agronomy

MDPI

Article

The Impact of the Growth Regulators and Cultivation Conditions of Temporary Immersion Systems (TISs) on the Morphological Characteristics of Potato Explants and Microtubers

Dias Daurov [1,2,*], Ainash Daurova [1], Zagipa Sapakhova [1,2], Rakhim Kanat [1,2], Dana Akhmetzhanova [1], Zhanar Abilda [1], Maxat Toishimanov [1], Nurgul Raissova [1], Murat Otynshiyev [1], Kabyl Zhambakin [1] and Malika Shamekova [1,2,*]

[1] Laboratory of Breeding and Biotechnology, Institute of Plant Biology and Biotechnology, Almaty 050040, Kazakhstan; a.daurova@ipbb.kz (A.D.); z.sapakhova@ipbb.kz (Z.S.); r.kanat@ipbb.kz (R.K.); d.akhmetzhanova@ipbb.kz (D.A.); z.abilda@ipbb.kz (Z.A.); m.toishimanov@ipbb.kz (M.T.); n.raissova@ipbb.kz (N.R.); m.otynshiev@ipbb.kz (M.O.); k.zhambakin@ipbb.kz (K.Z.)
[2] Tanir Research Laboratory, 75B Al-Farabi Avenue, Almaty 050060, Kazakhstan
* Correspondence: d.daurov@ipbb.kz (D.D.); m.shamekova@ipbb.kz (M.S.)

Abstract: Potatoes (*Solanum tuberosum* L.) constitute one of the most economically important annual crops. In terms of tissue culture, potato microtubers (MTs) have a number of advantages over conventional plants. These advantages include their small size, which greatly facilitates storage, transport, and germplasm exchange compared to in vitro plants. One effective solution for the production and mass propagation of healthy MTs is the use of temporary immersion systems (TISs). In this study, in a SETIS^{TM} system containing kinetin/gibberellic acid (GA)/indole-3-butyric acid (IBA) hormones, we investigated the effects of different nutrient media on the morphological characteristics of potato explants and MTs. We determined the optimal cycling duration (3 h) with an immersion frequency of 2 min. The results revealed that the optimal nutrient medium for culturing single-node potato explants in a SETIS^{TM} bioreactor was the M7 medium containing kinetin (2 mg/L), GA (0.5 mg/L), and IBA (0.5 mg/L). The optimal nutrient medium for obtaining potato MTs was the M1 medium (hormone-free) with a high concentration of sucrose (9%) at 18 °C under dark growing conditions. Thus, a universal nutrient medium, employed in a bioreactor, was selected for the mass propagation of potato MTs for both domestic and foreign potato varieties.

Keywords: potato (*Solanum tuberosum* L.); microtubers; temporary immersion system; varieties; nutrient medium; immersion frequency

Citation: Daurov, D.; Daurova, A.; Sapakhova, Z.; Kanat, R.; Akhmetzhanova, D.; Abilda, Z.; Toishimanov, M.; Raissova, N.; Otynshiyev, M.; Zhambakin, K.; et al. The Impact of the Growth Regulators and Cultivation Conditions of Temporary Immersion Systems (TISs) on the Morphological Characteristics of Potato Explants and Microtubers. *Agronomy* **2024**, *14*, 1782. https://doi.org/10.3390/agronomy14081782

Academic Editor: Valeria Cavallaro

Received: 10 July 2024
Revised: 30 July 2024
Accepted: 13 August 2024
Published: 14 August 2024

1. Introduction

According to the Food and Agriculture Organization (FAO) database, potatoes (*Solanum tuberosum* L.) are one of the most important food crops grown worldwide, with an annual global production of more than 470 million tons [1]. Potatoes play a key role in crop production, ensuring food security, notably in Kazakhstan, where up to 700 thousand tons of seed potatoes are required annually [2]. In addition to seed potatoes grown in the country, about 30 thousand tons of seed potatoes are imported each year, with about 80% of these coming from the Netherlands through private companies [3].

According to figures from 2023, potatoes occupied 187.8 thousand hectares in Kazakhstan, with the gross harvest amounting to 3788.1 thousand tons. At the same time, the 2023 yield was only 20.5 t/ha [2]. In industrial production, potatoes are vegetatively propagated using seed tubers, which makes them highly vulnerable to viral infections and seed-borne disease transmission [4].

Today, the use of healthy, virus-free seeds is one of the key objectives in successful potato production [5,6]. Certified seed systems typically start with minitubers, which are produced from healthy in vitro plants under controlled conditions. However, the main limiting factors for minituber seed potato production are the low multiplication rate and adaptation problems after transplantation into open fields [7]. Microtubers (MTs) are miniaturized seed materials weighing between 24 and 280 mg [8]. As MTs are produced under controlled conditions, the risk of contamination is very low, making them an ideal starting material for seed tuber production. MTs are suitable for medium-term storage and are therefore excellent multiplication materials [9]. They can also be planted directly into soil and grown all year round [10]. In vitro propagated potato seedlings are commonly used for tuber production in seed potato production programs [11]. In vitro MT production aids in addressing challenges such as virus-free seed potato storage, important variety conservation, and healthy genetic material sharing [10,11]. MTs can be produced, stored for a year, and then immediately transported to markets without the need for transplantation to a fresh environment and subsequent acclimatization [12]. Therefore, MTs have been proposed as an alternative source of first-generation seed potatoes derived from tissue culture; such an approach solves problems during the transplanting of vegetative seedlings from in vitro to in vivo conditions and addresses storage issues [13].

One of the solutions for producing healthy seed potatoes is the use of temporary immersion systems or bioreactors (TISs/TIBs), which are becoming increasingly popular among biotechnological methods for large-scale crop micropropagation [14]. These systems improve nutrient availability and prevent physiological changes and cell deformation [15]. This allows for optimizing the conditions for a specific culture and improving regeneration protocols by adjusting the immersion frequency, which is usually a few minutes. It is necessary to use bioreactors capable of providing adequate controlled conditions for the physiological growth and development of different types of cultures.

In recent years, many studies have been conducted using various immersion systems, including RITA® systems (Cirad, Vitropic, France) [16], temporarily immersed modular bioreactors BioMINT™ I and II (Getzersdorf, Austria) [17,18], the Monobloc Advance Temporary immersion bioreactor system MATIS® (CIRAD, Montpellier, France) [16], gravity immersion bioreactors (BIGs) [19], temporary immersion bioreactors (TIBs) [15,20], and SETIS™ systems (Vervit, Zelzate, Belgium) [21].

The SETIS™ system offers ample space for illumination and includes a silicone frame with a stainless-steel mesh (0.9 mm pore size), which facilitates the manipulation of different culture media. It also has a reservoir for storing up to 3 L of a culture medium [22]. These characteristics make this system an attractive option for potato cultivation and MT production [23]. The use of bioreactors presents significant opportunities. Among these, the temporary immersion system (TIS), which includes SETIS™ systems, has emerged as the most widely adopted choice [24,25]. TIS plant production can aid in addressing growing labor costs and worker shortages. It offers much faster and cheaper plant multiplication of a comparable or even better quality than agar cultures [19,26]. Additionally, the production time is much shorter. After four months of production using agar cultures, 195 plants can be produced starting from just 10 [27].

One of the key factors affecting the growth and development of potato plants in the bioreactor is the nutrient medium [20], various combinations and modifications of which contribute to the efficiency of potato plant microclonal propagation [20]. The formation of plants with a developed root system plays a key role in the process of clonal micropropagation. A strong root system has a positive effect, allowing more nutrients to be assimilated and MTs to be formed. An important aspect of developing a strong root system, key to the success of MT production, is the use of phytohormones to stimulate the rhizogenesis process in single-node potato cuttings. In one study, many MTs were generated on nutrient media containing a high concentration of sucrose [8]. Meanwhile, morphological characteristics such as plant height (PH), root length (RL), number of internodes (NNs), weight per

plant (WP), microtuber diameter (DM), number of microtubers per plant (NMP), and total microtuber weight (TW) were highly dependent on potato genotype [28].

Many studies have been carried out to determine explant types, as well as the influence of nutrient media on MT formation [29,30]. Practically speaking, for each variety, it is necessary to select the optimal conditions for cultivating potato plants. However, there are no studies describing the mass production of healthy potato seed material on a single optimized nutrient medium for cultivating explants and MT production.

Due to the low quality of domestic seed and planting material, as well as the inadequate national controls, 95% of potato seeds in Kazakhstan come from abroad [31].

The objective of this study was to develop an optimal protocol for the mass production of potato MTs in the SETIS^{TM} system for both domestic and foreign potato varieties and to evaluate their morphological characteristics.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The plants of 8 potato varieties were used as plant material: 4 varieties of Dutch origin (Minerva, Romano, Aladdin, and Soprano), 2 varieties of German origin (Gala and Natasha), and 2 varieties of domestic origin (Aksor and Tobol). Potato plants were obtained as described previously [32]. Potato plants were grown under the following conditions: 25 °C, 16 h/8 h (light/dark) light cycle, 244 µmol m^{-2} s^{-1} of warm white lights, and 50% relative humidity. Figure 1 shows a flowchart detailing the protocol's various steps.

