Pak. J. Bot., 58(15): December 2026.

ARTICLE | DOI: http://dx.doi.org/10.30848/PJB2026-12(15)

ESTABLISHMENT OF A REGENERATION SYSTEM FOR YAM (*DIOSCOREA POLYSTACHYA*) USING STEM TIPS

SHUQIN HAO, ZEHONG XU, YINUO BI, ZITONG LI, XINPENG WU AND JING SHU*

College of Agricultural Science and Technology, Shandong Agriculture and Engineering University, Jinan 250102, China

*Corresponding author's email: shujing79@163.com

Abstract

To enable rapid propagation of high-quality yam (*Dioscorea polystachya*) varieties and obtain virus-free plantlets, the stem tips of yam were used as explants to investigate the effects of different disinfection times and methods on the sterilization efficiency. Using MS medium as the basal medium, the influence of varying concentrations of plant hormones on seedling formation, virus-free plantlet induction rate, and rooting rate was explored. The results showed that the optimal sterilization method for yam tissue culture explants was soaking in 75% ethanol for 7 seconds, followed by immersion in 0.1% mercuric chloride solution for 6 minutes. The best primary culture medium was MS + 0.1 mg/L NAA + 0.5 mg/L 6-BA, achieving the highest seedling formation rate of 82.22%.The virus-free plantlet induction rate of the optimal system reached 80.00%, showing a satisfactory detoxification effect. For subculture proliferation, the optimal medium was MS + 0.3 mg/L IBA + 0.5 mg/L 6-BA, yielding the highest proliferation coefficient of 4.47. The best rooting medium was MS + 0.2 mg/L IBA + 0.1 mg/L NAA + 0.6 mg/L 6-BA, achieving a 100% rooting rate. This study provides theoretical and technical support for the rapid propagation of superior yam varieties, improving both yield and quality.

Key words: Yam stem tip; Detoxification; Tissue culture

Introduction

Yam (*Dioscorea polystachya* Turcz.) is a perennial climbing vine belonging to the Dioscoreaceae family. Its tubers are rich in starch, saponins, mucilage, protein, and other bioactive compounds, conferring significant edible, medicinal, and economic value (Butler & Lobley, 2016). Studies have shown that the high content of unsaturated fatty acids in yam is beneficial for preventing cardiovascular diseases and shows potential in managing diabetes and its complications (Gu *et al.,* 2025). With increasing market demand, developing large-scale yam cultivation has become an important strategy for enhancing yield and economic returns (Nagendra *et al.,* 2025). According to statistics from the FAO, global annual yam production has exceeded 73 million tons, of which China accounts for 38%, with the main production areas concentrated in the Yellow River and Huai River basins and the southwestern mountainous areas (Zhou *et al.,* 2024).

However, the sustainable development of the yam industry faces severe challenges. The traditional propagation method of asexual reproduction relying on tubers, while simple to operate, is highly susceptible to virus accumulation and transmission. Plants infected with viruses such as Dioscorea latent virus (DsLV) and yam mosaic virus (YMV) exhibit decreased photosynthetic rate, physiological dysfunction, germplasm degeneration, and sharp yield reduction (Hu *et al.,* 2023). Data indicate that the infection rate of compound viruses in continuously cropped fields is as high as 62–89%, which can cause a yield loss of 25–40% (McLeish *et al.,* 2022). In addition, traditional propagation methods have two major technical bottlenecks: firstly, apical dominance limits a single tuber to only 3–5 effective buds, with a biomass conversion efficiency of less than 40%; secondly, long-term asexual propagation leads to germplasm degeneration, with the quality of single plants in some production areas declining by up to 18.6% (Wang *et al.,* 2018). These problems seriously restrict the healthy development of the yam industry.

Plant tissue culture technology provides an effective solution to overcome these limitations. Among them, shoot tip meristem culture has become a key technology for obtaining virus-free seedlings due to its vigorous cell division and low or no virus content, with a virus clearance rate of over 95% (Su *et al.,* 2024). This technology enables rapid clonal propagation of superior genotypes, shortens breeding cycles, and facilitates year-round production independent of seasonal or environmental constraints (Kim *et al.,* 2020). The establishment of an efficient tissue culture system is critically dependent on medium composition, including the type, concentration, and ratio of plant growth regulators. An efficient and stable tissue culture system is also a powerful tool for realizing the in vitro preservation of yam germplasm resources and preventing varietal degeneration (Marconi *et al.,* 2018).

