Postprint: Induction of In Vitro Tubers of Potato 'Mira'Using a "Solid-Liquid"Double-Layer Culture Method

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Date: 2018-05-18T00:00:00+00:00

Abstract

A method for inducing microtubers in tissue culture rooms was established to obtain potato "seeds" for direct field planting, thereby compensating for the insufficient production of pre-basic seeds due to high temperatures in summer and expanding the quantity of pre-basic seeds. Using virus-free plantlets in vitro of the potato variety 'Mira' as experimental material and adopting a "solid-liquid double-layer" culture method, the culture media for the robust seedling growth stage and microtuber induction stage of 'Mira' plantlets were optimized through orthogonal experiments; simultaneously, the effects of sucrose concentration, light conditions, and CCC concentration on microtuber tuberization were investigated through single-factor experiments. The results indicated that in "solid-liquid" double-layer culture, the optimized medium for the robust seedling culture stage of 'Mira' was: modified MS medium (with ammonium nitrate changed to 2,000 mg · L ^{1} and potassium nitrate changed to 2,000 mg · L ^{1} in MS medium) + 500 mg · L ^{1} CCC + 0.1% activated charcoal + 0.1 mg · L ^{1} DA-6 + 1 mg · L ^{1} 6-BA + 0.1 mg · L ^{1} NAA + 3% sucrose + 6 g · L ^{1} agar, pH 5.8; the optimized medium for microtuber induction and growth stage was: MS1 medium (with microelements and iron salts at twice the amount of MS medium) + 1.5% activated charcoal + 4 mg · L ^{1} 6-BA + 8% sucrose; during the microtuber induction stage, microtubers induced under low light at 4 h · d ^{1} were superior to those under dark culture in terms of tuberization index, large tuber rate, and tuber weight.

Full Text

Induction of Microtuber of Potato Cultivar 'Mira' by "Solid + Liquid" Double Layer Culture

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Abstract

An efficient method for inducing microtubers in a tissue culture room was established to obtain potato 'seed' suitable for direct field planting, thereby compensating for the shortage of pre-elite seed potato production during summer high temperatures and increasing the quantity of pre-elite seed potatoes. Using virus-free plantlets of potato cultivar 'Mira' as experimental material, a "solid + liquid" double-layer culture system was employed. The culture media for both the vigorous seedling growth stage and the microtuber induction stage were optimized through orthogonal experiments. Additionally, single-factor experiments were conducted to investigate the effects of sucrose concentration, light conditions, and CCC concentration on microtuber formation. The results demonstrated that in the "solid + liquid" double-layer culture system, the optimal medium for the vigorous seedling stage was: modified MS medium (with NH4NO3 adjusted to 2,000 mg · L-1 and KNO3 to 2,000 mg · L-1) + 500 mg · L-1 CCC + 0.1% activated carbon + 0.1 mg · L-1 DA-6 + 1 mg · L-1 6-BA + 0.1 mg · L-1 NAA + 3% sucrose + 6 g · L-1 agar, pH 5.8. The optimal medium for the microtuber induction and growth stage was: MS1 medium (with double the concentration of trace elements and iron salts compared to standard MS) + 1.5% activated carbon + 4 mg · L-1 6-BA + 8% sucrose. During the microtuber induction stage, microtubers cultured under dim light for 4 h · d-1 exhibited superior performance in tuberization index, large tuber rate, and tuber weight compared to those cultured in darkness.

