enhance their viability during acclimatization in the screen house.

Fresh weight of the potato seed

The growth of potato explants differed significantly between the temporary immersion system (TIS) and the conventional system. Specifically, the fresh weight of plantlets cultured in TIS reached approximately twice that of seeds grown in the conventional system. After 35 days of incubation, the TIS + 30 g L^{-1} sucrose treatment resulted in the highest fresh weight (95.45 g), while the conventional system + 60 g L^{-1} sucrose treatment yielded the lowest (33.56 g) (Table 2). This outcome is likely attributable to the inhibitory effect of excessive carbon source concentrations on plant metabolism. The addition of 30 and 40 g L^{-1} sucrose in TIS did not result in substantial differences in fresh weight, a trend also observed in the conventional system. As shown in Table 2, plantlet growth was inversely related to sucrose concentration in the growth medium. This finding aligns with Dewir et al. (2023), who reported a decrease in the fresh weight of *Rubus fruticosus* shoots grown in media with 60 g L^{-1} sucrose (0.430 g) compared to those grown in media with 45 g L^{-1} sucrose (0.677 g). Similarly, Nasution et al. (2024) found that a 3% sucrose concentration was superior to a 4.5% concentration in terms of height, number of leaves, leaf length, and chlorophyll content of *Stevia rebaudiana*.

Several reports have documented increased hyperhydricity or vitrification of explants in liquid culture systems. In potato propagation using a bioreactor system, some seeds exhibited vitrification/hyperhydricity, characterized by clear, glassy, and transparent plantlets (Husen et al., 2024). This phenomenon may be due to the continuous immersion of explants in liquid media within the bioreactor system. In contrast, TIS involves a specific nutrient soaking period for the explants. Observations indicate that potato plantlets in TIS and conventional systems did not exhibit hyperhydricity. The duration and frequency of nutrient flow during the soaking period in TIS can influence vitrification or hyperhydricity (Mu et al., 2024)

Table 1. Effect of culture system and sucrose on chlorophyll content.

Treatment	Chlorophyll (mg L^{-1})		
	a	b	total
TIS + Sucrose 30 g L^{-1}	0.73 d	0.85 d	1.45 d
TIS + Sucrose 40 g L^{-1}	0.66 c	0.71 c	1.27 c
TIS + Sucrose 50 g L^{-1}	0.69 cd	0.70 c	1.39 d
TIS + Sucrose 60 g L^{-1}	0.64 c	0.57 bc	1.21 bc
Conventional + Sucrose 30 g L^{-1}	0.45 a	0.53 b	0.98 b
Conventional + Sucrose 40 g L^{-1}	0.55 b	0.43 a	0.98 b
Conventional + Sucrose 50 g L^{-1}	0.47 b	0.49 ab	0.96 b
Conventional + Sucrose 60 g L^{-1}	0.42 a	0.45 a	0.87 a

Numbers followed by the same letter are not significantly different from the DMRT test at the $p>0.05$ level.

Table 2. Effect of culture system and sucrose on fresh weight.

Treatment	Fresh weight (g)	
	Before incubation	After incubation
TIS + Sucrose 30 g L^{-1}	0.70	95.45
TIS + Sucrose 40 g L^{-1}	0.95	95.32
TIS + Sucrose 50 g L^{-1}	0.85	87.56
TIS + Sucrose 60 g L^{-1}	0.92	81.67
Conventional + Sucrose 30 g L^{-1}	0.87	45.18
Conventional + Sucrose 40 g L^{-1}	0.98	44.28
Conventional + Sucrose 50 g L^{-1}	0.86	40.50
Conventional + Sucrose 60 g L^{-1}	0.95	33.56

Fresh weight of plantlets is carried out when they are 35 DAI (Days After Inoculation).