Although scientists at home and abroad have made significant progress in the field of yam tissue culture, challenges remain in explant selection, shortening the culture cycle, cost control, and maintaining genetic stability (Smither *et al.,* 2019). Therefore, this study used yam shoot tips as explants and systematically optimized the entire process from surface disinfection, primary induction, subculture proliferation to rooting culture. The aim is to establish an efficient and stable rapid propagation technology system for yam virus elimination, providing theoretical basis and technical support for the large-scale production, yield, and quality improvement of superior yam varieties.

Received: 30-03-2026 | Revised: 20-05-2026 | Accepted: 25-05-2026 | Online: 22-07-2026

Materials and Methods

Plant materials and reagents: This study was conducted from January to May 2025 at the experimental facility of Jinan Wanwu Growth Technology Co., Ltd. The experimental material was robust *Dioscorea polystachya* Turcz. tubers purchased from the local market.

Reagents used included: 0.1% $HgCl_2$, 75% ethanol, MS basal medium, sucrose, agar, indole-3-butyric acid (IBA), 6-benzylaminopurine (6-BA), and naphthaleneacetic acid (NAA). All chemical reagents were of analytical grade.

Experimental Methods

Explant disinfection: The effects of different treatments on explant contamination rates were studied by setting disinfection time gradients. Material pretreatment: healthy, disease-free yam tubers were selected, rinsed under running water for 30 minutes to remove surface dirt, and then placed in a 4℃ refrigerator for 48 h to break dormancy and induce sprouting (Fig. 1).

Disinfection procedure: yam stem tips were used as explants. The stem tip meristems with a diameter of 0.3–0.8 mm were selected as experimental explants. Pretreated stem tips were transferred to a clean bench, immersed in 75% ethanol for 7 seconds, rinsed with sterile water, and then disinfected in 0.1% $HgCl_2$ solution for 6, 8, and 10 minutes (Table 1). The solution was continuously shaken during disinfection to ensure thorough contact. After disinfection, the tips were rinsed 6 times with sterile water and placed in sterile petri dishes. The stem tip meristem was observed and dissected under a stereomicroscope (SZM-41/42/T1/T2) (Figs. 2, 3), and then inoculated onto induction medium.

For each treatment, 5 bottles were inoculated with 3 explants per bottle, and each treatment was repeated three times. After 15 days of culture, the explant contamination rate and browning rate were recorded to determine the optimal disinfection time.

Primary culture: MS medium was used as the basal medium, supplemented with 6 g/L agar and 30 g/L sucrose. Different concentrations of NAA and 6-BA were then introduced (Table 2). The pH of the medium was adjusted to 5.8-6.0. For each treatment, 5 bottles were inoculated with 3 shoot tip explants in each bottle, and the treatment was repeated three times. The seedling survival rate of the explants was observed and recorded after 25 days of culture, and statistical analysis was performed.

Subculture: To obtain a large number of aseptic seedlings, robust aseptic seedlings obtained from primary culture were subcultured. MS medium was used as the basal medium, with 6 g/L agar and 30 g/L sucrose added, and different concentrations of IBA and 6-BA were set (Table 3). The pH of the medium was adjusted to 5.8-6.0. For each treatment, 5 bottles were inoculated with 3 aseptic seedlings in each bottle, and the reaction was repeated three times. After 30 days of culture, the growth of the seedlings was observed, and the proliferation coefficient was calculated to determine the optimal hormone concentration for aseptic seedling differentiation.