Keywords: potato, microtuber, 6-BA, CCC, activated carbon, DA-6, tissue culture

Introduction

Liangshan Prefecture is located in southwestern Sichuan Province and features a typical plateau mountainous terrain with abundant sunlight and large diurnal temperature variations, creating favorable conditions for potato cultivation. As a major potato-producing region in Sichuan, particularly in Yi ethnic areas above 1,500 m elevation, potato serves as both a primary food source and economic staple for local farmers. Developing the potato industry in these regions represents a crucial pathway for poverty alleviation and wealth creation among impoverished Yi communities. In 2015, the average potato yield in Liangshan was 22.5 t · hm-2, significantly lower than the 46.9–48.7 t · hm-2 achieved in countries such as the United States, Belgium, and New Zealand (FAO data). Prac-

tical experience has demonstrated that adopting virus-free seed potatoes can substantially improve both yield and quality. Currently, virus-free seed potato production in Liangshan relies on virus-free plantlets that undergo laborious and complex processes including acclimatization, washing, and transplanting, resulting in high production costs. Moreover, high temperatures in greenhouse facilities during summer prevent pre-elite seed production, leading to a severe shortage of virus-free seed potatoes to meet production demands.

Potato microtubers are tiny tubers, 2–10 mm in diameter, formed in the leaf axils of in vitro plantlets through induction culture (Vander Zaag, 1988). As an innovative approach for germplasm conservation and virus-free seed potato production following virus-free plantlet technology, microtubers offer advantages of small size, light weight, and ease of storage, transport, and preservation. They can be used as "seed" for large-scale field planting, accelerating virus-free seed potato propagation, shortening the production cycle, and enabling year-round multiplication, with increasingly widespread applications. Additionally, microtubers serve as receptors for genetic transformation in potato genetic engineering research.

Microtuber formation in potatoes is influenced by numerous factors, including genotype (Khalil et al., 2017; Gopal et al., 2004), temperature (Jiang and Guo, 2007; Ma et al., 2010), culture method (Shuai et al., 2004; Bai et al., 2002), light conditions (Hussain et al., 2006), carbon source (Gopal et al., 2004), mineral nutrition (Radouani and Lauer, 2015; Ma et al., 1999), and plant growth regulators (Gopal et al., 2004). Pelacho and Mingo-Castel (1991) emphasized that robust plantlets with thick stems, well-developed root systems, and dark green leaves are essential prerequisites for obtaining high-quality, high-yield microtubers. Consequently, microtuber induction typically employs a two-step process: first cultivating virus-free potato plantlets on a vigorous seedling medium, followed by microtuber induction. Studies comparing different culture methods have shown that liquid medium generally yields superior results in tuberization rate, tuber weight, and tuber diameter compared to solid medium under equivalent concentrations (Piao et al., 2003; Bai et al., 2002). However, liquid culture involves complex operations, high contamination rates, and fragile plantlets, leading to elevated sterilization costs. Although the "solid + liquid" double-layer culture method exhibits slightly lower tuberization rates, it offers advantages of simpler operation, reduced contamination, and lower sterilization energy consumption, making it suitable for large-scale production (Shuai et al., 2004). This study employed a "solid + liquid" double-layer culture system, utilizing orthogonal experimental design to optimize medium components for both the vigorous seedling stage and microtuber induction stage, while also investigating the effects of light conditions, sucrose concentration, and CCC concentration on microtuber formation. The objective was to develop efficient culture and induction techniques for robust plantlets and microtubers with simplified operations and shortened growth cycles, providing valuable references for microtuber cultivation.

Materials and Methods

1.1 Experimental Materials

The experimental material consisted of virus-free 'Mira' plantlets maintained in our laboratory. Under sterile conditions, the plantlets were cut into nodal segments bearing 1–2 leaf nodes and inoculated onto subculture medium at a density of 15–20 plantlets per vessel. The cultures were maintained at 25±2 °C under a light intensity of 2,000–4,000 lx with a 16 h · d-1 photoperiod until the plantlets reached approximately 10 cm in height for subsequent experiments.

1.2.1 Vigorous Seedling Culture

Virus-free 'Mira' plantlets were excised into single-node segments under sterile conditions and inoculated onto vigorous seedling culture medium at 10 segments per vessel. The basal medium composition consisted of: base medium + CCC + activated carbon + DA-6 + 1 mg · L-1 6-BA + 0.1 mg · L-1 NAA + 3% sucrose + 6 g · L-1 agar, pH 5.8. A four-factor, three-level orthogonal experimental design was employed to determine the optimal composition and concentrations of base medium, CCC, activated carbon, and DA-6 (Table 1), comprising nine treatment combinations with five replicates each. The cultures were incubated at 25±2 °C under 2,000–4,000 lx illumination with a 16 h · d-1 photoperiod for 20 days. Parameters including root number, stem diameter, internode length, fresh weight, and leaf area were recorded for each treatment to identify the optimal vigorous seedling medium for 'Mira' plantlets.