Primary roots of the potato seed

The primary root length of potato plantlets differed significantly between the two systems. The TIS + 30 g L^{-1} sucrose treatment resulted in a root length of 15.00 cm, a significant increase compared to the Conventional + 30 g L^{-1} sucrose treatment, which measured 6.76 cm (Table 3). These results are consistent with those reported by Hwang et al. (2022), who observed root lengths of chrysanthemum (14.5 cm), strawberry (6 cm), and *Cnidium officinale* (5 cm) in TIS, while semi-solid culture yielded 11 cm, 4 cm, and 2 cm, respectively. In the present study, a significant difference in root length was observed between the TIS and conventional systems, with TIS roots exhibiting greater length and density, as well as a healthier appearance (Figure 2e). Nutrient supply intervals and gas exchange within the TIS system are important factors influencing plant root growth (Hwang et al., 2022).

Morphological differences in the primary roots of Granola Lembang cultivar potato plantlets were also observed between TIS and conventional culture. In addition to primary and secondary roots, potato plantlets also developed adventitious roots at the nodes. In TIS, adventitious roots emerged more rapidly than in the conventional system, appearing two weeks after planting in TIS compared to three weeks in the conventional system. The impact of TIS and conventional systems, with varying sucrose concentrations in the media, on root growth of potato plantlets was notable (Figures 2a-2d). Consistent with these results, Siregar et al. (2023) reported that the culture system (TIS) and potato cultivars (Atlantic Malang, Granola L., Dayang Sumbi, Maglia) significantly impacted primary root length of seedlings. A key advantage of TIS is the removal of volatile compounds such as ethylene through increased ventilation via forced aeration, resulting in a superior growth rate compared to plants in semi-solid culture (Hwang et al., 2022)

Potato seed leaves

Leaf development was observed beginning at 4 days after inoculation (DAI), with leaf number increasing weekly. During the first week, the TIS + 30 g L^{-1} sucrose treatment group exhibited the highest mean leaf count (3.45), while the Conventional + 30 g L^{-1} sucrose treatment group exhibited the lowest (2.45) (Table 4). From weeks two through five (35 DAI), the TIS + 30 g L^{-1} sucrose treatment consistently presented the highest mean leaf count, and the Conventional + 60 g L^{-1} sucrose treatment, the lowest. These results suggest that both conventional and TIS systems, when combined with elevated sucrose concentrations, negatively impact leaf number in potato seeds, potentially due to carbon oversupply and subsequent metabolic inhibition of potato explants.

The culture system significantly influenced potato seed leaf development up to 35 DAI. Leaf number was generally lower in the conventional system compared to the TIS system. Furthermore, visual assessment of potato seed leaves in the TIS system indicated a more intense green color, increased thickness, and larger size (Figure 2g), suggesting enhanced nutrient uptake in the TIS environment. The number of leaves of 4 potato cultivars differed between the conventional system and the TIS bioreactor: Atlantic Malang (9.11), Dayang Sumbi (8.28), Granola L. (8.78), Maglia (8.86) in the TIS bioreactor, compared to 5.79, 7.67, 5.87, 6.39, respectively, in the conventional system (Siregar et al., 2023). Similarly, Hwang et al. (2022) reported that *Cnidium officinale*, Chrysanthemum, and Strawberry plants grown in a TIS exhibited the highest number of leaves when compared to semi-solid and liquid culture systems. The TIS system's cyclical immersion and aeration phases optimize gas exchange and nutrient absorption (Martínez-Estrada et al., 2019). Consistent with these findings,

Table 3. Effect of culture system and sucrose on root length at 35 DAI.

Treatment	Root length (cm)
TIS + Sucrose 30 g L^{-1}	15.00 d
TIS + Sucrose 40 g L^{-1}	14.12 d
TIS + Sucrose 50 g L^{-1}	13.78 cd
TIS + Sucrose 60 g L^{-1}	11.23 c
Conventional + Sucrose 30 g L^{-1}	6.76 b
Conventional + Sucrose 40 g L^{-1}	6.21 ab
Conventional + Sucrose 50 g L^{-1}	5.86 a
Conventional + Sucrose 60 g L^{-1}	6.10 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 4. Effect of culture system and sucrose on the number of potato plantlet leaves.