Rooting culture: Healthy aseptic seedlings (approximately 3-4 cm in height) after 30 days of subculture were transferred to rooting medium to promote root development. The basal medium was MS, supplemented with 6 g/L agar and 30 g/L sucrose, and the pH was adjusted to 5.8-6.0. An $L_4(2^3)$ orthogonal experimental design was used to study the effects of IBA, NAA, and 6-BA at different levels on rooting (Table 4). A blank control without any plant growth regulators was also included. For each treatment 5 bottles were inoculated with 3 adventitious buds in each bottle, and the treatment was repeated three times. After 30 days of cultivation, the rooting rate and number of roots were recorded to determine the optimal rooting medium formula:

Table 1. Explant disinfection time treatment.

Dispose	75% C_2H_5OH (s)	0.1% $HgCl_2$ (min)
A1	7	6
A2	7	8
A3	7	10

Table 2. Growth regulators in primary culture medium for sterile seedlings.

Dispose	NAA (mg/L)	6-BA (mg/L)
B1	0.1	1.0
B2	0.2	1.0
B3	0.1	0.5
B4	0.2	0.5

Table 3. Growth regulators in subculture medium for sterile seedlings.

Dispose	IBA (mg/L)	6-BA (mg/L)
C1	0.3	0.5
C2	0.3	1.0
C3	0.6	0.5
C4	0.6	1.0

Table 4. Plant growth regulators in rooting culture medium for sterile seedlings.

Test number hormone treatment	IBA (mg/L)	NAA (mg/L)	6-BA (mg/L)
D1	0.2	0.05	0.3
D2	0.2	0.1	0.6
D3	0.4	0.05	0.6
D4	0.4	0.1	0.3

Data analysis and processing

Experimental data were calculated using the following formulas:

$$\text{Contamination rate (\%)} = \frac{\text{Number of contaminated explants}}{\text{Total number of inoculated explants}} \times 100$$

$$\text{Browning rate (\%)} = \frac{\text{Number of browned explants}}{\text{Total number of inoculated explants}} \times 100$$

$$\text{Seedling rate (\%)} = \frac{\text{Number of seedling explants}}{\text{Total number of inoculated explants}} \times 100$$

$$\text{Proliferation coefficient} = \frac{\text{Number of stem segments after proliferation}}{\text{Number of inoculated stem segments}}$$

$$\text{Rooting rate (\%)} = \frac{\text{Total number of rooting plants}}{\text{Total number of inoculations}} \times 100$$

All data from the experiment were collated and analyzed using Microsoft Excel 2021. SPSS version 25.0 was employed to assess significant differences in univariate analyses of the relevant test data. The Least Significant Difference (LSD) method and Duncan's multiple range test were utilized to evaluate the significance of differences between treatments. Additionally, SPSSAU was used to conduct multivariate and multi-level orthogonal range analysis, as well as multivariate analysis of variance. Significant analyses were performed to identify differences among individual treatments.

Results

Explant disinfection: Disinfection duration significantly affected the contamination and browning rates of yam shoot tip explants (Table 5). Treatment A1 exhibited the lowest browning rate, with only 15.5% contamination, while A1 had the same browning rate as A2 (22.22%) and A3 (24.45%). This disinfection method appears to be more damaging to the cells, which is detrimental to the subsequent regeneration process. A3 demonstrated the highest contamination rate, with A1 treatment showing a contamination rate of 0%, significantly lower than that of A2 (4.45%) and A3 (22.22%). This showed that A1 provided the most effective disinfection. The contamination rate of A3 was markedly higher than that of A1 and A2, and there was a significant difference in browning rates between A1 and A3. These results suggest that as disinfection time increases, both the contamination rate and browning rate of yam explants also increase. Ultimately, it was concluded that the optimal disinfection protocol for yam shoot tips was a 75% ethanol treatment for 7 seconds, followed by a 0.1% $HgCl_2$ treatment for 6 minutes.

Primary culture for sterile seedlings: It can be concluded that the effects of different hormone ratios on the primary culture of yam shoot tips vary significantly (Table 6 and Fig. 4). Among the treatments, the seedling rate for B3 reached the highest level at 82.22%, which was significantly higher than that of the other treatments. B3 exhibited the best growth, characterized by dense bud clusters, robust plant bodies, and vibrant green leaves, indicating that this hormone ratio effectively promoted bud cluster differentiation and leaf vitality. In contrast, the low seedling rates observed in the B2 (68.89%) and B4 (64.45%) treatments may be attributed to the high concentration of NAA, which inhibited bud differentiation while promoting callus and root development. Excessive NAA can also lead to leaf yellowing. The seedling formation rate for the B1 (75.55%) treatment was lower than that of the B3 treatment, likely due to uneven nutrient distribution resulting from the slight inhibition of bud elongation caused by the high concentration of 6-BA. In conclusion, the optimal hormone ratio for yam shoot tip culture is MS + NAA 0.1 mg/L + 6-BA 0.5 mg/L.