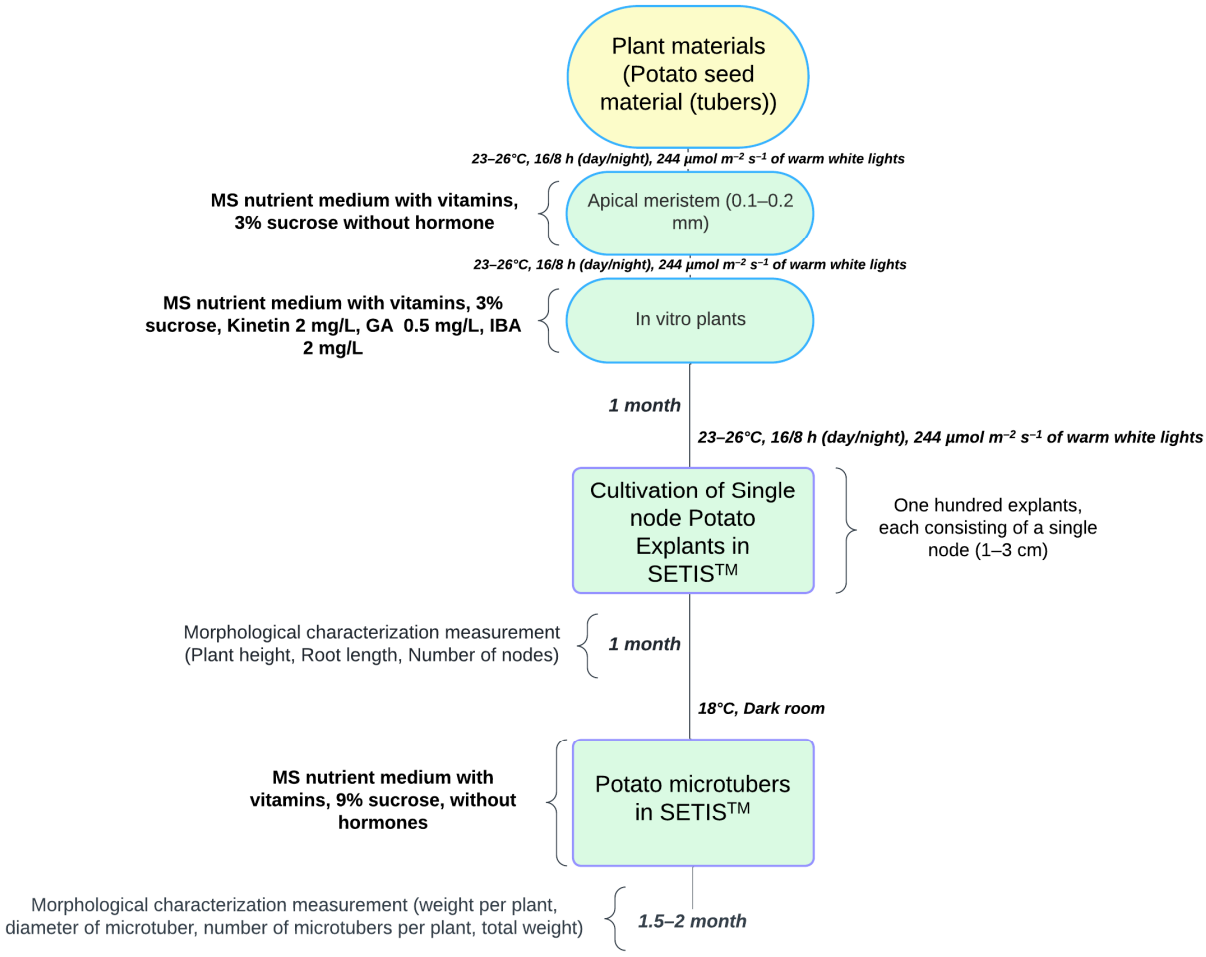

Figure 1. Flowchart detailing the different production steps for potato microtubers in a SETIS^{TM} system.

2.2. Apical Meristem Cultivation

Potato tubers were grown under controlled conditions in a greenhouse at 23–26 °C with a 16/8 (day/night) light regime, 244 µmol m^{-2} s^{-1} of warm white lights, and 50–60% humidity. After the plants reached 20 cm, the shoot tops were cut off, washed under running water, and disinfected with 1:1 bleach solution (2.5% active chlorine) for 10 min in a laminar flow chamber. The explants were then washed with 70% ethyl alcohol (EtOH) for 5 s, after which the explants were washed 5 times with sterile distilled water. The shoot apical meristem, consisting of the apical cone and one to two leaf primordia, was isolated under a microscope (Premiere SMZ-05, USA) in a laminar flow chamber (ESCO, Changi, Singapore). Isolated apical meristems of 0.1–0.2 mm were transplanted onto 4.43 g/L of a Murashige and Skoog (MS) nutrient medium with vitamins (PhytoTechnology Lab, Lenexa, KS, USA) [33], containing 0.8% agar (Santa Cruz Biotechnology, Dallas, TX, USA), 3% sucrose (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 2 mg/L kinetin (AppliChem GmbH, Darmstadt, Germany), and 40 mg/L ribavirin (PhytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany); the pH was adjusted to 5.8–6.0 before autoclaving. Apical meristems underwent weekly subculturing onto the MS nutrient medium as described above. After one month, the obtained regenerants were transplanted onto 4.43 g/L of the MS nutrient medium with vitamins and 3% sucrose without hormones; the pH was adjusted to 5.8–6.0 before autoclaving. Apical meristems were grown under the following conditions: 25 °C, 16 h/8 h (light/dark) light cycle, 244 µmol m^{-2} s^{-1} of warm white lights, and 50% relative humidity. After one month, the obtained regenerants were cloned for the further propagation and cultivation of single-node explants in the SETIS^{TM} system.

2.3. Cultivation of Single-Node Potato Explants in SETIS^{TM}

One hundred explants, each consisting of a single node (1–3 cm) obtained from cloned regenerants, were cultured for 1 month in sterile SETIS^{TM} system (Vervit, Zelzate, Belgium) vessels consisting of two parts (culture and nutrient), each with 1000 mL of the nutrient medium. The medium contained 4.43 g/L of the Murashige and Skoog medium with vitamins [33], 3% sucrose, 2 mg/L kinetin, 0.5 mg/L IBA (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and 0.5 mg/L GA (PhytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany); the pH was adjusted to 5.8–6.0 before autoclaving (Table 1). A SETIS^{TM} bioreactor connected to an automatic air pressure regulator (0.5 bar) was placed under temperature conditions of 23–26 °C, 16/8 h (day/night) light cycle, and 244 µmol m^{-2} s^{-1} of warm white lights. The nutrient medium was replaced three times a month. To select the optimal nutrient conditions for PH, RL, and NN, the Gala variety, which is widely cultivated in Kazakhstan and abroad, was selected. The Gala variety was cultured using seven different nutrient media of MS, with different cycles (1, 3, and 5 h) at immersion frequencies of 2 min, and the addition of kinetin, GA, and IBA.

Table 1. Nutrient media for potato explant cultivation.

Media	Hormones (mg/L)			TIS—Immersion Frequency (2 min Immersion)
	Kinetin	GA	IBA	
M1	0	0	0	1 h 3 h 5 h
M2	0	0.5	0	1 h 3 h 5 h
M3	2	0	0	1 h 3 h 5 h

Table 1. *Cont.*

Media	Hormones (mg/L)			TIS—Immersion Frequency (2 min Immersion)
	Kinetin	GA	IBA	
M4	2	0.5	0	1 h 3 h 5 h
M5	0	0	0.5	1 h 3 h 5 h
M6	0	0.5	0.5	1 h 3 h 5 h
M7	2	0.5	0.5	1 h 3 h 5 h

2.4. Potato MTs in SETIS™

After 1 month of culturing, the nutrient medium was replaced in order to obtain potato MTs (Table 2). The SETIS™ bioreactor was placed in a dark room at 18 °C and connected to an automatic air pressure regulator (0.5 bar). The explants were cultured for 1.5–2 months until MTs formed. The nutrient medium was changed three times per month. After optimizing the immersion frequency (3 h) and selecting the optimal nutrient medium for cultivating single-node explants (M7) and forming potato microtubers in a SETIS™ bioreactor (M1), we introduced seven more potato varieties.

Table 2. Nutrient media for obtaining potato microtubers.

Media	Hormones (mg/L)		TIS—Immersion Frequency (2 min Immersion)
	Kinetin	IBA	
M1	0	0	1 h 3 h 5 h
M2	0	2	1 h 3 h 5 h
M3	2	0	1 h 3 h 5 h
M4	2	2	1 h 3 h 5 h

2.5. Statistical Analysis

Significant results were tested using two-way analysis of variance (ANOVA) and a post hoc Duncan's Multiple Range Test [34]. The a priori significance level was established at $p < 0.05$. A principal component analysis (PCA) was performed using the JMP pro 17 software (JMP Statistical Discovery LLC, Cary, NC, USA) and SPSS Statistics 27 software (IBM) software packages. A Pearson's correlation analysis was completed using the ggcorr function, prepared using the GGally package Ver. 2.2.1 in RStudio version 2024.04.2+764 software (www.rstudio.com, accessed on 13 August 2024) developed by the R Core Team [35]. All of the data analyses were performed for three biological repeats ($n = 100$) per treatment (media/immersion frequency), and the values shown in the figures represent the average values of these. Sample variability is given as the mean ± SD.

3. Results

3.1. Selection of the Optimal Nutrient Medium for Cultivating Single-Node Potato Explants in SETIS™

As shown in Figure 2, higher values for plant PH, RL, and NN were observed when immersed in the nutrient medium every 3 h (Figure 2D–F). Thus, with the M7 nutrient medium, the PH reached 17.23 ± 1.2 cm, the RL was 29.9 ± 1.8 cm, and the NN was 12.6 ± 0.5 pcs. This was followed by the M4 and M6 nutrient media, where the PH was 12.7 ± 0.9 cm and 14.6 ± 0.6 cm, the RL was 26.2 ± 1.5 cm and 26.2 ± 1.4 cm, and the NN was 9.0 ± 0.0 pcs and 8.6 ± 0.5 pcs, respectively.