1.2.2 Microtuber Induction Culture

Microtuber induction was performed using the solid-liquid double-layer culture method. After 20 days of vigorous seedling culture, 20 mL of sterile liquid induction medium was aseptically added to each culture vessel. The liquid medium composition consisted of: base medium + sucrose + 6-BA + activated carbon, pH 5.8. A four-factor, three-level orthogonal experimental design was used to optimize the combinations and concentrations of base medium, 6-BA, activated carbon, and sucrose (Table 2), with nine treatment combinations and five replicates each. The cultures were incubated at 25±2 °C under dim light (4 h · d-1) to induce tuberization. After 40 days of induction culture, parameters including tuberization index (number of tubers per plantlet), tuber weight (g), large tuber rate (>0.1 g), and tuber diameter were measured to determine the optimal medium for 'Mira' microtuber induction.

Table 1. Factors and levels related to the best medium for virus-free plantlets vigorous growth

Factors	Levels	Concentration
A. CCC concentration (mg • L-1)	A1: 100, A2: 200, A3: 500	100(A1), 200(A2), 500(A3)
B. Activated carbon concentration (%)	B1: 0.05, B2: 0.1, B3: 0.15	0.05(B1), 0.1(B2), 0.15(B3)
C. Kind of culture medium	C1: MS, C2: Modified MS, C3: 2MS	MS(C1), Modified MS(C2), 2MS(C3)
D. DA-6 concentration (mg • L-1)	D1: 0, D2: 0.1, D3: 1	0(D1), 0.1(D2), 1(D3)

Note: Modified MS medium means that NH4NO3 has been changed from 1,650 to 2,000 mg • L-1 and KNO3 has been changed from 1,900 to 2,000 mg • L-1, compared to MS medium; 2MS medium means that the dosage was two times of MS medium.

Table 2. Factors and levels related to the best medium for induction of potato microtuber

Factors	Levels	Concentration
A. Sucrose concentration (%)	A1: 8, A2: 10, A3: 12	8(A1), 10(A2), 12(A3)
B. 6-BA concentration (mg • L-1)	B1: 2, B2: 4, B3: 6	2(B1), 4(B2), 6(B3)
C. Activated carbon concentration (%)	C1: 0.5, C2: 1.0, C3: 1.5	0.5(C1), 1.0(C2), 1.5(C3)
D. Kind of culture medium	D1: MS, D2: MS1, D3: MS2	MS(D1), MS1(D2), MS2(D3)

Note: MS1 medium means that two times of FeSO4 (Na2EDTA) and trace elements of MS medium; MS2 means that KH2PO4 has been changed from 170 to 340 mg • L-1.

1.2.3 Single-Factor Experiments

1.2.3.1 Effect of Light on Microtuber Formation Using the optimized vigorous seedling medium, plantlets were cultured for 20 days before adding 20 mL of optimized microtuber induction medium to each vessel. The cultures were then incubated at 25±2 °C under three different light conditions: dim light for 4 h • d-1, dim light for 8 h • d-1, or complete darkness, with five replicates per

treatment. Observations were made after 40 days to identify the most favorable light condition for microtuber formation.

1.2.3.2 Effect of Sucrose Concentration on Microtuber Formation Following 20 days of culture on optimized vigorous seedling medium, 20 mL of sterile microtuber induction medium containing sucrose at concentrations of 2%, 4%, 6%, or 8% was added to each vessel. The cultures were maintained at 25±2 °C under dim light (4 h · d-1) for 40 days before evaluation to determine the optimal sucrose concentration for microtuber induction.