Subculture for sterile seedlings: There are significant differences in the subculture of yam shoot tips when using different plant growth regulators, specifically IBA and 6-BA (Table 7). Among the 4 treatments, the C1 treatment group exhibited the highest value-added ability, with a proliferation coefficient of 4.47. Although the C2 treatment maintained a high proliferation rate, it resulted in yellowing leaves and excessive bud cluster density, likely due to the high concentration of cytokinin (6-BA), which promotes lateral bud differentiation (Fig. 5). The proliferation coefficients for C1 (4.47) and C2 (4.34) were significantly higher than those for C3 (3.53) and C4 (3.87). Therefore, it can be concluded that the optimal hormone ratio for yam subculture is MS + IBA 0.3 mg/L + 6-BA 0.5 mg/L.

Table 5. Effects of different disinfection durations on yam explants.

Dispose	Contamination rate (%)	Browning rate (%)	Explant status
A1	0.00±0.000b	15.55±3.85a	The explants remain green, while a few turn brown and form colonies
A2	4.45±3.85b	22.22±10.18a	A small number of explants have white colonies attached to them, while some of the explants have turned brown
A3	22.22±3.85a	24.45±3.85a	A small number of explants have white colonies attached to them, while some of the explants have turned brown

Note: Different lowercase letters in the same column indicate a significant difference between treatments at the 0.05 level

Table 6. Effects of hormone ratios on the primary culture of yam shoot tips.

Dispose	Seedling rate (%)	Growth
B1	75.55 ± 3.85ab	The growth is robust, with relatively dense bud clusters. The leaves are tender and green, the buds are strong and vibrant, and the leaves are large
B2	68.89 ± 3.85bc	The growth is average; the buds are green, and the leaves are yellow
B3	82.22 ± 7.70a	The growth is robust, the buds are plentiful, and the leaves are vibrant green
B4	64.45 ± 3.85c	The growth is average, the leaves are large, and they exhibit a yellow-green color

Note: Different lowercase letters in the same column indicate a significant difference between treatments at the 0.05 level

Table 7. Effect of hormone ratios on subculture of yam shoot tip.

Dispose	Proliferation coefficient	Growth
C1	4.47 ± 0.07a	The growth is robust, the buds are vibrant and green, and the leaves are large
C2	4.34 ± 0.11a	The growth is robust, the buds are plentiful, and the leaves are yellowing
C3	3.53 ± 0.07c	The growth is weak, the bud clusters are sparse, the stems are thin, and the leaves are yellow
C4	3.87 ± 0.07b	The growth is weak, the bud clusters are sparse, the stems are thin, and the leaves are yellowish-green

Note: Different lowercase letters in the same column indicate a significant difference between treatments at the 0.05 level

Fig. 1. Stem segment sprouting.

Fig. 2. Explant disinfection.

Fig. 3. Stem tip dissection procedure under stereomicroscope.