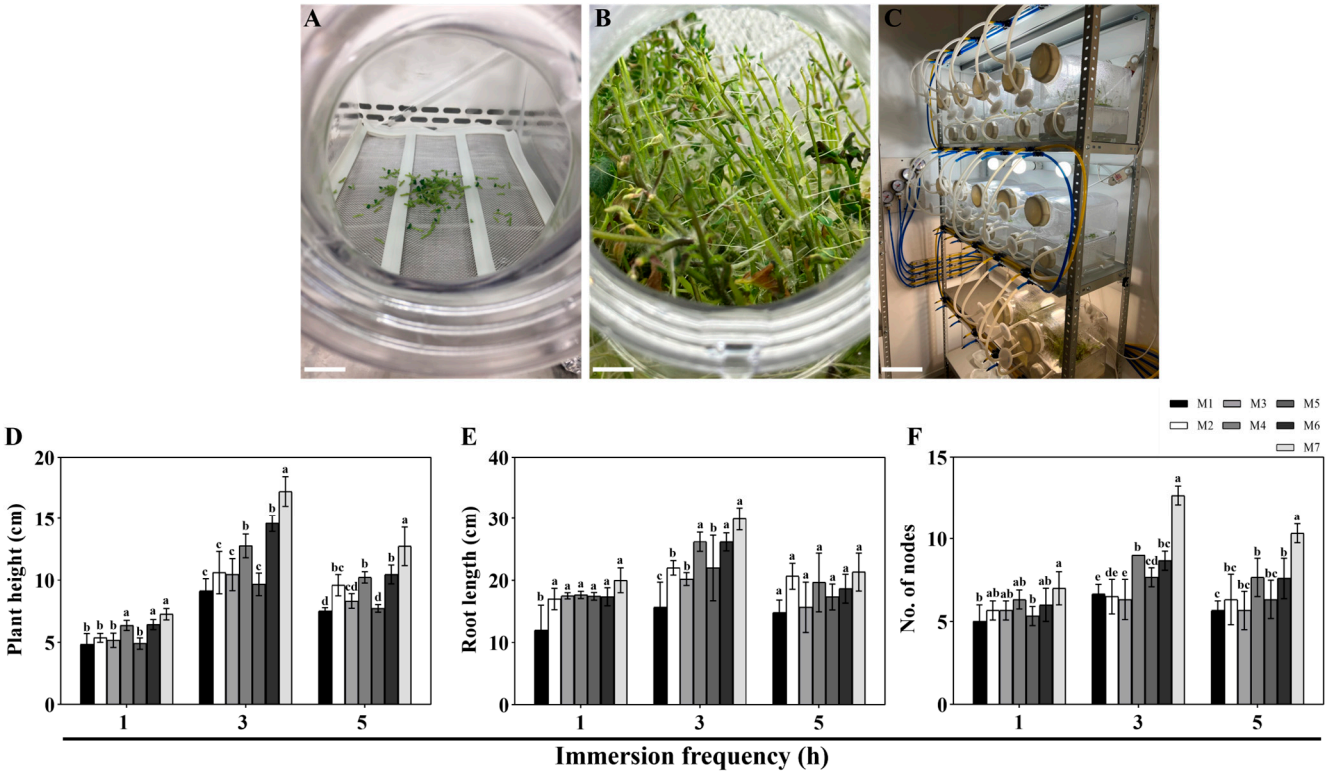

Figure 2. Morphological characteristics of potato plants depending on different nutrient media and immersion frequencies of Gala potato variety. (**A**) Single-node potato explants obtained from one-month cloned regenerants (1–3 cm). (**B**) Active growth of potato plants (2–3 weeks). (**C**) Rack with SETIS™ bioreactors (3–4 weeks). (**D**) Plant height. (**E**) Root length. (**F**) Number of nodes. Data are means ± standard deviations of three biological replicates. M1 (without hormones), M2 (kinetin 0 mg/L, GA 0.5 mg/L, IBA 0 mg/L), M3 (kinetin 2 mg/L, GA 0 mg/L, IBA 0 mg/L), M4 (kinetin 2 mg/L, GA 0.5 mg/L, IBA 0 mg/L), M5 (kinetin 0 mg/L, GA 0 mg/L, IBA 0.5 mg/L), M6 (kinetin 0 mg/L, GA 0.5 mg/L, IBA 0.5 mg/L), and M7 (kinetin 2 mg/L, GA 0.5 mg/L, IBA 0.5 mg/L). Scale bar: 1 cm. According to the Duncan test, different letters above the bars indicate significant differences ($p < 0.05$).

The results showed that immersion for 1 and 5 h resulted in low PH, RL, and NN. In comparison, after 3 h of immersion, the PH value of the M7 nutrient medium was 57.8 and 26.1% higher than those after 1 and 5 h of immersion, respectively. The RL after 3 h immersion was about 30% higher than that after 1 and 5 h of immersion, respectively, whereas the NN was 44.7 and 18.4% higher than that at 1 and 5 h of immersion, respectively.

The lowest PH, RL, and NN of all the nutrient media were in the hormone-free (control) medium M1 regardless of immersion time.

The results of the two-factor analysis of variance revealed a strong significance ($p < 0.001$) in terms of the nutrient media (M) and immersion frequency (I) regarding PH (26.7 and 61.4%), RL (34.9 and 34.7%), and NN (48.3 and 24.5%). The M × I interaction

showed a significant relationship ($p < 0.001$) with PH (5.9%) and NN (11.2%), but there was no significant difference in RL (9.2%) (Table S1).

The principal component analysis (PCA) confirmed that, after 3 h of immersion, nutrient medium M7 resulted in the maximum potato explant growth and development. Figure 3A shows that the PCA explained 96.5% of the total variation, with PC1 explaining 91.1% and PC2 explaining 5.4%. The Pearson's correlation analysis showed that nutrient media and immersion frequency were positively correlated with PH, RL, and NN (Figure 3B). A strong correlation was observed between PH and RL (r = 0.714, $p < 0.001$), PH and NN (r = 0.789, $p < 0.001$), and RL and NN (r = 0.686, $p < 0.001$).

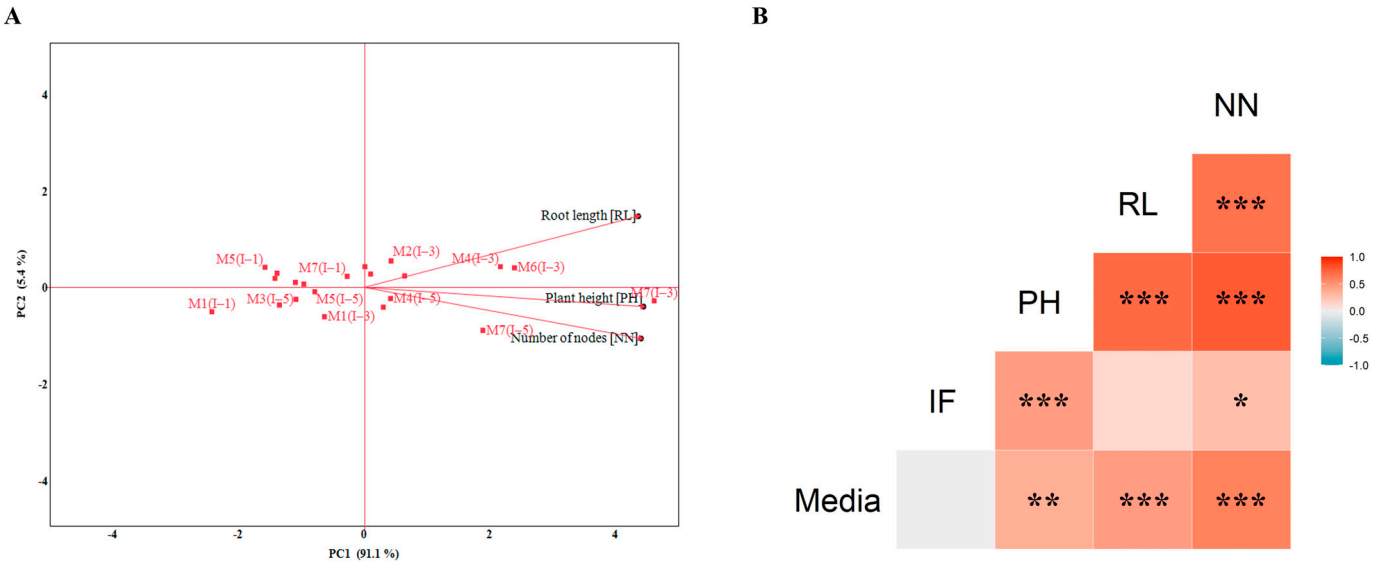

Figure 3. Principal component analysis (PCA) (**A**). (**B**) Pearson's correlation analysis examining morphological characteristics under different nutrient media and immersion frequencies. The blue and red colors correspond to positive and negative correlations, respectively. IF—immersion frequency; PH—plant height; RL—root length; and NN—number of nodes. *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

Thus, we concluded that, by immersing potato explants every 3 h and adding kinetin/GA/IBA hormones, it is possible to obtain full-grown plants with a well-developed root system.

3.2. Selection of the Optimal Nutrient Medium for Potato MT Formation in SETIS^{TM}

Similarly to the optimization of nutrient media for cultivating single-node potato cuttings, we conducted an experiment to select the optimal nutrient media for potato MT formation in a SETIS^{TM} bioreactor. Microtuber WP, DM, NMP, and TW were selected as the main morphological characteristics to be analyzed. Again, we used the Gala variety and cultivated it on four different MS nutrient media with different cycles (1, 3, and 5 h) and 2 min immersions with the addition of kinetin and IBA (Figure 4).

It was found that the best microtuber WP, DM, NMP, and TW of the MTs were achieved with a 3 h immersion frequency and M1 nutrient medium (Figure 4C–F). With nutrient medium M1 (without hormones), the WP reached 1.6 ± 0.03 g, the NMP was 8.33 ± 0.5 pcs, and the TW of the MTs was 34.0 ± 1.7 g. However, the DM was the smallest, amounting to 1.3 ± 0.3 cm in M1 and M2, whereas DM values of 1.7 ± 0.05 cm and 1.8 ± 1.1 cm were observed in M3 and M4, respectively.