Treatment	Number of leaves				
	7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
TIS + Sucrose 30 g L^{-1}	3.45 c	4.85 d	7.86 d	10.43 d	14.55 d
TIS + Sucrose 40 g L^{-1}	3.10 bc	4.30 c	6.55 cd	10.35 d	14.23 c
TIS + Sucrose 50 g L^{-1}	3.53 d	4.50 d	6.78 c	10.26 c	13.87 bc
TIS + Sucrose 60 g L^{-1}	2.89 b	3.20 ab	6.00 bc	10.23 bc	11.56 b
Conventional + Sucrose 30 g L^{-1}	2.45 a	3.15 a	5.20 b	7.45 ab	8.55 ab
Conventional + Sucrose 40 g L^{-1}	3.21 c	4.00 bc	5.80 b	8.10 b	10.87 b
Conventional + Sucrose 50 g L^{-1}	2.65 ab	3.40 b	4.45 a	7.10 a	8.18 a
Conventional + Sucrose 60 g L^{-1}	2.54 a	3.00 a	4.40 a	7.00 a	8.10 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 5. Effect of culture system and sucrose on plantlet shoots.

System/Treatment	Number of shoots				
	7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
TIS + Sucrose 30 g L^{-1}	1.18 a	1.56 b	2.65 b	4.22 b	7.22 c
TIS + Sucrose 40 g L^{-1}	1.25 b	1.66 bc	3.20 c	4.27 c	7.34 c
TIS + Sucrose 50 g L^{-1}	2.10 d	2.85 d	4.67 d	7.20 d	8.95 d
TIS + Sucrose 60 g L^{-1}	1.20 b	1.45 ab	2.55 b	4.20 b	6.55 bc
Conventional + Sucrose 30 g L^{-1}	1.00 a	1.23 a	2.23 a	3.70 a	5.75 b
Conventional + Sucrose 40 g L^{-1}	1.85 c	2.25 c	4.21 cd	6.45 c	7.77 cd
Conventional + Sucrose 50 g L^{-1}	1.34 ab	1.55 b	2.20 a	4.00 ab	4.33 a
Conventional + Sucrose 60 g L^{-1}	1.12 a	1.33 a	2.24 a	3.85 a	4.10 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).

Husen et al. (2024) previously demonstrated a significant difference in leaf number in Amudra variety potato seeds developed in a bioreactor system.

Number of shoots of the potato seed

The number of shoots did not significantly differ between the temporary immersion system (TIS) and the conventional system. In both systems, potato shoot growth increased throughout the observation period. Elevated sucrose levels correlated with an increased number of potato shoots in both the conventional and TIS systems. Specifically, the TIS system with 50 g L^{-1} sucrose yielded 8.95 shoots, while the conventional system with 40 g L^{-1} sucrose produced 7.77 shoots (Table 5). These results align with Reisdörfer-Schorr et al. (2023), who reported that a medium with 45 g L^{-1} sucrose produced 23.9 shoots compared to 20.0 shoots in a medium with 30 g L^{-1} sucrose in *Eucalyptus saligna.*

The culture system significantly impacted the number of potato shoots at 35 days after inoculation (DAI). The TIS system produced nearly twice as many shoots as the conventional system, potentially due to the optimized oxygen supply in the TIS system, which facilitates explant utilization for new shoot formation. This observation is consistent with Agisimanto et al. (2023), who found that a partial immersion bioreactor (PIB) system produced 18-24 new potato shoots from 2-4 explants, while semi-solid culture yielded only 1-3 new shoots per explant. Furthermore, optimal nutrient absorption in liquid media compared to semi-solid media may contribute to this difference. The nutrient spray bioreactor (NSB) has also been shown to enhance potato shoot and microtuber growth compared to solid culture systems, attributed to improved gas exchange and nutrient absorption within the vessel (Rahman et al., 2015). Siregar et al. (2023) reported variations in the number of axillary shoots among four potato cultivars in conventional and TIS bioreactor systems: Atlantic Malang (1.83), Dayang Sumbi (1.89), Granola L. (1.39), and Maglia (1.61) in the TIS bioreactor, compared to 1.56, 0.33, 0.53, and 0.98, respectively, in conventional systems. These findings suggest that the TIS can optimize the growth of *S. tuberosum* L. by

Table 6. Effect of culture system and sucrose on plantlet height.