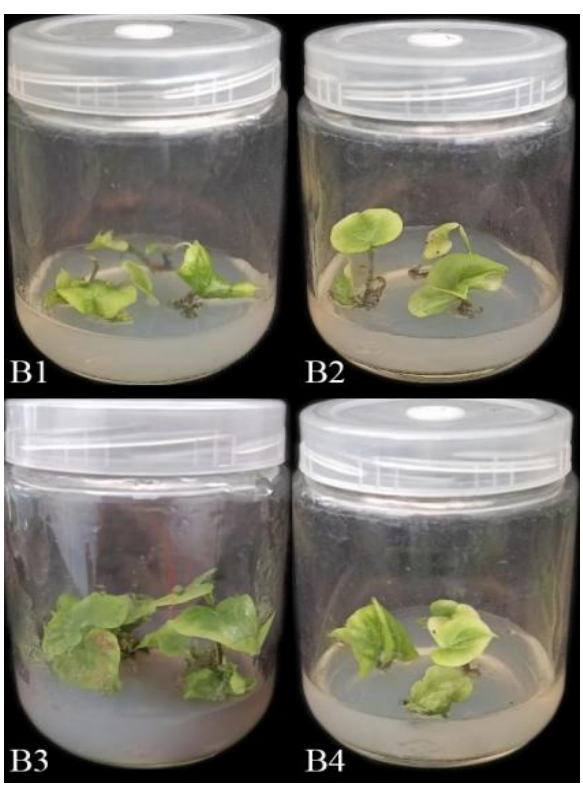

Fig. 4. Budding of yam shoot tip.

Fig. 5. Effect of different concentrations of hormone ratios on yam subculture.

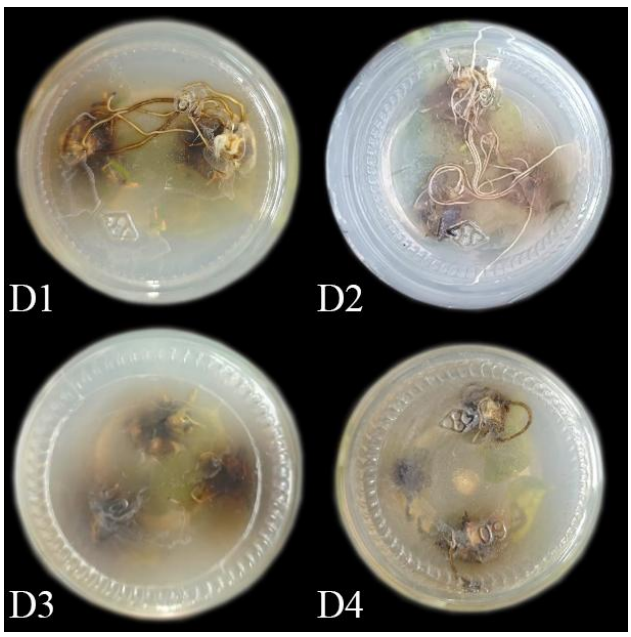

Fig. 6. Effect of different concentrations of hormone ratios on yam rooting culture.

Rooting culture for sterile seedlings: Various plant growth regulators—specifically IBA, NAA, and 6-BA—exhibited significant differences in the subculture of yam shoot tips (Fig. 6). Among the 4 treatments, D2 exhibited the highest rooting success, achieving a rooting rate of 100% (Table 8). The combination of IBA and NAA significantly enhanced root structure; IBA promoted root differentiation, while NAA influenced root elongation. Although 6-BA at a concentration of 0.6 mg/L did not show significant effects, it may indirectly support the rooting process. The results of the range analysis presented in Table 7 revealed that the impact of various factors on the rooting rate followed the order: A (IBA) > B (NAA) > C (6-BA) during the rooting process of yam. The optimal hormone ratio for yam rooting culture was determined to be A1B2C2. The F ratios for factors A and B were significantly high, measuring 15,304.61 and 5,927.77, respectively (Table 9). The corresponding P values for these factors were substantially less than 0.05, indicating that factors A and B had a significant impact on the rooting rate of yam. In contrast, the F ratio for factor C was only 1, with a P value of 0.5, suggesting that its effect was not significant. Therefore, the optimal hormone concentrations for yam rooting culture were 0.2 mg/L IBA, 0.1 mg/L NAA, and 0.6 mg/L 6-BA. In summary, the combination of 0.2 mg/L IBA, 0.1 mg/L NAA, and 0.6 mg/L 6-BA produced the most effective rooting results among the various hormone concentrations tested. Thus, the optimal hormone ratio for yam rooting culture was MS + IBA 0.2 mg/L + NAA 0.1 mg/L + 6-BA 0.6 mg/L.

Table 8. Orthogonal design and results for rooting culture.