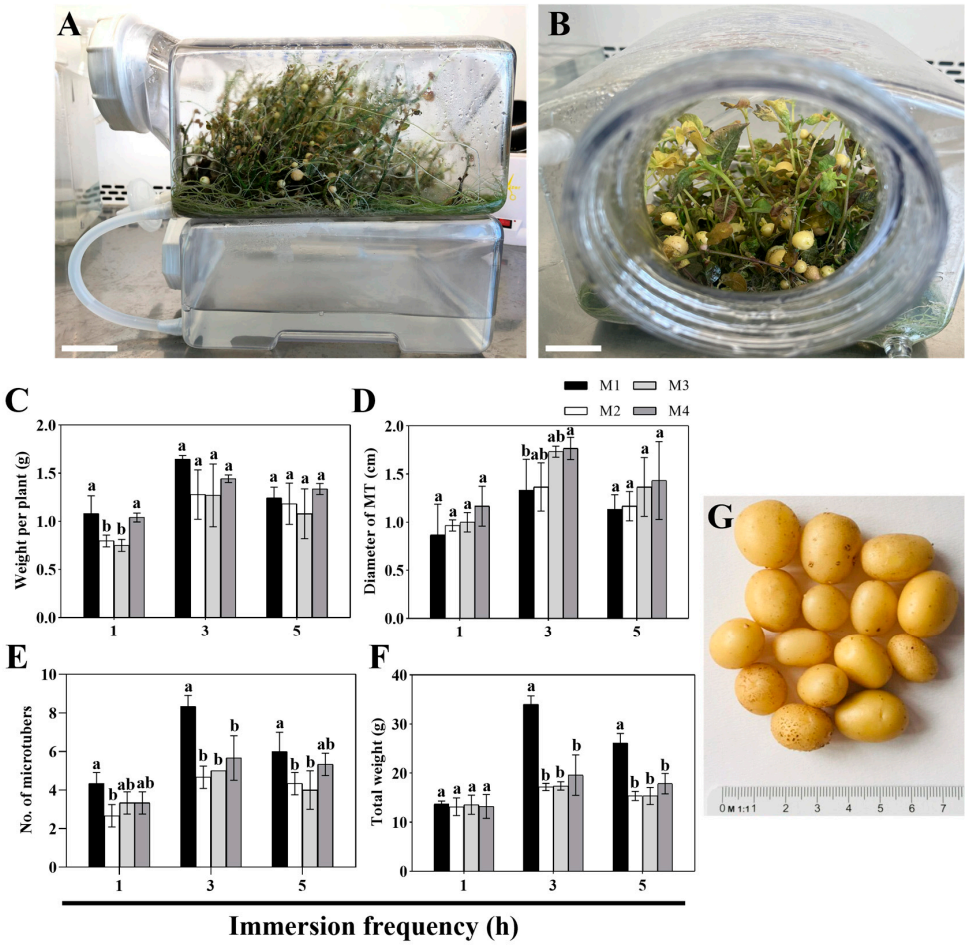

Figure 4. Morphological characteristics of potato plants depending on different nutrient media and immersion frequencies of Gala potato variety. (**A**) Potato microtubers in SETIS^{TM} bioreactor (2–3 weeks). (**B**) Microtuber formation in SETIS^{TM} bioreactor (3–4 weeks). (**C**) Weight of microtubers per plant. (**D**) Diameter of MT. (**E**) Number of microtubers per plant. (**F**) Total weight. (**G**) Harvested microtubers (4 weeks). Data are means ± standard deviations of three biological replicates. M1 (without hormones), M2 (kinetin 0 mg/L, IBA 2 mg/L), M3 (kinetin 2 mg/L, IBA 0 mg/L), and M4 (kinetin 2 mg/L, IBA 2 mg/L). Scale bar: 1 cm. According to the Duncan test, different letters above the bars indicate significant differences ($p < 0.05$).

In the M1 nutrient medium, the microtuber WP values at immersion frequencies of 1 and 5 h were 24–34% lower than that at 3 h immersion. The NMP in M1 was 28 and 48% higher compared with 1 and 5 h of immersion. The TW of the MTs for a 3 h immersion was 23 and 60% higher. The DM was 0.8–1.3 cm in most varieties.

The two-factor analysis revealed a strong significance between the nutrient media and microtuber WP (19.2%) and DM (18.2%) ($p < 0.01$), as well as between the nutrient media and the NMP (34.4%) and TW (39%) of the MTs ($p < 0.001$). Immersion frequency also showed a strong significance ($p < 0.001$) with all the parameters, namely microtuber WP (52.5%), DM (46.2%), NMP (43.8%), and TW (33.3%) (Table S2). However, nutrient media (M) and immersion frequency (I) did not have a significant effect on the microtuber WP (3.6%), DM (2.6%), and NMP (7.9%), although they had a strongly significant effect on the TW (20.9%) of the MTs. Thus, we concluded that, with an immersion frequency of 3 h and the addition of kinetin/IBA hormones, potato MTs can be produced en masse in a SETIS^{TM} bioreactor.

The principal component analysis (PCA) confirmed that, among the four media, nutrient medium M1 at an immersion frequency of 3 h was the most effective in producing potato MTs. Figure 5A shows that the PCA explained 96.9% of the total variation, with PC1

explaining 75.5% and PC2 explaining 21.4%. The Pearson's correlation analysis showed a negative correlation between the nutrient media and microtuber WP, NMP, and TW, whereas immersion frequency showed a positive correlation with all the parameters studied (Figure 5B). A strong correlation was observed between the WP and NMP (r = 0.756, $p < 0.001$) and the NMP and TW (r = 0.828, $p < 0.001$).

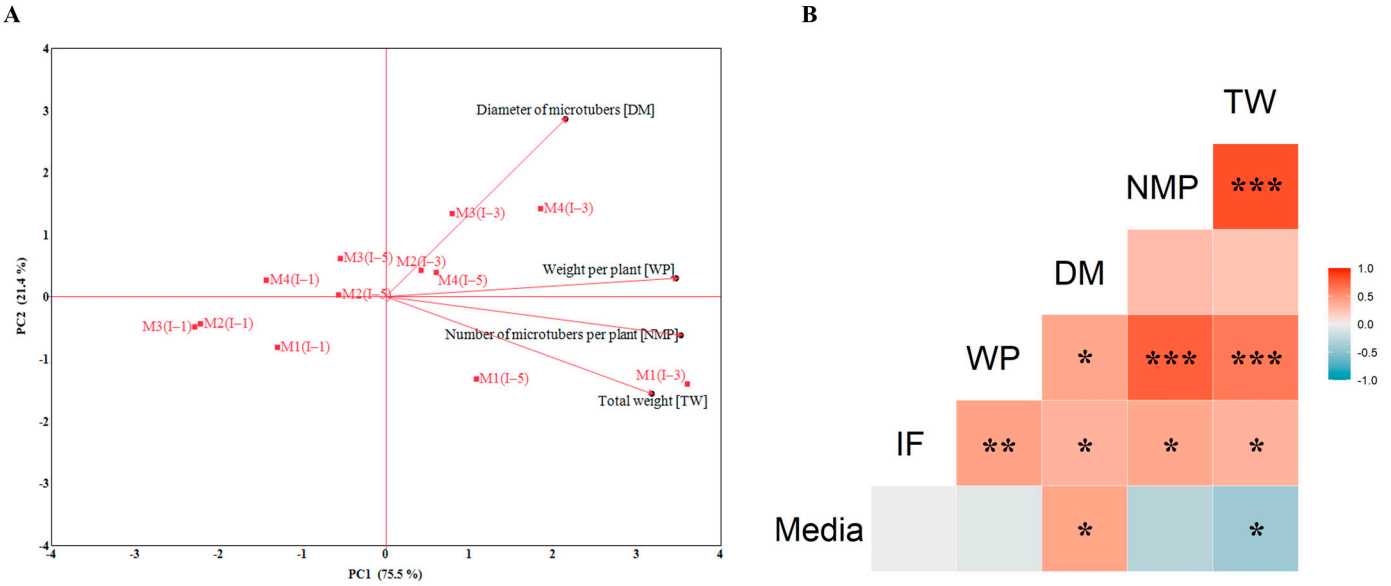

Figure 5. Principal component analysis (PCA) (**A**). (**B**) Pearson's correlation analysis between morphological characteristics under different nutrient media and immersion frequencies. The blue and red colors correspond to positive and negative correlations, respectively. IF—immersion frequency; WP—weight of microtubers per plant; DM—diameter of microtuber; NMP—number of microtubers per plant; and TW—total weight. *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

As a result of our work on MT production, we found that the hormone-free nutrient medium was the best for MT formation.

3.3. Introduction of Potato Varieties into SETIS^{TM}

As can be seen from Figure 6, almost all potato varieties had similar PH, RL, and NN. PH and RL were the highest in the Romano variety, at 17.2 ± 1.0 cm and 31.4 ± 1.4 cm, respectively (Figure 6A,B). However, the maximum number of internodes (NN) was observed in the Natasha variety, with 14.3 ± 0.5 pcs (Figure 6C), while the minimum PH was found in the Minerva and Aladdin potato varieties, with 14.5 ± 0.7 cm and 15.2 ± 0.6 cm, respectively. An RL of 26.2 ± 1.9 cm and 26.7 ± 2.6 cm was observed in the Aladdin and Natasha varieties, respectively.

The microtuber WP values did not differ significantly among all potato varieties (Figure 6D). There was variation in the DM values; this was largest in the Aksor variety (1.6 ± 0.2 cm), followed by the Soprano and Natasha varieties at 1.5 ± 0.15 cm and 1.4 ± 0.2 cm, respectively (Figure 6E). There was also a slight difference in the NMP, whereby the highest values were for the Aladdin variety (9.33 ± 0.5 pcs) and the lowest values for the Tobol and Minerva varieties (7.33 ± 0.5 pcs) (Figure 6F). The TW in all varieties ranged from 31.9 to 37.5 g (Figure 6G).

According to the PCA, the first two components, PC1 (34.9%) and PC2 (25.4%), made the most significant contribution, accounting for 60.3% of the total variance in the potato morphological parameters and thus confirming the trend in the results (Figure 7A). The results revealed that the optimized nutrient medium and immersion frequency had the most significant effect on varieties such as Aksor, Soprano, Natasha, Romano, and Minerva; they had a less significant effect on the Aladdin and Tobol varieties. A high positive correlation was observed between PH and DM (r = 0.483), RL and TW (r = 0.501), and NN

and WP (r = 0.734), whereas a negative correlation was identified between RL and NMP (r = −0.515), WP and NMP (r = −0.533), and DM and TW (r = −0.547) (Figure 7B).