1.2.3.3 Effect of CCC Concentration on Microtuber Formation The vigorous seedling medium consisted of modified MS medium + CCC + 1% activated carbon + 0.1 mg · L-1 DA-6 + 1 mg · L-1 6-BA + 0.1 mg · L-1 NAA + 3% sucrose + 6 g · L-1 agar, pH 5.8, with CCC concentrations of 100 mg · L-1 or 500 mg · L-1. After 20 days of seedling culture, 20 mL of sterile optimized microtuber induction medium was added to each vessel, and the cultures were incubated at 25±2 °C under dim light (4 h · d-1) with five replicates per treatment. Observations after 40 days were used to assess the impact of different CCC concentrations in the seedling medium on subsequent microtuber formation.

1.3 Data Collection and Processing

All data were analyzed using SPSS 22.0 statistical software. Range analysis and analysis of variance (ANOVA) were employed for optimizing the vigorous seedling medium and microtuber induction medium, while single-factor experiments were analyzed using ANOVA followed by LSD tests ($p<0.05$) for significance testing.

Results

2.1.1 Effect of Different Treatments on Root Number of Plantlets

Roots are essential plant organs for absorbing water and nutrients from the environment, making root number a key parameter for evaluating plantlet growth. As shown in Table 3 , treatment combination A1B2C2D2 produced the maximum root number of 4.9 roots per plantlet, with desirable root vigor. Range analysis of factor levels (Table 4) revealed that the factors influencing root number, in descending order of importance, were: culture medium type > activated carbon > DA-6 > CCC. Root number was relatively higher on modified MS medium, while plantlets cultured on 2MS medium exhibited fewer roots and weaker growth. The optimal combination for root induction was A1B2C2D1.

2.1.2 Effect of Different Treatments on Stem Diameter of Plantlets

Stem diameter reflects the robustness of plantlet growth. Table 3 shows that treatment combination A1B2C2D2 produced the most vigorous plantlets with

a stem diameter of 1.62 mm · plantlet-1, while the remaining eight treatment combinations showed no significant differences in stem diameter. Range analysis results (Table 4) indicated that CCC, activated carbon, culture medium type, and DA-6 had approximately equal influence on stem diameter.

2.1.3 Effect of Different Treatments on Internode Length of Plantlets

Table 3 shows that treatment combination A3B3C1D2 produced the shortest internode length of 2.90 mm, while combination A1B2C2D2 yielded an internode length of 3.87 mm, though the difference was not statistically significant. The longest internode length (8.27 mm) was observed in treatment combination A1B3C3D3. Range analysis (Table 4) demonstrated that the factors affecting internode length, in order of importance, were: culture medium type > CCC > DA-6 > activated carbon. The optimized medium formulation for minimizing internode length was A3B2C2D2.

2.1.4 Effect of Different Treatments on Leaf Area of Plantlets

Leaves are the primary organs for photosynthesis, and leaf area correlates with plantlet vigor. Table 3 reveals significant differences in leaf area among treatment combinations. Combination A1B2C2D2 achieved the maximum leaf area of 0.27 cm2, while combinations A2B3C2D1, A3B1C2D3, A1B3C3D3, and A2B1C3D2 produced the smallest, most slender leaves. Range analysis (Table 4) indicated that the factors influencing leaf area, in descending order, were: culture medium type CCC > DA-6 activated carbon. The optimal combination for maximizing leaf area was A1B2C2D2.

2.1.5 Effect of Different Treatments on Fresh Weight of Plantlets

Fresh weight directly reflects nutrient accumulation during plantlet growth and serves as the most reliable indicator of vigor given the limited space in culture vessels. Table 3 shows that treatment combination A1B2C2D2 achieved the highest fresh weight of 0.20 g per plantlet, while combinations A2B3C2D1, A2B2C1D3, and A3B3C1D2 yielded the lowest fresh weights with slender plantlets. Range analysis (Table 4) revealed that appropriate DA-6 concentration most significantly promoted fresh weight increase, followed by CCC, while culture medium type and activated carbon had minimal effects. The optimized medium formulation for maximizing fresh weight was A1B2C2D2.