Treatment	Plantlet height (cm)				
	7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
TIS + Sucrose 30 g L^{-1}	4.23 d	6.87 d	8.45 d	10.22 d	13.43 d
TIS + Sucrose 40 g L^{-1}	4.18 c	6.13 c	7.90 c	9.43 cd	11.54 c
TIS + Sucrose 50 g L^{-1}	3.55 b	5.10 bc	7.00 b	9.12 c	11.33 c
TIS + Sucrose 60 g L^{-1}	4.12 c	6.15 c	7.45 bc	9.55 cd	11.11 c
Conventional + Sucrose 30 g L^{-1}	2.32 ab	4.85 b	5.21 ab	6.85 b	8.23 b
Conventional + Sucrose 40 g L^{-1}	1.85 a	3.45 a	4.33 a	5.55 a	6.66 a
Conventional + Sucrose 50 g L^{-1}	2.10 a	4.44 a	5.13 a	6.10 ab	8.20 b
Conventional + Sucrose 60 g L^{-1}	2.22 ab	4.76 ab	5.10 a	6.18 ab	8.16 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).

Table 7. Virus detection on potato plantlet.

Treatment	Jenis Pengujian			
	Metoda ELISA (Visual)			
	PVX	PVY	PVS	PLRV
TIS + Sucrose 30 g L^{-1}	negatif (-)	negatif (-)	negatif (-)	negatif (-)
TIS + Sucrose 40 g L^{-1}	negatif (-)	negatif (-)	negatif (-)	negatif (-)
TIS + Sucrose 50 g L^{-1}	negatif (-)	negatif (-)	negatif (-)	negatif (-)
TIS + Sucrose 60 g L^{-1}	negatif (-)	negatif (-)	negatif (-)	negatif (-)
Conventional + Sucrose 30 g L^{-1}	negatif (-)	negatif (-)	negatif (-)	negatif (-)
Conventional + Sucrose 40 g L^{-1}	negatif (-)	negatif (-)	negatif (-)	negatif (-)
Conventional + Sucrose 50 g L^{-1}	negatif (-)	negatif (-)	negatif (-)	negatif (-)
Conventional + Sucrose 60 g L^{-1}	negatif (-)	negatif (-)	negatif (-)	negatif (-)

(-) = Negative reaction not infected, (+) = Positive reaction to infection; Tested using antiserum PVX, PVY, PVS, and PLRV.

promoting shoot development through consistent contact with the nutrient medium and enhanced gas exchange within the vessel (Alexander et al., 2025).

Potato seed height

In the first week, the TIS + Sucrose 30 g L^{-1} treatment resulted in the greatest seed height (4.23 cm) compared to all other treatments. Conversely, the Conventional + Sucrose 40 g L^{-1} treatment exhibited the lowest height in the first week (1.85 cm) and maintained the smallest value (6.66 cm) until the final observation at 35 DAI. At this time, the TIS + Sucrose 30 g L^{-1} treatment exhibited the highest value (13.43 cm). The height of potato seeds in TIS was twice that of the conventional system, which aligns with findings by Siregar et al. (2023). Their research indicated that seed height of four potato cultivars was greater in the bioreactor system (liquid media) than in the conventional system (semi-solid medium): Atlantic Malang cultivar (12.64 cm), Dayang Sumbi (13.39 cm), Granola L. (13.83 cm), and Maglia (11.09 cm) in the bioreactor system, compared to Atlantic Malang (7.59 cm), Dayang Sumbi (8.27 cm), Granola L. (8.25 cm), and Maglia (6.46 cm) in the conventional system. This difference may be attributed to the enhanced nutrient absorption by explants in liquid media (TIS) compared to semi-solid media (conventional). Liquid media offer advantages over agar culture, including improved contact between the nutrient media and the explants, as well as a greater surface area of the explants involved in media absorption (Agisimanto et al., 2023). The use of liquid media can lead to improved plant seed production compared to solid media for various plant species due to the extensive contact between nutrients and explants (Mosqueda Frómeta et al., 2017).