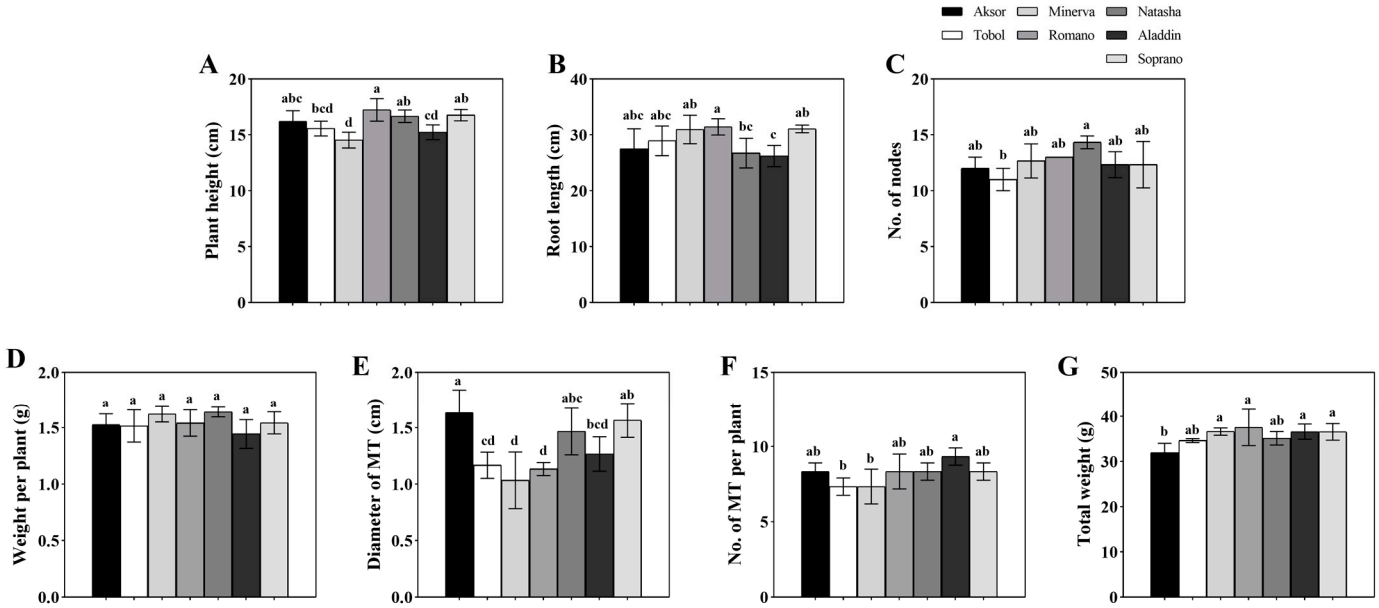

Figure 6. Morphological characteristics of plants of seven potato varieties. (**A**) Plant height. (**B**) Root length. (**C**) Number of nodes. (**D**) Weight of microtubers per plant. (**E**) Diameter of microtuber. (**F**) Number of microtubers per plant. (**G**) Total weight. All morphological characterization data were collected after 4–5 weeks of culturing in the SETIS^{TM} system. Data are means ± standard deviations of three biological replicates. According to the Duncan test, different letters above the bars indicate significant differences ($p < 0.05$).

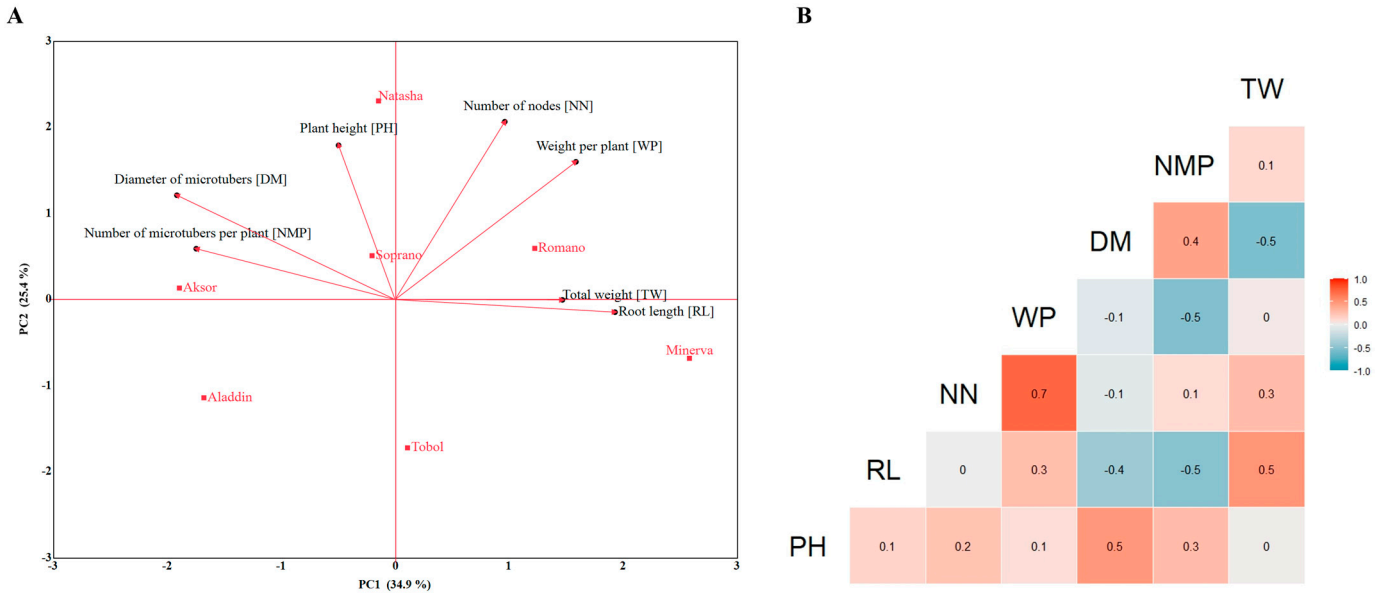

Figure 7. Principal component analysis (PCA) (**A**). (**B**) Pearson's correlation analysis between morphological characteristics under different nutrient media and immersion frequencies. The blue and red colors correspond to positive and negative correlations, respectively. IF—immersion frequency; PH—plant height; RL—root length; NN—number of nodes; WP—weight of microtubers per plant; DM—diameter of microtuber; NMP—number of microtubers per plant; and TW—total weight.

4. Discussion

4.1. Selection of the Optimal Nutrient Medium for Cultivating Single-Node Potato Explants in SETIS™

Potatoes (*Solanum tuberosum* L.) are considered the fourth largest staple crop after rice, wheat, and corn. As such, potato growing is one of the key branches of crop production dictating food security in Kazakhstan [31,36]. In vitro culture has become of great importance in potato maintenance and selection and in creating a healthy bank of varieties [37–40]. As is widely known, the basis of modern, elite potato seed production in developed economy countries is the production of high-quality pre-basic healthy material [41]. The use of isolated apical meristems and clonal micropropagation in in vitro methods allows us to ensure potato varieties are free from viral infections [42].

Time immersion bioreactors are a valuable option for producing potato microtubers. This method not only induces more tubers than using a solid medium but also increases tuber size and weight [43]. Using time immersion bioreactors improves the quality and reduces the costs of seed potato cultivation [44]; yields and productivity in seed and commercial potato farms can thus be increased. Temporary immersion systems can also be used for the mass multiplication of regenerant plants during the planting season. This technology opens up new possibilities for commercial laboratories involved in potato seed production, as the resulting microtubers support long-term storage and can be transplanted without the need for acclimatization. SETIS™ bioreactors offer many advantages over other methods of culturing plant tissue. These advantages include reducing the risk of contamination, saving costs on gelling agents, and providing a homogeneous nutrient medium. Erol et al. reported that bioreactor systems performed better at the rooting stage compared to solid culture [45]. However, this technology requires further modification in order to improve and increase potato tuber yield by optimizing the nutrient media, cultivation conditions, and immersion frequencies.

Liquid cultures are currently considered more effective for a number of species, including potato MT production, compared to semi-solid nutrient media [46–48]. Recent studies have shown that bioreactors can be successfully used for potato micropropagation [10,49] and that they can also be easily used in liquid cultivation for the mass propagation of vegetable, woody, fruit, ornamental, and medicinal plants [50,51].

In this study, we took single-node potato segments and placed them in a SETIS™ bioreactor. A previous study [9] showed that single-node potato segments are more effective than double-node segments in potato MT formation. We optimized various nutrient media and examined their effects on plant growth and development in a SETIS™ bioreactor. Thus, we studied the effects of seven MS nutrient media with kinetin, GA, and IBA hormones, aiming to explore their influence on morphological characteristics like PH, RL, and NN.

As a result, it was revealed that potato plants cultivated with the M7 nutrient medium and kinetin/GA/IBA hormones had well-developed aerial parts and root systems. In general, and based on our results, it was found that kinetin/GC or GC/IMC had a positive effect on the growth and development of potato explants. In their absence, however, few morphological characteristics were observed. According to the results of the two-factor analysis of variance, morphological indices of PH, RL, and NN on the M7 nutrient medium showed a strong significance ($p < 0.001$) compared to others. In this study, we used IBA as an auxin, facilitating the obtaining of complete potato plants in a SETIS™ bioreactor. In comparison to IAA (indole-3-acetic acid), IBA is much more effective in inducing lateral and adventitious plant roots [52], stimulating the accelerated growth and elongation of the stem. Previous studies confirm that the presence of various auxins and cytokinins in nutrient media has a positive effect on potato plant root formation, growth, and development [53,54]. Currently, research in this field is focused predominantly on the effects of kinetin [55], BAP [29], GA [56], chlorochlorine chloride (CCC) [57], zeatin [58], or a combination thereof [59,60].

For the purpose of discovering the optimal growth and explant culture in the bioreactor, we used different immersion frequencies (1, 3, and 5 h), with the results showing

that an immersion frequency of 3 h significantly influenced all of the studied morphological characteristics ($p < 0.001$). Our data are consistent with previous research on the different immersion frequencies in various crops [15,61,62], including potatoes [26], and the explant immersion time was found to have influenced the morphological characteristics. It is known that overly long immersion causes hyperhydricity in plants [63], while overly brief immersion has been shown to be insufficient for potato explant growth and development [15].

4.2. Selection of Optimal Nutrient Medium for Potato MT Formation in SETIS^{TM}

We carried out work to optimize four nutrient media for the Gala potato variety. The hormones kinetin and IBA were used to optimize potato MT production, in addition to different immersion cycles (1, 3, and 5 h) with an immersion frequency of 2 min, in order to obtain MTs in a SETIS^{TM} bioreactor. Based on the results, it was revealed that a hormone-free nutrient medium (M1) with a high sucrose concentration (9%) was most effective for MT production. An immersion cycle of 3 h was optimal for MT formation. The studied morphological characteristics were microtuber WP, DM, NMP, and TW, and a strong correlation was revealed between the nutrient medium, and WP and DM ($p < 0.01$) and between the nutrient medium, and NMP and TW ($p < 0.001$).