Based on comprehensive evaluation, treatment combination A1B2C2D2 was selected as the optimized vigorous seedling medium: modified MS + 100 mg · L-1 CCC + 0.1% activated carbon + 0.1 mg · L-1 DA-6 + 1 mg · L-1 6-BA + 0.1 mg · L-1 NAA + 3% sucrose + 6 g · L-1 agar, pH 5.8. This formulation produced plantlets with maximum root number, stem diameter, fresh weight, and leaf area, along with relatively short internode length, fully meeting the requirements for vigorous seedling cultivation.

2.2.1 Effect of Different Treatments on Microtuberization Index

Table 5 demonstrates that different treatment combinations significantly affected the tuberization index. Combination A1B2C3D2 achieved the highest tuberization index of 1.23, followed by A3B3C3D1 with an index of 0.87, while remaining combinations showed no significant differences. Range analysis of factor levels (Table 6) revealed that the factors influencing tuberization index, in descending order, were: 6-BA > activated carbon > sucrose > culture medium type. Appropriate 6-BA concentration most significantly increased the tuberization index, followed by activated carbon, while sucrose and culture medium type had relatively minor effects. The medium combination yielding the maximum tuberization index was A1B2C3D2.

2.2.2 Effect of Different Treatments on Microtuber Weight

Table 5 shows that combination A1B2C3D2 produced the maximum tuber weight of 0.25 g per tuber, significantly different from other treatments. Range analysis (Table 6) indicated that the factors affecting tuber weight, in order of importance, were: 6-BA > activated carbon > culture medium type > sucrose, suggesting that tuber weight could be increased by appropriately adjusting 6-BA and activated carbon concentrations. The optimal medium combination for maximizing tuber weight was A1B2C3D2.

2.2.3 Effect of Different Treatments on Large Tuber Rate

Table 5 reveals that treatment combination A1B2C3D2 achieved the highest large tuber rate of 66.15%, though not significantly different from combinations A2B3C2D2, A3B3C3D1, and A1B3C2D3. Combination A1B1C1D1 produced the lowest large tuber rate of 9.34%. Range analysis (Table 6) demonstrated that the factors influencing large tuber rate, in descending order, were: 6-BA > culture medium type > activated carbon > sucrose. Appropriate 6-BA concentration most significantly increased the large tuber rate, followed by culture medium type; increased KH2PO4 concentration enhanced the large tuber rate, while activated carbon and sucrose had relatively minor effects. The medium combination yielding the highest large tuber rate was A1B3C3D2.

2.2.4 Effect of Different Treatments on Microtuber Diameter

Table 5 shows that treatment combination A1B2C3D2 produced the maximum tuber diameter of 6.3 mm, significantly different from other combinations, while A1B1C1D1 yielded the minimum diameter of only 2.2 mm. Range analysis (Table 6) indicated that the factors affecting tuber diameter, in order of importance, were: activated carbon > culture medium type 6-BA > sucrose. Increased activated carbon concentration most effectively enhanced tuber diameter, followed by culture medium type and 6-BA, while sucrose concentration had the least influence. The medium combination producing the largest tuber diameter was A1B2C3D2.

Based on comprehensive evaluation, combination A1B2C3D2 was selected as the optimal microtuber induction medium: MS1 + 1.5% activated carbon + 4 mg · L-1 6-BA + 8% sucrose.

2.3.1 Effect of Light on Tuberization Rate

Light is a major factor influencing potato microtuber formation. Table 7 shows that extending the photoperiod to 8 h · d-1 of dim light significantly increased the tuberization index and maximum tuber weight. However, no significant difference in average tuber weight was observed between the 8 h · d-1 dim light treatment and dark culture. The large tuber rate under 8 h · d-1 dim light was significantly lower than that under both 4 h · d-1 dim light and dark conditions. Tuber diameter was greater under both 4 h · d-1 and 8 h · d-1 dim light treatments compared to dark culture. Since large tuber rate represents a crucial indicator in microtuber induction, the optimal light condition should balance this parameter with average tuber weight, tuber diameter, and tuberization index. Therefore, this study concludes that dim light treatment at 4 h · d-1 provides the optimal condition for microtuber formation.