Virus detection

Based on ELISA (Enzyme-Linked Immunosorbent Assay) testing, all treatments were free of the primary potato viruses (Table 7). This outcome is significant for the production of virus-free potato seeds, which is essential to minimize the propagation of low-quality seeds. The generation of virus-free plants is critical for effective disease management, the preservation of potato germplasm, and the control of virus-mediated potato disease dissemination (Campos and Ortiz, 2019). Potato Virus X (PVX), among other viruses, can diminish potato tuber yield (Verchot, 2022). Potato Virus S (PVS) infection negatively affects the in vitro production of "Dunluce" potato microtubers; infected plantlets yield only 12.6 microtubers, whereas virus-free plantlets produce 19.4 microtubers (Bettoni et al., 2022).

Virus-free plants can be obtained through in vitro propagation, initiated via meristem culture. A study by Naddaf et al. (2021), comparing the propagation of sweet cherry (*Prunus avium* cv. Siahe-Mashhad) through meristem culture and micro-grafting, demonstrated that meristem culture yielded a higher proportion of Plum Pox Virus (PPV)-free plants. Similarly, Benke et al. (2023) reported that meristem culture could produce garlic (*Allium sativum* L.) free of Onion yellow dwarf virus (OYDV), Garlic common latent virus (GCLV), and Shallot latent virus (SLV). Propagation of 14 potato cultivars through meristem culture resulted in potato plantlets free of Potato Virus A (PVA), Potato Virus Y (PVY), and Potato Leaf Roll Virus

(PLRV) (Mishra et al., 2024). In that experiment, explants were derived from seeds developed through meristem culture to ensure the production of virus-free potato seeds (plantlets).
The production of virus-free potato seeds is of paramount importance, as viral diseases can substantially reduce potato yields. Several potato cultivars in Moscow experienced yield reductions following viral infection. Specifically, Potato Virus Y reduced the yield of the Red Scarlet cultivar by 54.9%, Potato Virus S reduced the yield of the Adretta cultivar by 52.3%, and Potato Virus M reduced the yield of the Ilyinsky cultivar by 48.8% (Kolychikhina et al., 2021). Kereša et al. (2022) reported that potato plantlets infected with PVM+PVS exhibited a 31% reduction in tuber weight per plant and a 64% reduction in average tuber weight. Furthermore, the R1 clone infected with both viruses produced a significantly lower tuber yield, reduced by 59%. Therefore, producing virus-free potato seeds is crucial for achieving significantly improved field yields.

Materials and Methods

In vitro culture
Potato explants were cultured using two distinct propagation systems: a conventional culture system utilizing culture bottles with semi-solid media, and a temporary immersion system (TIS). The primary differences between these systems relate to the media type and oxygen availability within the TIS. The TIS employed liquid media without agar, whereas the conventional system utilized semi-solid media with agar.

Plant material
Plant explants were obtained from potato plantlets of the Granola Lembang cultivar, sourced from the Vegetable Plant Assembly and Modernization Center (BRMP), located at Jalan Tangkuban Perahu No. 517, Lembang, West Java. The Granola Lembang cultivar is derived from the West German Granola Clone. Explants, measuring 1 cm in length (Figure 1a), were used, with a total of 80 explants per TIS vessel and conventional culture. A 2x4 factorial Completely Randomized Design was employed, with two factors: propagation system (TIS and conventional) and sucrose concentration in the media, with four levels (30, 40, 50, and 60 g/L^{-1}). This resulted in 8 treatment combinations, each with 5 replicates.

Experimental site
The experiment was conducted in the in vitro culture laboratory at the University of Muhammadiyah Malang, located at Jalan Raya Sengkaling No. 188, Dau, Malang, East Java, over a 6-month period from March to July 2025. Potato explants were incubated in a controlled environment maintained at an average temperature of 20 °C, 60% humidity, and a 16-hour light/8-hour dark photoperiod with a light intensity of 4000 lux.

TIS preparation
The temporary immersion system (TIS) tool comprises a vessel, a filter, a rubber stopper, a silicone tube, and a syringe filter. Prior to use, all components must be sterilized. The vessel, filter, rubber stopper, and silicone tube are washed thoroughly with soap, soaked in a 10% sodium hypochlorite solution for 30 minutes, rinsed with distilled water, and allowed to dry. All components, including the syringe filter, are then placed in a polypropylene (PP) plastic bag and sterilized via autoclaving at 121°C for 40 minutes (Kryukov et al., 2022).