Today, many researchers are studying the effects of hormones on MT formation, with hormones having a significant influence on tuber initiation and growth in potato plants [58,64,65]. Therefore, the effects of various growth regulators on MT reproduction have been extensively studied, showing that the number of MTs increases significantly in the presence of kinetin [66], 6-Benzylaminopurine (BAP) [67], and chlorochlorine chloride (CCC) [68] when rapid MT production is the goal. Moreover, the addition of succinic acid with BAP [64,69], zeatin [70], and thidiazuron [71] has similar effects on plant development to that of kinetin [72], thus promoting MT formation and growth. In our study on the Gala variety, MT formation did not require the addition of hormones. The data in this study are consistent with that of previous research, confirming that a hormone-free nutrient medium promotes an increase in the number of MTs in potatoes [73]. Many studies indicate that low concentrations of sucrose reduce the MT growth rate [74], while a high sucrose concentration, on the contrary, has a positive effect on growth and the amount of potato MT formation [75]. Some studies reported a significant difference in the immersion time of potato plants in obtaining MTs, which significantly affected the morphological characteristics [76]. In our case, the immersion cycle also had a strong correlation ($p < 0.001$) with WP, DM, NMP, and TW. There are many studies on the optimization of the immersion frequency in SETIS^{TM} bioreactors, both in potatoes [77,78] and in various other crops [21,79,80].

4.3. Introduction of Potato Varieties into SETIS^{TM}

An optimized nutrient medium that allows potatoes to grow regardless of their genotype and origin is needed for the mass propagation of potatoes. Previous studies have mainly examined the effect of growing media on a specific potato genotype [81,82]. Thus, after optimizing nutrient media for cultivating single-node explants and optimizing potato MT production in a bioreactor, we introduced seven genotypes into a SETIS^{TM} bioreactor. Thereafter, the morphological characteristics of potatoes of various origins were studied. According to the results, it was revealed that the PH of the Romano, Soprano, and Natasha genotypes was the highest at 17.2 ± 1.0 cm and 16.7 ± 0.5 cm. The highest RL values were demonstrated by the genotypes Romano, Soprano, and Minerva at 31.4 ± 1.4 cm, 31.0 ± 0.7 cm, and 31.0 ± 2.5 cm, respectively. With respect to the NN indicator, the Natasha genotype was identified as having a value of 14.3 ± 0.5 pcs. Many researchers report the use of sucrose for enhanced in vitro potato tuberization, especially in high concentrations [83,84]. In this study, we used nutrient media with 9% sucrose to obtain MTs. These were then studied for their morphological characteristics: WP, DM, NMP, and TW. The WP value for all genotypes was in the range of 1.4–1.6 g. DM varied and ranged between

1.0 and 1.6 cm across all genotypes; similar results are reported in other studies [85–87]. The maximum NMP value was recorded for the Aladdin genotype, with approximately nine pieces, while the minimum number was in the Tobol genotype (seven pieces). The highest TW value was 37.5 ± 4.0 g in the Romano genotype; this was about 36.6 g in the Minerva, Aladdin, and Soprano genotypes. These results are in agreement with recent studies where a high sucrose content (8%) increased the germination rate, size, and number of microtubers compared to low concentrations of sucrose (non-osmotic) [88]. According to the PCA, the lowest indicator values for all studied morphological characteristics were in the Aladdin and Tobol genotypes. The Pearson's correlation analysis showed a high positive correlation among the potato genotypes, which had r = 0.734 between the number of internodes and the WP. RL was also positively correlated (r = 0.501) with TW. NN and RL are important for obtaining potato MTs [89]. According to our results, a negative correlation was identified between the NMP and RL (r = −0.515), as well as the WP (r = −0.533). Some studies found no significant difference between NMP and RL, including TW [90,91].

5. Conclusions

The obtained results indicate that, for most genotypes, SETIS^{TM} is an effective system for producing mass quantities of potato MTs. Our research outcomes show that it is possible to obtain fully fledged plants with a well-developed root system in 2–3 months by using a nutrient medium, the M7 medium (kinetin/GA/IBA) and the M1 medium (without hormones), with a high sucrose concentration (9%), which is effective for mass MT production. A 2 min immersion frequency and a 3 h nutrient cycle are optimal for most potato genotypes. Thus, for both domestic and foreign potato varieties, the obtained results can be used as a universal method for mass MT production. In the future, further studies will provide an alternative to the commercial cultivation of not only potatoes but also other economically important plants.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14081782/s1, Table S1: Two-way ANOVA indicating effect of different nutrient media (M), immersion frequency (I), and their interactions (M × I) on plant height (PH), root length (RL), and number of nodes (NN) of Gala potato variety; Table S2: Two-way ANOVA indicating effect of different nutrient media (M), immersion frequency (I), and their interactions (M × I) on weight per plant (WP), diameter of microtuber (DM), number of microtubers per plant (NMP), and total weight (TW) of Gala potato variety.

Author Contributions: Conceptualization, K.Z. and M.S.; data curation, R.K., Z.A. and M.T.; formal analysis, D.D., A.D., R.K., M.T. and M.O.; funding acquisition, K.Z.; investigation, Z.S., D.A., Z.A., N.R. and M.O.; methodology, D.D., A.D., D.A., Z.S. and N.R.; software, M.T.; project administration, M.S.; resources, Z.S.; supervision, M.S.; visualization, D.D., D.A. and M.S.; writing—original draft, D.D.; writing—review and editing, A.D. and K.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan (grant No. AP14870410 and program No. BR18574099).

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Food and Agriculture Organization of the United Nations. Available online: https://www.fao.org (accessed on 3 July 2024).
2. Bureau of National Statistics. Agency for Strategic Planning and Reforms of the Republic of Kazakhstan. Available online: https://stat.gov.kz (accessed on 3 July 2024).
3. Im, J.S.; Seo, S.G.; Kim, M.O.; Cheon, C.G.; Park, Y.E.; Cho, J.H.; Cho, K.S.; Chang, D.C.; Choi, J.K.; Lee, J.N.; et al. Recent trend and prospects of potato industry in Kazakhstan. *J. Korean Soc. Int. Agric.* **2018**, *30*, 177–183. [CrossRef]
4. Jones, R.A.C. Global Plant Virus Disease Pandemics and Epidemics. *Plants* **2021**, *10*, 233. [CrossRef] [PubMed]