2.3.2 Effect of Sucrose Concentration on Microtuber Formation

Sucrose concentration exerts a significant inductive effect on microtuber formation. Table 8 demonstrates that increasing sucrose concentration significantly enhanced both tuberization index and large tuber rate. At 8% sucrose, the tuberization index reached 1.25, the large tuber rate achieved 95.63%, and the maximum tuber weight attained 0.491 g · tuber-1. For tuber weight and diameter, 8% sucrose showed no significant difference from 4% and 6% concentrations but was significantly higher than 2% (Table 8). At elevated sucrose concentrations of 10% and 12%, the tuberization index, tuber weight, large tuber rate, and tuber diameter all decreased markedly (Table 6). Therefore, 8% sucrose represents the optimal concentration for microtuber induction.

2.3.3 Effect of CCC Concentration on Microtuber Formation

Table 9 shows that compared to 100 mg · L-1 CCC treatment, the 500 mg · L-1 CCC treatment increased the tuberization index and maximum tuber weight by 29.72% and 14.71%, respectively. Therefore, a CCC concentration of 500 mg · L-1 is recommended for the initial vigorous seedling stage.

Discussion

In potato microtuber research, Qiu et al. (2008) found that plantlet vigor showed significant or highly significant positive correlation with average microtuber weight, and robust plantlets tuberized more readily than juvenile ones (Yan and Guo, 2004). Therefore, cultivating vigorous plantlets constitutes a fundamental prerequisite for producing larger microtubers. The solid-liquid double-layer culture method has become a common approach for microtuber induction due

to its capacity to regulate both the plantlet growth stage and the microtuber induction and growth stages (Bai et al., 2002; Shuai et al., 2004; Wang et al., 2006).

In vigorous seedling culture of potato plantlets, modifying the standard MS medium by adjusting NH4NO3 to 2,000 mg · L-1 and KNO3 to 2,000 mg · L-1 increased plantlet fresh weight, leaf area, and overall vigor while reducing internode length compared to standard MS and double-strength MS media. These results align with findings by Ma et al. (1999). However, other studies have reported that double-strength MS medium promoted robust growth in potato cultivars 'Kexin 4' and 'Kexin 2' (Jin et al., 1995), which contradicts our results. This discrepancy may be attributed to varietal and genotypic differences (Khalil et al., 2017).

CCC, a plant growth retardant commonly used in vigorous seedling culture, reduces internode length while increasing stem diameter, leaf number, and effective propagation segments. However, excessive concentrations can cause toxicity symptoms including excessive dwarfing, leaf curling, and chlorosis followed by abscission (Wu et al., 2015). Our study found that 100 mg · L-1 CCC maximized plantlet fresh weight, while 500 mg · L-1 CCC inhibited growth and markedly reduced internode length without exhibiting obvious toxicity. DA-6 (diethyl aminoethyl hexanoate), a broad-spectrum cytokinin-type plant growth regulator, has been shown to enhance leaf area, photosynthetic rate, and chlorophyll content while increasing strawberry yield at appropriate concentrations (Miao et al., 2007). Although rarely reported for potato plantlet propagation, our experiments demonstrated that 0.1 mg · L-1 DA-6 increased fresh weight, leaf area, and stem diameter while reducing internode length in 'Mira' plantlets, producing robust growth. Therefore, DA-6 can be applied in potato microtuber propagation. Activated carbon powder in culture medium promotes plantlet growth and rooting (Xia et al., 2011). In our experiments, 0.1% activated carbon enhanced 'Mira' plantlet growth by increasing root number, stem diameter, fresh weight, and leaf area while reducing internode length, making it suitable for vigorous seedling culture.