Media and sterilization
Murashige and Skoog (MS) media was used in this experiment, supplemented with agar for the conventional system and without agar for the temporary immersion system (TIS). MS media contains macronutrients, micronutrients, and vitamins to support explant growth during incubation. The media was sterilized by autoclaving at 121°C for 15 minutes (Hwang et al., 2022).

Explant inoculation
Explant inoculation was performed in a laminar air flow (LAF) cabinet. Prior to use, the LAF cabinet was sterilized by UV irradiation for 60 minutes and wiped down with 70% ethanol. Sterilized dissection tools (scissors and forceps), petri dishes, and sterile wipes were placed inside the LAF cabinet. Explants were cut into single-segment sections using the sterilized scissors and forceps, then 80 explants were transferred into each vessel. 200 mL of sterile media was added to the vessel, which was then sealed. The TIS was assembled in the incubation room by connecting the system to a pump and timer. The nutrient flow was programmed for 15-minute intervals every 3 hours for a duration of 35 days.

Chlorophyll analysis
Chlorophyll content analysis of plantlets was performed at the Biotechnology Laboratory, University of Muhammadiyah Malang, located at Jalan Raya Tlogomas No. 246, Malang, East Java. This analysis was conducted to determine the chlorophyll a, b, and total chlorophyll content of plantlets developed in temporary immersion systems (TIS) and conventional systems. Chlorophyll was extracted from leaf tissue using ethanol. Specifically, 0.5 g of potato plantlet leaves were placed in a mortar, macerated, and then combined with 10 mL of acetone before being filtered through filter paper. The resulting leaf extract was then analyzed using a spectrophotometer at wavelengths of 663 nm and 645 nm (Dullah et al., 2025). The data obtained were then applied to the following equation:
Chlorophyll b content = ((22.9 x A645)-(4.68 x A663)) x 0.02

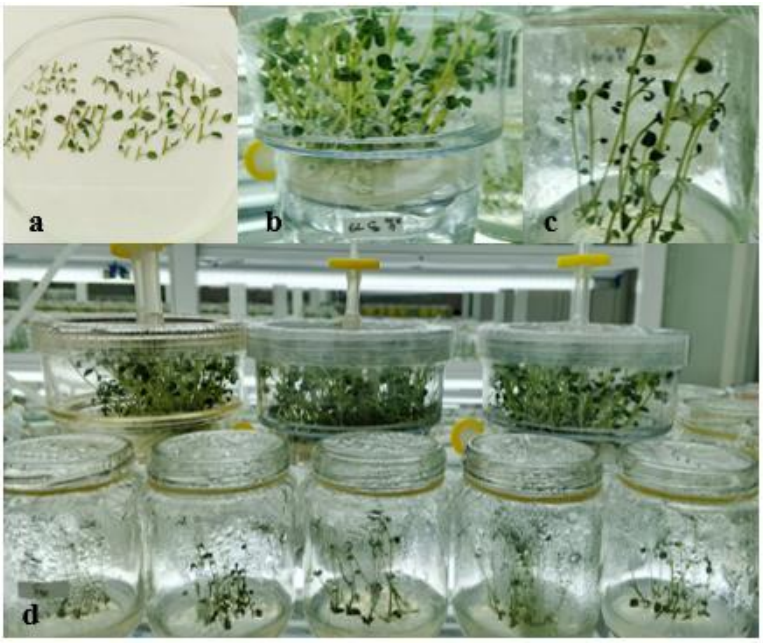

Fig 1. Potato plantlet growth; (a) Potato explants; (b) TIS; (c) Conventional system (semi-solid); (d) Comparison of growth in TIS with conventional system.