5. Lal, P.; Tiwari, R.K.; Behera, B.; Yadav, M.R.; Sharma, E.; Altaf, M.A.; Jena, R.; Ahmad, A.; Dey, A.; Kumar, A.; et al. Exploring potato seed research: A bibliometric approach towards sustainable food security. *Front. Sustain. Food Syst.* **2023**, *7*, 1229272. [CrossRef]
6. Bettoni, J.C.; Mathew, L.; Pathirana, R.; Wiedow, C.; Hunter, D.A.; McLachlan, A.; Khan, S.; Tang, J.; Nadarajan, J. Eradication of Potato Virus S, Potato Virus A, and Potato Virus M from Infected in vitro-Grown Potato Shoots Using In vitro Therapies. *Front. Plant Sci.* **2022**, *13*, 878733. [CrossRef] [PubMed]
7. Silva Filho, J.B.; Fontes, P.C.R.; Ferreira, J.F.d.S.; Cecon, P.R.; Santos, M.F.S.d. Best Morpho-Physiological Parameters to Characterize Seed-Potato Plant Growth under Aeroponics: A Pilot Study. *Agronomy* **2024**, *14*, 517. [CrossRef]
8. Abu Zeid, I.M.; Soliman, H.I.A.; Metwali, E.M.R. In vitro evaluation of some high yield potato (*Solanum tuberosum* L.) cultivars under imposition of salinity at the cellular and organ levels. *Saudi J. Biol. Sci.* **2022**, *29*, 2541–2551. [CrossRef] [PubMed]
9. Mamiya, K.; Tanabe, K.; Onishi, N. Production of potato (*Solanum tuberosum*, L.) microtubers using plastic culture bags. *Plant Biotechnol.* **2020**, *37*, 233–238. [CrossRef] [PubMed]
10. Rahman, M.Z.; Islam, S.S.; Chowdhury, A.N.; Subramaniam, S. Efficient microtuber production of potato in modified nutrient spray bioreactor system. *Sci. Hortic.* **2015**, *192*, 369–374. [CrossRef]
11. Mohamed, A.E.S.; Girgis, N.D. Factors affecting in vitro tuberization of potato. *Bull. Natl. Res. Cent.* **2023**, *47*, 80. [CrossRef]
12. Wojtania, A.; Mieszczakowska-Frąc, M. In Vitro Propagation Method for Production of Phenolic-Rich Planting Material of Culinary Rhubarb 'Malinowy'. *Plants* **2021**, *10*, 1768. [CrossRef]
13. Engels, J.M.M.; Ebert, A.W. A Critical Review of the Current Global Ex Situ Conservation System for Plant Agrobiodiversity. I. History of the Development of the Global System in the Context of the Political/Legal Framework and Its Major Conservation Components. *Plants* **2021**, *10*, 1557. [CrossRef] [PubMed]
14. Mirzabe, A.H.; Hajiahmad, A.; Fadavi, A.; Rafiee, S. Temporary immersion systems (TISs): A comprehensive review. *J. Biotechnol.* **2022**, *357*, 56–83. [CrossRef]
15. Uma, S.; Karthic, R.; Kalpana, S.; Backiyarani, S.; Saraswathi, M.S. A novel temporary immersion bioreactor system for large scale multiplication of banana (Rasthali AAB-Silk). *Sci. Rep.* **2021**, *11*, 20371. [CrossRef]
16. Kikowska, M.; Danek, K.; Gornowicz-Porowska, J.; Thiem, B. Application of temporary immersion system RITA® for efficient biomass multiplication and production of artificial seeds for ex situ conservation of *Linnaea borealis* L. *Plant Cell Tissue Organ Cult.* **2022**, *151*, 673–680. [CrossRef]
17. Monja-Mio, K.M.; Ojeda, G.; Herrera-Alamillo, M.Á.; Sánchez-Teyer, L.F.; Rescalvo-Morales, A. BioMINT: A Temporary Immersion System for Agave Micropropagation. *Methods Mol. Biol.* **2024**, *2759*, 77–88. [CrossRef]
18. Robert, M.L.; Herrera-Herrera, J.L.; Herrera-Herrera, G.; Herrera-Alamillo, M.A.; Fuentes-Carrillo, P. A new temporary immersion bioreactor system for micropropagation. *Methods Mol. Biol.* **2006**, *318*, 121–129. [CrossRef]
19. De Carlo, A.; Tarraf, W.; Lambardi, M.; Benelli, C. Temporary Immersion System for Production of Biomass and Bioactive Compounds from Medicinal Plants. *Agronomy* **2021**, *11*, 2414. [CrossRef]
20. Murthy, H.N.; Joseph, K.S.; Paek, K.Y.; Park, S.Y. Bioreactor systems for micropropagation of plants: Present scenario and future prospects. *Front. Plant Sci.* **2023**, *14*, 1159588. [CrossRef] [PubMed]
21. Méndez-Hernández, H.A.; Galaz-Ávalos, R.M.; Quintana-Escobar, A.O.; Pech-Hoil, R.; Collí-Rodríguez, A.M.; Salas-Peraza, I.Q.; Loyola-Vargas, V.M. In Vitro Conversion of *Coffea* spp. Somatic Embryos in SETIS™ Bioreactor System. *Plants* **2023**, *12*, 3055. [CrossRef]
22. SETIS. Available online: https://setis-systems.be (accessed on 3 July 2024).
23. Gautam, S.; Solis-Gracia, N.; Teale, M.K.; Mandadi, K.; da Silva, J.A.; Vales, M.I. Development of an in vitro Microtuberization and Temporary Immersion Bioreactor System to Evaluate Heat Stress Tolerance in Potatoes (*Solanum tuberosum* L.). *Front. Plant Sci.* **2021**, *12*, 700328. [CrossRef]
24. Steingroewer, J.; Bley, T.; Georgiev, V.; Ivanov, I.; Lenk, F.; Marchev, A.; Pavlov, A. Bioprocessing of differentiated plant in vitro systems. *Eng. Life Sci.* **2013**, *13*, 26–38. [CrossRef]
25. Werner, S.; Maschke, R.; Eibl, D.; Eibl, R. Bioreactor Technology for Sustainable Production of Plant Cell-Derived Products. In *Bioprocessing of Plant In Vitro Systems; Reference Series in Phytochemistry*; Pavlov, A., Bley, T., Eds.; Springer: Cham, Switzerland, 2017; pp. 413–432.
26. Escalona, M.; Samson, G.; Borroto, C.; Desjardins, Y. Physiology of Effects of Temporary Immersion Bioreactors on Micropropagated Pineapple Plantlets. *In Vitro Cell. Dev. Biol. Plant.* **2003**, *39*, 651–656. [CrossRef]
27. Pożoga, M.; Olewnicki, D.; Latocha, P. A Temporary Immer-sion System as a Tool for Lowering Planting Material Production Costs Using the Example of *Pennisetum* × *advena* 'Rubrum'. *Agriculture* **2024**, *14*, 1177. [CrossRef]
28. Volkov, D.V.; Daurov, D.L.; Daurova, A.K.; Abay, Z.S.; Zhapar, K.K.; Zhambakin, K.Z.; Shamekova, M.K. Obtaining potato microtubers in a liquid nutrient medium. *Proc. Natl. Acad. Sci. Belarus* **2020**, *58*, 432–442. [CrossRef]
29. Hajare, S.T.; Chauhan, N.M.; Kassa, G. Effect of Growth Regulators on In Vitro Micropropagation of Potato (*Solanum tuberosum* L.) Gudiene and Belete Varieties from Ethiopia. *Sci. World J.* **2021**, *2021*, 5928769. [CrossRef] [PubMed]
30. Abeuova, L.S.; Kali, B.R.; Rakhimzhanova, A.O.; Bekkuzhina, S.S.; Manabayeva, S.A. High frequency direct shoot regeneration from Kazakh commercial potato cultivars. *PeerJ* **2020**, *8*, e9447. [CrossRef]
31. Daurov, D.; Argynbayeva, A.; Daurova, A.; Zhapar, K.; Sapakhova, Z.; Zhambakin, K.; Shamekova, M. Monitoring the Spread of Potato Virus Diseases in Kazakhstan. *Am. J. Potato Res.* **2023**, *100*, 63–70. [CrossRef]

32. Daurov, D.; Daurova, A.; Karimov, A.; Tolegenova, D.; Volkov, D.; Raimbek, D.; Zhambakin, K.; Shamekova, M. Determining Effective Methods of Obtaining Virus-Free Potato for Cultivation in Kazakhstan. *Am. J. Potato Res.* **2020**, *97*, 367–375. [CrossRef]
33. Murashige, T.; Skoog, F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
34. Duncan, O.D.; Duncan, B. A methodological analysis of segregation indexes. *Am. Sociol. Rev.* **1955**, *20*, 210. [CrossRef]
35. RStudio Team. RStudio: Integrated Development for R. RStudio, PBC: Boston, MA, USA. 2020. Available online: http://www.rstudio.com/ (accessed on 13 August 2024).
36. Sharipova, D.S.; Aitbayev, T.E.; Tazhibayev, T.S.; Nacheva, E.K. The impact of new and improved elements of agricultural technologies on potato productivity in the south-east of Kazakhstan. *Biosci. Biotechnol. Res. Asia* **2016**, *13*, 1031–1036. [CrossRef]
37. Vollmer, R.; Villagaray, R.; Cárdenas, J.; Castro, M.; Chávez, O.; Anglin, N.L.; Ellis, D. A large-scale viability assessment of the potato cryobank at the International Potato Center (CIP). *In Vitro Cell. Dev. Biol.-Plant* **2017**, *53*, 309–317. [CrossRef]
38. Kushnarenko, S.; Romadanova, N.; Aralbayeva, M.; Zholamanova, S.; Alexandrova, A.; Karpova, O. Combined ribavirin treatment and cryotherapy for efficient Potato virus M and Potato virus S eradication in potato (*Solanum tuberosum* L.) in vitro shoots. *In Vitro Cell. Dev. Biol.-Plant* **2017**, *53*, 425–432. [CrossRef]
39. Yuorieva, N.; Sinetova, M.; Messineva, E.; Kulichenko, I.; Fomenkov, A.; Vysotskaya, O.; Osipova, E.; Baikalova, A.; Prudnikova, O.; Titova, M.; et al. Plants, Cells, Algae, and Cyanobacteria In Vitro and Cryobank Collections at the Institute of Plant Physiology, Russian Academy of Sciences—A Platform for Research and Production Center. *Biology* **2023**, *12*, 838. [CrossRef] [PubMed]
40. Espinosa-Leal, C.A.; Puente-Garza, C.A.; García-Lara, S. In vitro plant tissue culture: Means for production of biological active compounds. *Planta* **2018**, *248*, 1–18. [CrossRef]
41. Pandey, S.K.; Singh, S.V.; Sarkar, D. Potato (*Solanum tuberosum*) for sustaining food and nutrition security in developing world. *Indian J. Agric. Sci.* **2005**, *75*, 9043.
42. Purohit, S.D.; Teixeira da Silva, J.A.; Habibi, N. Current approaches for cheaper and better micropropagation technologies. *Int. J. Plant Dev. Biol.* **2011**, *5*, 1–36.
43. Andriani, S.; Siregar, L.A.M.; Safni, I. Microtubers production by using Temporary Immersion System (TIS) bioreactor to potato varieties. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *886*, 012005. [CrossRef]
44. Kämäräinen-Karppinen, T.; Virtanen, E.A.; Rokka, V.; Pirttilä, A.M. Novel bioreactor technology for mass propagation of potato microtubers. *Plant Cell Tissue Organ Cult. (PCTOC)* **2010**, *101*, 245–249. [CrossRef]
45. Erol, M.H.; Dönmez, D.; Biçen, B.; Şimşek, Ö.; Kaçar, Y.A. Modern Approaches to In Vitro Clonal Banana Production: Next-Generation Tissue Culture Systems. *Horticulturae* **2023**, *9*, 1154. [CrossRef]
46. Shukla, M.R.; Piunno, K.; Saxena, P.K.; Jones, A.M.P. Improved in vitro rooting in liquid culture using a two piece scaffold system. *Eng. Life Sci.* **2019**, *20*, 126–132. [CrossRef] [PubMed]
47. Pati, P.K.; Kaur, J.; Singh, P. A liquid culture system for shoot proliferation and analysis of pharmaceutically active constituents of *Catharanthus roseus* (L.) G. Don. *Plant Cell Tissue Organ Cult.* **2011**, *105*, 299–307. [CrossRef]
48. Latawa, J.; Shukla, M.R.; Saxena, P.K. An efficient temporary immersion system for micropropagation of hybrid hazelnut. *Botany* **2016**, *94*, 1–8. [CrossRef]
49. Pérez-Alonso, N.; Wilken, D.; Gerth, A.; Jähn, A.; Nitzsche, H.M.; Kerns, G.; Capote-Perez, A.; Jiménez, E. Cardiotonic glycosides from biomass of Digitalis purpurea L. cultured in temporary immersion systems. *Plant Cell Tissue Organ Cult.* **2009**, *99*, 151–156. [CrossRef]
50. Coetser, E.; du Toit, E.S.; Prinsloo, G. An Investigation into Using Temporary Immer-sion Bioreactors to Micropropagate Moringa oleifera Lam. Callus, Roots, and Shoots. *Agronomy* **2022**, *12*, 2672. [CrossRef]
51. Hwang, H.-D.; Kwon, S.-H.; Murthy, H.N.; Yun, S.-W.; Pyo, S.-S.; Park, S.-Y. Temporary Immersion Bioreactor System as an Efficient Method for Mass Production of In Vitro Plants in Horticulture and Medicinal Plants. *Agronomy* **2022**, *12*, 346. [CrossRef]
52. Frick, E.M.; Strader, L.C. Roles for IBA-derived auxin in plant development. *J. Exp. Bot.* **2018**, *69*, 169–177. [CrossRef]
53. Kazan, K. Auxin and the integration of environmental signals into plant root development. *Ann. Bot.* **2013**, *112*, 1655–1665. [CrossRef] [PubMed]
54. Saidi, A.; Hajibarat, Z. Phytohormones: Plant switchers in developmental and growth stages in potato. *J. Genet. Eng. Biotechnol.* **2021**, *19*, 89. [CrossRef]
55. Šimko, I. Effects of kinetin, paclobutrazol and their interactions on the microtuberization of potato stem segments cultured in vitro in the light. *Plant Growth Regul.* **1993**, *12*, 23–27. [CrossRef]
56. Rahman, M.H.; Islam, M.J.; Mumu, U.H.; Ryu, B.R.; Lim, J.D.; Azad, M.O.K.; Cheong, E.J.; Lim, Y.S. Effect of Light Quality on Seed Potato (*Solanum tuberose* L.) Tuberization When Aeroponically Grown in a Controlled Greenhouse. *Plants* **2024**, *13*, 737. [CrossRef]
57. Sakha, B.M.; Bhatia, A.K.; Batra, V.K.; Chaudhary, V.K.; Batra, P.; Khurana, S.C. In vitro microtuberization in potato (*Solanum tuberosum* L.) cultivars. *Indian J. Exp. Biol.* **2004**, *42*, 1245–1247.
58. Kaur, A.; Reddy, M.S.; Kumar, A. Efficient, one step and cultivar independent shoot organogenesis of potato. *Physiol. Mol. Biol. Plants* **2017**, *23*, 461–469. [CrossRef] [PubMed]