Treatment	Factor A	Factor B	Factor C	Rooting rate (%)
D1	1	1	1	83.64
D2	1	2	2	100.00
D3	2	1	2	57.90
D4	2	2	1	73.84
K1	183.64	141.54	157.48	
K2	131.74	173.84	157.90	
k1	91.82	70.77	78.74	
k2	65.87	86.92	78.95	
Extreme difference (R)	25.95	16.15	0.21	
Primary and secondary factors	A>B>C			
Optimal combination	A1B2C2			

Note: K1 and K2 are the sum of the levels of each factor, and K1 and K2 are the average values of each level of each factor.

Table 9. One-way ANOVA of rooting rates.

Factor	Sum of squares of deviations	Degree of freedom	Mean square	F-ratio	P-value	Distinctiveness
A	673.403	1	673.403	15304.614	0.005146	*
B	260.822	1	260.822	5927.773	0.008268	*
C	0.044	1	0.044	1.000	0.5	
Error	0.04	1	0.04			

Discussion

This study successfully established an efficient in vitro regeneration and rapid propagation system using yam shoot tips as explants and successfully obtained virus-free seedlings (Kereša *et al.,* 2021). The results confirmed the effectiveness of shoot tip meristem culture in virus clearance, mainly due to the active cell division characteristics and low virus load in this region (Xue *et al.,* 2020). Through systematic optimization of culture conditions, we established a complete in vitro regeneration technology system for yam, providing reliable technical support for the preservation of yam germplasm resources and the promotion of superior varieties (Florentino *et al.,* 2022).

Explant disinfection is the primary key step in establishing an in vitro culture system (Jia *et al.,* 2023). This study found that disinfection time had a decisive impact on the culture effect by setting different disinfection time gradients. The experimental results showed that although extending the disinfection time could effectively reduce the microbial contamination rate, it will significantly increase the browning rate of explants due to the phytotoxic effect of disinfectants. When the treatment time of 0.1% $HgCl_2$ was extended to 10 minutes, the contamination rate was decreased to 22.22%, but the browning rate was increased to 24.45%, which seriously affected the viability of explants. This browning phenomenon is closely related to the oxidation of phenolic substances and is one of the main limiting factors in the tissue culture process of yam (Gogile *et al.,* 2024). Through systematic optimization, the two-step disinfection method used in this study (treatment with 75% ethanol for 7 seconds, followed by treatment with 0.1% $HgCl_2$ for 6 minutes) achieved the best balance between controlling contamination and reducing physiological damage. The explant contamination rate was 0%, and the browning rate

was 15.55%, thus ensuring the highest explant survival rate (Van *et al.,* 2021).

The formulation of the culture medium, especially the balance of plant growth regulators, had a profound impact on all stages of micropropagation. In primary culture, the combination of 0.1 mg/L NAA and 0.5 mg/L 6-BA in MS medium achieved the highest seedling rate (82.22%). This indicates that a relatively low ratio of auxin to cytokinin is beneficial for breaking the dormancy state of meristematic tissues and promoting the initial proliferation of shoots. Notably, higher concentrations of NAA (0.2 mg/L) inhibited bud differentiation and promoted callus formation, consistent with the known role of auxin in inducing cell division and root development, but potentially at the expense of direct organogenesis (Salgado *et al.,* 2025). Experimental observations revealed that the B3 treatment (0.1 mg/L NAA + 0.5 mg/L 6-BA) not only achieved the highest seedling rate but also produced dense bud clusters, robust seedlings, and deep green leaves, exhibiting optimal growth and development.

In the subculture stage aimed at achieving rapid propagation, the combination of 0.3 mg/L IBA and 0.5 mg/L 6-BA showed the best proliferation effect, with a proliferation coefficient of 4.47. Continuous culture observations showed that this formulation not only maintained a high proliferation rate but also ensured seedling quality, avoiding physiological abnormalities. Although higher concentrations of 6-BA (1.0 mg/L) also induced vigorous proliferation, it triggered cytokinin stress symptoms such as vitrification and leaf yellowing. The superior performance of the C1 formulation highlights the importance of balanced cytokinin levels for maintaining healthy, highly proliferating seedlings. The difference in proliferation efficiency observed in this study compared to previous reports may stem from subtle variations in genotype differences among yam cultivars, explant physiological states, and culture conditions, highlighting the necessity of conducting genotype-specific optimization studies (Ping *et al.,* 2016).