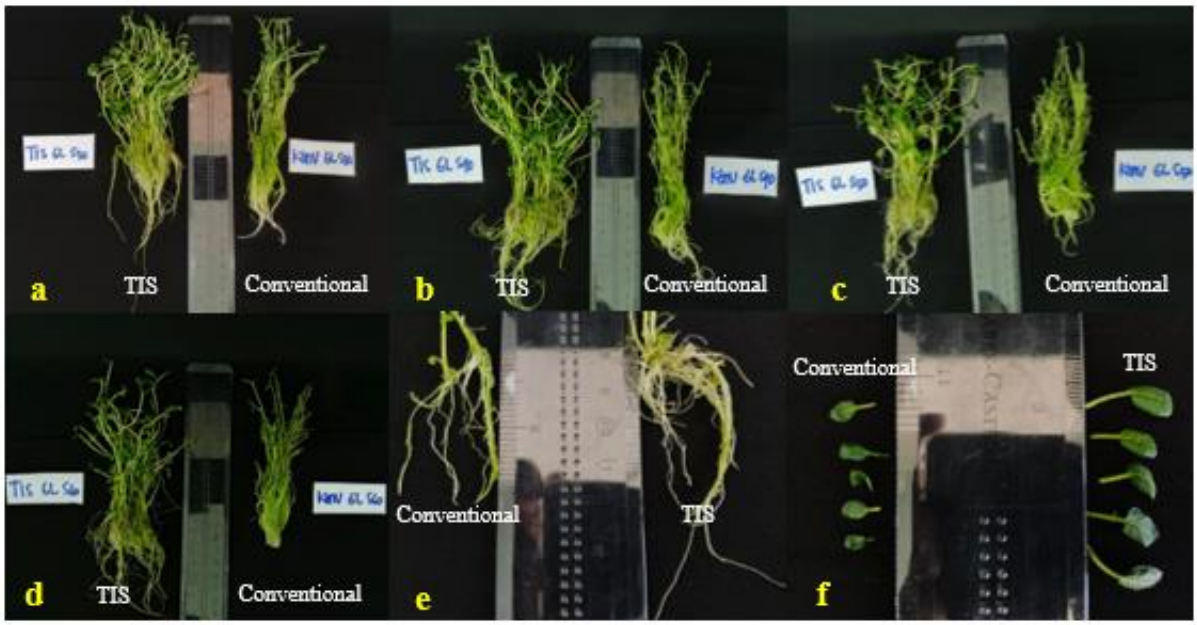

Fig 2. Morphology of potato plantlets in TIS and conventional systems; (a) 30 g L^{-1} sucrose treatment; (b) 40 g L^{-1} sucrose treatment; (c) 50 g L^{-1} sucrose treatment; (d) 60 g L^{-1} sucrose treatment; (e) Roots; (f) Leaves.

Total chlorophyll content = ((20.2 x A645)+(8.02 x A663)) x 0.02
In the equation, the symbol "A" stands for the wavelength used in the spectrophotometer.

Virus detection test

To detect viral presence, samples were tested using an enzyme-linked immunosorbent assay (ELISA). This assay was conducted to determine whether the resulting potato seed plantlets were infected with Potato Virus Y (PVY), Potato Virus S (PVS), Potato Virus X (PVX), or Potato Leaf Roll Virus (PLRV). The assay was performed at the Vegetable Plant Assembly and Modernization Center (BRMP) Testing Laboratory, located at Jalan Tangkuban Perahu No. 517, Lembang, West Java.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) at a 95% confidence level to determine significant differences between treatments. Where significant differences were indicated, a Duncan Multiple Range Test (DMRT) was performed at the 5% confidence level. The data are presented in tabular and figure format.

Conclusion

The results of this study indicate that potato seeds cultured in temporary immersion systems (TIS) exhibit superior quality compared to those developed in conventional culture systems. The addition of 30 g L^{-1} sucrose yielded significantly better results than higher sucrose concentrations. For applications requiring a greater number of shoots, the TIS + 50 g L^{-1} sucrose treatment is recommended for propagation. Potato seed plantlets propagated in both temporary immersion bioreactor systems (TIBS) and conventional culture systems were found to be free of major potato viruses (Potato Virus X, Potato Virus Y, Potato Virus S, Potato Leaf Roll Virus). Mass propagation of potato seed plantlets can be effectively achieved using temporary immersion systems (TIS).

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