In microtuber induction culture, doubling the concentrations of trace elements and iron salts in MS medium improved tuberization index, tuber weight, large tuber rate, and tuber diameter. Other studies have reported that quadrupling trace elements can induce tuberization (Liu, 2001), and doubling iron salts can increase tuber weight (Zhang, 2004). In our modification of MS medium, increasing KH2PO4 to 340 mg · L-1 decreased the tuberization index but increased the large tuber rate. Research indicates that excessive potassium dihydrogen phosphate reduces tuber number while increasing tuber weight, with recommendations to adjust phosphorus concentration to 255 mg · L-1 (Zhang, 2004). Therefore, further optimization of trace elements, iron salts, and KH2PO4 concentrations in MS medium is warranted.

In potato microtuber induction culture, exogenous hormones are necessary to shorten production cycles and enhance yield and quality, with 6-BA being the

most commonly used and effective regulator (Hu and Jiang, 1989; Yang et al., 2008). Our study found that 6-BA had the greatest impact on tuberization index, tuber weight, and large tuber rate under the tested factor levels, confirming its essential role in microtuber formation for 'Mira' potato plantlets. High sucrose concentrations (6%–10%) are indispensable for microtuber induction (Hu and Jiang, 1989; Lü et al., 2004; Gopal et al., 2004). The optimal sucrose concentration for 'Mira' microtuber induction was 8% (Elaleem et al., 2015; Hu and Jiang, 1989; Khalil et al., 2017), as both higher and lower concentrations adversely affected microtuber induction and growth, consistent with our findings. As an adsorbent, activated carbon demonstrates clear benefits in potato microtuber induction by promoting tuber formation. At 1.5% concentration, activated carbon increased tuberization index, tuber weight, large tuber rate, and tuber diameter. Additionally, activated carbon reduced light intensity in the medium, facilitated nutrient translocation, and adsorbed harmful metabolic byproducts, thereby promoting tuberization.

Light represents a primary factor affecting potato microtuber formation. Most studies indicate that microtuber induction and growth require dark conditions (Hussain et al., 2006; Cui et al., 2001; Ali et al., 2018; Elaleem et al., 2015). However, some research suggests that 'Mira' potato is insensitive to light for tuberization (Cui et al., 2001), with diffuse light being optimal for induction (Zhao, 2005). Our results demonstrate that culturing under dim light for 4 h · d-1 yielded higher tuberization index, tuber weight, large tuber rate, maximum tuber weight, and tuber diameter compared to dark culture. This may be attributed to light delaying leaf senescence (Slimmon et al., 1989) and continuously providing energy for microtuber growth and development. Microtubers cultured under 4 h · d-1 dim light extended beyond the liquid medium during the expansion phase, resulting in higher germination rates at harvest, whereas dark-induced microtubers exhibited lower germination rates and dormancy periods, consistent with findings by Shuai et al. (2004). Therefore, this characteristic allows for selective application: dim light induction can be used for microtubers requiring immediate planting, while dark induction is suitable for those intended for storage and transport.

Since most microtubers develop at the base of plantlets with fewer forming in upper portions, reducing internode length allows more nodes to be immersed in induction medium for increased tuber production. Our experiments revealed that using 500 mg · L-1 CCC during the initial vigorous seedling stage increased subsequent tuberization index and maximum tuber weight by 29.72% and 14.71%, respectively. Large microtubers exhibit higher germination rates and produce uniform seedlings. In production practice, microtubers exceeding 0.5 g can be used directly as "seed" (Park et al., 2009). Therefore, the cultivation of large microtubers···

This study determined the optimal medium formulations and culture conditions for both vigorous seedling cultivation and microtuber induction stages of 'Mira' potato using the "solid + liquid" double-layer culture method, achieving high

tuberization index, tuber weight, large tuber rate, and tuber diameter. These findings provide valuable technical references for rapid seed potato propagation of this cultivar and other major potato varieties, holding significant scientific and practical importance.

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