59. Kolachevskaya, O.O.; Myakushina, Y.A.; Getman, I.A.; Lomin, S.N.; Deyneko, I.V.; Deigraf, S.V.; Romanov, G.A. Hormonal Regulation and Crosstalk of Auxin/Cytokinin Signaling Pathways in Potatoes In Vitro and in Relation to Vegetation or Tuberization Stages. *Int. J. Mol. Sci.* **2021**, *22*, 8207. [CrossRef] [PubMed]
60. Lomin, S.N.; Myakushina, Y.A.; Kolachevskaya, O.O.; Getman, I.A.; Savelieva, E.M.; Arkhipov, D.V.; Deigraf, S.V.; Romanov, G.A. Global View on the Cytokinin Regulatory System in Potato. *Front. Plant Sci.* **2020**, *11*, 613624. [CrossRef] [PubMed]
61. Ramírez-Mosqueda, M.A.; Cruz-Cruz, C.A.; Cano-Ricárdez, A.; Bello-Bello, J.J. Assessment of different temporary immersion systems in the micropropagation of anthurium (*Anthurium andreanum*). *3 Biotech* **2019**, *9*, 307. [CrossRef] [PubMed]
62. Bello-Bello, J.J.; Schettino-Salomón, S.; Ortega-Espinoza, J.; Spinoso-Castillo, J.L. A temporary immersion system for mass micropropagation of pitahaya (*Hylocereus undatus*). *3 Biotech* **2021**, *11*, 437. [CrossRef]
63. Polivanova, O.B.; Bedarev, V.A. Hyperhydricity in Plant Tissue Culture. *Plants* **2022**, *11*, 3313. [CrossRef]
64. Sonnewald, S.; Sonnewald, U. Regulation of potato tuber sprouting. *Planta* **2014**, *239*, 27–38. [CrossRef]
65. Suttle, J.C. Physiological regulation of potato tuber dormancy. *Am. J. Potato Res.* **2004**, *81*, 253–262. [CrossRef]
66. Mashhad, S.; Moeini, M. The effect of cytokinin and coumarin on in vitro micrrotuberization of potato (*Solanum tuberosum* L.) cv. Marfona. *Ludus Vitalis* **2015**, *11*, 165–170.
67. Siregar, L.A.M.; Turnip, L.; Damanik, I.R. Immersion in 6-benzylaminopurine for dormancy release and initiation of potato sprouts at various tuber weight and storage duration. *J. Agron. Indones.* **2021**, *49*, 60–67. [CrossRef]
68. Peng, M.; Wang, X.; Li, L. The effect of plant growth regulator and active charcoal on the development of microtubers of potatoes. *Am. J. Plant Sci.* **2012**, *3*, 1535–1540. [CrossRef]
69. Dhital, S.P.; Lim, H.T. Microtuberization of potato (*Solanum tuberosum* L.) as influenced by supplementary nutrients, plant growth regulators, and in vitro culture conditions. *Potato Res.* **2012**, *55*, 97–108. [CrossRef]
70. Suttle, J.C. Involvement of endogenous gibberellins in potato tuber dormancy and early sprout growth: A critical assessment. *J. Plant Physiol.* **2004**, *161*, 157–164. [CrossRef] [PubMed]
71. Mohamed, M.F.; Abdalla, M.M.A.; Damarany, A.A.M. Differential axillary-bud proliferation responses of two sweet potato cultivars to benzyl adenine and thidiazuron. *Ass. Univ. Bull. Environ. Res.* **2007**, *10*, 21–30.
72. Kumlay, A.M.; Ercisli, S. Callus induction, shoot proliferation and root regeneration of potato (*Solanum tuberosum* L.) stem node and leaf explants under long-day conditions. *Biotechnol. Equip.* **2015**, *29*, 1075–1084. [CrossRef]
73. Naqvi, B.; Abbas, H.; Ali, H. Evaluation of in vitro tuber induction ability of two potato genotypes. *Pak. J. Agric. Sci.* **2019**, *56*, 77–81.
74. Shukla, S.R.; Zala, H.N.; Solanki, S.D.; Ant, H.M. Optimizing Microtubers Production for Sustainable Potato Cultivation in Gujarat, India. *Biol. Life Sci. Forum* **2023**, *27*, 2. [CrossRef]
75. Fufa, M.; Diro, M. The effects of sucrose on in vitro tuberization of potato cultivars. *Adv. Crop Sci. Tech.* **2013**, *1*, 2.
76. Etienne, H.; Berthouly, M. Temporary immersion systems in plant micropropagation. *Plant Cell Tissue Organ Cult.* **2002**, *69*, 215–231. [CrossRef]
77. Tapia, M.D.L.; Arbizu, C.; Beraún, F.; Lorenzo, J.; Escalona, M. Pre-basic seed potato (*Solanum tuberosum* L.) production using temporary immersion bioreactors. *Peruv. J. Agron.* **2018**, *2*, 9. [CrossRef]
78. Jova, M.C.; Kosky, R.G.; Cabrera, R.; Feria, M.D.; Perez, M.B.; Vega, V.M.; Torres, J.L. Performance of yam microtubers from temporary immersion system in field conditions. *Afr. J. Biotechnol.* **2011**, *10*, 9268–9271.
79. Ashraf, M.F.; Abd Aziz, M.; Stanslas, J.; Kadir, M.A. Optimization of immersion frequency and medium substitution on microtuberization of Chlorophytum borivilianum in RITA system on production of saponins. *Process Biochem.* **2013**, *48*, 73–77. [CrossRef]
80. Nongdam, P.; Beleski, D.G.; Tikendra, L.; Dey, A.; Varte, V.; El Merzougui, S.; Pereira, V.M.; Barros, P.R.; Vendrame, W.A. Orchid Micropropagation Using Conventional Semi-Solid and Temporary Immersion Systems: A Review. *Plants* **2023**, *12*, 1136. [CrossRef] [PubMed]
81. Novikov, O.O.; Romanova, M.S.; Leonov, N.I.; Kosinova, E.I. Influence of various phytohormones on the growth and development of the Solnechny potato variety in vitro. *BIO Web Conf.* **2021**, *36*, 05008. [CrossRef]
82. Guade, Y.F. The effect of plant growth hormones (auxins and cytokinins) on in-vitro shooting and rooting ability of potato nodal culture. *Eur. J. Biomed.* **2017**, *4*, 489–493.
83. Miri, M.; Janakirama, P.; Held, M.; Ross, L.; Szczyglowski, K. Into the root: How cytokinin controls rhizobial infection. *Trends Plant Sci.* **2016**, *21*, 178–186. [CrossRef]
84. Gong, H.L.; Dusengemungu, L.; Igiraneza, C.; Rukundo, P. Molecular Regulation of Potato Tuber Dormancy and Sprouting: A Mini-Review. *Plant Biotechnol. Rep.* **2021**, *15*, 417–434. [CrossRef]
85. Viola, R.; Roberts, A.G.; Haupt, S.; Gazzani, S.; Hancock, R.D.; Marmiroli, N.; Machray, G.C.; Oparka, K.J. Tuberization in Potato Involves a Switch from Apoplastic to Sym-plastic Phloem Unloading. *Plant Cell* **2001**, *13*, 385–398. [CrossRef]
86. Boubaker, H.; Saadaoui, W.; Dasgan, H.Y.; Tarchoun, N.; Gruda, N.S. Enhancing Seed Potato Production from In Vitro Plantlets and Microtubers through Biofertilizer Application: Investigating Effects on Plant Growth, Tuber Yield, Size, and Quality. *Agronomy* **2023**, *13*, 2541. [CrossRef]
87. Diémé, A.; Ba, O.; Sagna, M.; Sy, M. Influence of the Size of Potato Microtubers (*Solanum tuberosum* L.) on the Yield of Plants under Semi-Axenic Conditions. *Adv. Biosci. Biotechnol.* **2021**, *12*, 65–77. [CrossRef]

88. Herrera-Isidron, L.; Valencia-Lozano, E.; Rosiles-Loeza, P.Y.; Robles-Hernández, M.G.; Napsuciale-Heredia, A.; Cabrera-Ponce, J.L. Gene Expression Analysis of Microtubers of Potato *Solanum tuberosum* L. Induced in Cytokinin Containing Medium and Osmotic Stress. *Plants* **2021**, *10*, 876. [CrossRef]
89. Otroshy, M. Utilization of Tissue Culture Techniques in a Seed Potato Tuber Production Scheme. Ph.D. Thesis, Wageningen University and Research, Wageningen, The Netherlands, 2006.
90. Pour, M.S.; Omidi, M.; Majidi, I.; Davoodi, D.; Tehrani, P.A. In-vitro plantlet propagation and microtuberization of meristem culture in some of wild and commercial potato cultivars as affected by NaCl. *Afr. J. Agric. Res.* **2010**, *5*, 268–274.
91. Long, J.; Yu, F.; Wu, Y.; Xu, Z.; Liu, X. Regulation of Different Lights on Energy Acquisitions, Microtuber Formation, and Growth of In Vitro-Grown *Solanum tuberosum* L. *Agronomy* **2024**, *14*, 1232. [CrossRef]