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Postprint: Study on In Vitro Rapid Propagation of 'Yang' s Jinhong 50' Kiwifruit

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Abstract

'Yangshi Jinhong 50' kiwifruit is a superior yellow-fleshed red-centered cultivar of Actinidia chinensis. This study utilized its stem segments with axillary buds as explants and employed tissue culture methods for in vitro culture to investigate suitable sterilization methods for explants, optimal plant growth regulator types and concentration ratios for its rapid in vitro propagation, and established two in vitro regeneration pathways: Pathway I involved direct induction of axillary bud sprouting from stem segments; Pathway II involved initial callus formation at the base of stem segments, followed by differentiation into adventitious buds. The results demonstrated that the optimal sterilization method for stem segments with axillary buds of 'Yangshi Jinhong 50' kiwifruit was 75% ethanol for 30s + 15% Ca(ClO)2 for 5min + 0.1% mercuric chloride for 8min; in Pathway I, the optimal medium for inducing axillary bud sprouting from stem segments was MS + 4.0 mg · L-1 6-BA + 0.1 mg · L-1 NAA; in Pathway II, the optimal medium for inducing callus formation at the base of stem segments and producing adventitious buds was MS + 3.0 mg · L-1 6BA + 0.3 mg · L-1 NAA; the optimal plant growth regulator combination for culturing cluster buds was MS + 4.0 mg · L-1 6-BA + 0.4 mg · L-1 NAA; the optimal plant growth regulator combination for adventitious bud rooting culture was 1/2 MS + 0.9 mg · L-1 IBA, with adventitious roots differentiating after approximately 20 d and complete plantlets obtained after approximately 40 d; tissue-cultured seedlings after rooting could achieve a 96% transplant survival rate in a substrate with garden soil: fine sand = 1:1. This study established an in vitro rapid propagation system for 'Yangshi Jinhong 50' kiwifruit, providing a foundation for subsequent genetic transformation and genetic improvement research.

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Full Text

Preamble

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Title: Study on Micropropagation in vitro of Actinidia chinensis ‘Yangshi Jinhong 50’

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Abstract

Actinidia chinensis ‘Yangshi Jinhong 50’is a superior yellow-fleshed, red-centered cultivar of Actinidia chinensis Planch. This study utilized stem segments with axillary buds as explants for in vitro culture via tissue culture methods to investigate optimal explant sterilization protocols and the best combinations of plant growth regulators for rapid micropropagation. Two in vitro regeneration pathways were established: Pathway I involved direct induction of axillary bud sprouting from stem segments, while Pathway II involved initial callus formation at the stem base followed by adventitious bud differentiation. The results demonstrated that the optimal sterilization method for stem segments with axillary buds of ‘Yangshi Jinhong 50’ kiwifruit was 75% ethanol for 30 seconds + 15% Ca(ClO) for 5 minutes + 0.1% mercuric chloride for 8 minutes. In Pathway I, the optimal medium for inducing axillary bud germination was MS + 4.0 mg · L^{1} 6-BA + 0.1 mg · L^{1} NAA. In Pathway II, the optimal medium for inducing callus formation and subsequent adventitious bud production at the stem base was MS + 3.0 mg · L^{1} 6-BA + 0.3 mg · L^{1} NAA. The best plant growth regulator combination for cluster bud culture was MS + 4.0 mg · L^{1} 6-BA + 0.4 mg · L^{1} NAA. The optimal combination for adventitious bud rooting was 1/2 MS + 0.9 mg · L^{1} IBA, with adventitious roots differentiating after approximately 20 days and complete plants obtained after about 40 days. Rooted plantlets achieved a 96% survival rate when transplanted to a substrate mixture of garden soil and fine sand at a 1:1 ratio. This study establishes an in vitro rapid propagation system for ‘Yangshi Jinhong 50’ kiwifruit, providing a foundation for subsequent genetic transformation and gene improvement research.

Keywords: Actinidia chinensis ‘Yangshi Jinhong 50’, stems with axillary buds, tissue culture, plant growth regulators, regeneration system

Introduction

Kiwifruit represents one of the four most successful artificially domesticated and cultivated wild fruit crops of the 20th century (Warrington et al., 1990). Var-

ious explants including stem segments (Wang LQ, 2017), anthers (Wang GF, 2017), leaves (Wei PF, 2016), petioles (Ge XL, 2009), and endosperm (Lin Y et al., 2012) have been utilized for tissue culture studies, establishing tissue culture technology as a crucial pathway for elite kiwifruit cultivar propagation. Actinidia chinensis, a species endemic to China, offers unique flavor characteristics and high economic value, with cultivation areas expanding in recent years (Wang RL et al., 2018). 'Yangshi Jinhong 50' kiwifruit (Actinidia chinensis 'Yangshi Jinhong 50') is a superior cultivar selected from Actinidia chinensis by the Yangshi Kiwifruit Research Institute in Yangzhou, Jiangsu Province in 2013. The cultivar produces fruits averaging over 100 g each, featuring golden flesh with a red center, high sugar content, fine texture, and excellent taste, positioning it as a promising new variety that may surpass 'Hayward' . Xixia County represents a major kiwifruit production region in China where 'Yangshi Jinhong 50' is extensively cultivated, demonstrating good market performance, positive farmer evaluations, and broad market prospects. Currently, seedling grafting on seedling rootstocks is the primary propagation method in Xixia County, requiring one year to develop seedling rootstocks and an additional year to manage grafted plant growth, resulting in a two-year propagation cycle (Tang LL et al., 2016). This lengthy process is also susceptible to canker disease, limiting industrial development. Although numerous reports on kiwifruit tissue culture exist, kiwifruit is dioecious with highly heterozygous genotypes (Ye KY et al., 2012), and tissue culture systems are genotype-dependent (Huang HW et al., 2013). No reports have documented tissue culture protocols for 'Yangshi Jinhong 50' . Therefore, this study employed stem segments with axillary buds from 'Yangshi Jinhong 50' for tissue culture to provide technical support for scientific cultivation in Xixia County.