The most significant results were observed during the rooting induction phase, where the synergistic application of auxins played a crucial role. The optimal rooting medium was MS medium supplemented with 0.2 mg/L IBA, 0.1 mg/L NAA, and 0.6 mg/L 6-BA, achieved a rooting rate of 100%. This result can be explained by the complementary effects of IBA and NAA: IBA excels at promoting robust root development, while NAA is significantly effective in inducing more adventitious roots (Yang *et al.,* 2017). Range analysis of the orthogonal experiment further showed that the order of influence of each factor on the rooting rate was IBA > NAA > 6-BA, with IBA showing a significant F-value of 15304.61. The addition of low concentrations of cytokinin (6-BA) may help maintain seedling vigor, thereby indirectly supporting root initiation. In the D2 treatment, not only did the rooting rate reach 100%, but the root system also developed well, with robust taproots and well-developed lateral roots, laying a good foundation for subsequent transplanting and acclimatization.

Furthermore, the system established in this study showed good potential in terms of genetic stability. Morphological observation of the regenerated plants revealed no significant phenotypic variation, indicating that this propagation system could maintain genetic consistency well. However, long-term genetic stability still needs further verification using techniques such as molecular markers.

Conclusion

In this study, yam shoot tips were utilized as explants. During the disinfection process, the explants were immersed in 75% ethanol for 7 seconds and subsequently rinsed with sterile water. The highest survival rate of yam shoot tips was observed when they were soaked in a sterile bottle containing a 0.1% mercuric chloride solution for 6 minutes. In the primary culture, the optimal formulation was MS medium supplemented with 0.1 mg/L NAA and 0.5 mg/L 6-BA, resulting in a seedling rate of 82.22%. For subculturing, the most effective formulation was MS medium with 0.3 mg/L IBA and 0.5 mg/L 6-BA. Additionally, the best rooting effect was achieved with MS medium containing 0.2 mg/L IBA, 0.1 mg/L NAA, and 0.6 mg/L 6-BA. This study provides both theoretical and technical support for enhancing yam yield, rapidly propagating superior yam varieties, and improving yam quality. With the ongoing advancements in biotechnology, the integration of tissue culture techniques with other innovative technologies will create new opportunities for the enhancement of yam varieties and the growth of the industry.

Author's Contributions: Conceptualization, S.H.; Data curation, S.H., Z.X. and Y.B.; Formal analysis, S.H. and Z.L.; Funding acquisition, J.S.; Investigation, S.H.; and X.W.; Methodology, S.H. and Z.X.; Project administration, J.S.; Resources, J.S.; Validation, S.H.; Visualization, S.H.; Writing—original draft, S.H.;Writing—review and editing, S.H. and J.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (32302518).

Data Availability Statement: The datasets that support this study are available from the corresponding author on reasonable request.

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

Ethics declaration: The plant material used in this study appears to be of wild origin.

References

Butler, A. and M. Lobley. 2016. Training as a social purpose: Are economic and social benefits delivered? *Int. J. Train. Dev.*, 20(4): 249-261.

Florentino, H.O., D.F. Jones, C.A. Irawan, D. Ouelhadj, B. Khosravi and D.R. Cantane. 2022. An optimization model for combined selecting, planting and harvesting sugarcane varieties. *Ann. Oper. Res.*, 314(2): 451-469.

Gogile, A., T. Markos, M. Kebede, D. Kidanemariam and A. Abraham. 2024. Elimination of yam mosaic virus from yam using an optimized combination of meristem culture and thermotherapy. *Australas. Plant Pathol.*, 53(2): 185-197.

Gu, Y., F. Zhong, T. Sun and Z. Chang. 2025. Active ingredients and mechanisms of peony seed blended oil in diabetes treatment using Kunming mice: Insights from GC–MS, network pharmacology, and experimental verification. *J. Funct. Foods*, 125: 106652.

Hu, W., M. Zhao, S. Zhang, Y. Li, J. Dai, C. Gu and X. Liao. 2023. Optimized leaf storage and photosynthetic nitrogen trade-off promote synergistic increases in photosynthetic rate and photosynthetic nitrogen use efficiency. *Physiol. Plant.*, 175(5): e14013.

Jia, W., J. Rong, M. Zhang, W. Peng, X. He, X. Lei and Y. Wang. 2023. *In vitro* adventitious regeneration and plantlet transplantation of *Atractylodes chinensis* (DC.) Koidz., a valuable medicinal plant. *Plant Cell, Tiss. Organ Cult.*, 155(1): 209-220.

Kereša, S., K. Kurtović, S.G. Ban, D. Vončina, I.H. Jerčić, S. Bolarić and A.B. Mihovilović. 2021. Production of virus-free garlic plants through somatic embryogenesis. *Agronomy*, 11(5): 876.

Kim, K., F. Li, J.W. Yoo and C.Y. Kim. 2020. The relationships among environments, external knowledge acquisition, and innovation. *Sustainability*, 12(14): 5541.

Marconi, G., N. Ferradini, L. Russi, F. Veronesi and E. Albertini. 2018. Genetic characterization of the apple germplasm collection in central Italy: The value of local varieties. *Front. Plant Sci.*, 9: 1460.

McLeish, M.J., A.D. Zamfir, B.M. Babalola, A. Peláez, A. Fraile and F. García-Arenal. 2022. Metagenomics show high spatiotemporal virus diversity and ecological compartmentalisation: Virus infections of melon, *Cucumis melo*, crops, and adjacent wild communities. *Virus Evol.*, 8(2): veac095.

Nagendra, L., M. Chandran and M. Hiligsmann. 2025. The economic benefits of fracture prevention. *Expert Rev. Pharmacoecon. Outcomes Res.*, 25(8): 1143-1148.

Ping, A., L. Hou and G. Xing. 2016. Study on stem tip tissue culture and stem segment side bud seedling formation of iron stick yam. *Heilongjiang Agri. Sci.*, 9: 6-10.

Salgado, M.G., P.J. Maity, D. Lundin and K. Pawlowski. 2025. The auxin phenylacetic acid induces NIN expression in the actinorhizal plant *Datisca glomerata*, whereas cytokinin acts antagonistically. *PLoS One*, 20(2): e0315798.

Smither, S.J., L.S. Eastaugh, J.S. Findlay, L.M. O'Brien, R. Thom and M.S. Lever. 2019. Survival and persistence of Nipah virus in blood and tissue culture media. *Emerg. Microbes Infect.*, 8(1): 1760-1762.

Su, W., C. Deng, W. Wei, X. Chen, H. Lin, Y. Chen and J. Jiang. 2024. Double-Heading produces larger fruit via inhibiting EjFWLs expression and promoting cell division at the early stage of loquat fruit development. *Horticulturae*, 10(8): 793.

Van Woesik, R., R.B. Banister, E. Bartels, D.S. Gilliam, E.A. Goergen, C. Lustic and D. Lirman. 2021. Differential survival of nursery-reared *Acropora cervicornis* outplants along the Florida reef tract. *Restor. Ecol.*, 29(1): e13302.

Wang, J., P. Xu, G. Zhou, X. Li, Q. Lu, X. Liu and C. Wang. 2018. Genetic improvement and breeding practices for Chinese mitten crab, *Eriocheir sinensis*. *J. World Aquac. Soc.*, 49(2): 292-301.

Xue, Z., L. Liu and C. Zhang. 2020. Regulation of shoot apical meristem and axillary meristem development in plants. *Int. J. Mol. Sci.*, 21: 2917.

Yang, Z.B., G. Liu, J. Liu, B. Zhang, W. Meng, B. Müller and Z. Ding. 2017. Synergistic action of auxin and cytokinin mediates aluminum-induced root growth inhibition in Arabidopsis. *EMBO Reports*, 18(7): 1213-1230.

Zhou, L., Y. Zhou, Z. Fu, J. Lü, C. Lu and H. Xiao. 2024. Vertical structures and microphysical mechanisms of winter precipitation over the southwestern mountainous area of China. *Atmosp. Res.*, 303: 107346.