Materials and Methods

1.1 Experimental Materials

Experimental materials were collected from the experimental field of Xixia County Kiwifruit Research Institute in May. Healthy, tender new shoot stem segments of 'Yangshi Jinhong 50' kiwifruit were selected as explants.

1.2 Culture Medium Composition and Conditions

MS medium served as the basal medium for axillary bud induction, callus induction, bud differentiation, and cluster bud culture, while 1/2 MS medium was used for rooting culture. All media contained 36 g·L^{1} sucrose and 6 g·L^{1} agar, with pH adjusted to 5.8-6.2. Plant growth regulators including 6-BA, NAA, and IBA were utilized. Media were sterilized at 121 °C and 0.1 MPa for 20 minutes. Cultures were maintained at 25 ± 2 °C under a light intensity of 1500–2000 lx with a 12-hour photoperiod.

1.3 Experimental Procedures

1.3.1 Explant Sterilization Tender branches of 'Yangshi Jinhong 50' kiwifruit were defoliated, washed with detergent, and rinsed under running tap water for 40 minutes before being cut into 1-1.5 cm stem segments with axillary buds. Four sterilization treatments were applied, with 30 explants per treatment. Contamination and browning rates were recorded after 21 days, and survival rate was calculated as 1 - contamination rate - browning rate. Sterilized stem segments were rinsed 5–6 times with sterile water to remove residual sterilant and secretions. Due to the dense fine hairs on the stem surface, sterile forceps were used to repeatedly agitate the segments during sterilization to ensure adequate contact between the sterilant and epidermal tissue.

1.3.2 Axillary Bud Culture Sterilized stem segments with axillary buds were trimmed at both ends to remove wound tissue in contact with the sterilant before inoculation onto MS media supplemented with various plant growth regulators, with axillary buds oriented upward. After 35 days of culture, axillary bud germination rate and number were recorded. Nine culture media were tested, with eight explants per medium and three replicates. Germination rate was calculated as (number of explants with sprouted buds / total number of inoculated explants) × 100%.

1.3.3 Callus and Adventitious Bud Induction During axillary bud induction, callus tissue formed at the stem segment base. Changes in callus development were recorded weekly for each medium, and the induction rate of callus redifferentiation into buds was calculated. The callus-to-bud induction rate (reported as germination rate in Table 3) was calculated as (number of explants with buds emerging from callus / total number of inoculated explants) × 100%.

1.3.4 Cluster Bud Proliferation Individual robust adventitious buds of uniform growth were selected and inoculated onto proliferation media for cluster bud culture. Bud growth was observed every 10 days, and proliferation rate and coefficient were recorded after 30 days. Nine culture media were tested, with seven adventitious buds per medium and three replicates. The proliferation coefficient represented the average number of buds regenerated per explant.

1.3.5 Adventitious Bud Rooting Individual robust adventitious buds measuring 2–3 cm with uniform growth were selected for rooting culture. Six culture media were tested, with seven adventitious buds per medium and three replicates. Data were recorded every 10 days. Rooting rate was calculated as (number of rooted adventitious buds / total number of inoculated adventitious buds) × 100%.

1.3.6 Transplanting of Rooted Plantlets Rooted sterile plantlets were transferred to a greenhouse for acclimatization. Bottle caps were loosened for 2 days, then opened for approximately 5 days. Plantlets were washed to remove residual medium from the roots before transplanting to a substrate mixture of garden soil and fine sand at a 1:1 ratio.

1.4 Data Analysis

Experimental data were initially processed using Excel 2013 and subsequently analyzed statistically with SPSS 22.0 software. Duncan' s multiple range test was employed for significance comparison among means.

Results

2.1 Effects of Different Sterilization Methods on Obtaining Sterile Stem Segments with Buds

After 20 days of observation, contamination primarily occurred within the first 9 days, while browning appeared gradually between days 7 and 20. The four sterilization treatments showed substantial differences in effectiveness for 'Yangshi Jinhong 50' kiwifruit stem segments with buds (Table 1). Treatment C, using only Ca(ClO) , exhibited the highest contamination rate at 70.00% and the lowest survival rate. The other three treatments incorporating mercuric chloride all achieved contamination rates below 50%, indicating superior sterilization efficacy. Treatment A (mercuric chloride for 5 minutes) showed 13.34% higher contamination and 10.02% lower survival compared to Treatment B (mercuric chloride for 8 minutes), demonstrating that 8-minute mercuric chloride treatment was more effective. Treatment D (combined Ca(ClO) and mercuric chloride) achieved 10.00% lower contamination and 3.24% higher survival than Treatment B, representing marginally superior performance. Explant browning results from phenol oxidation by phenol oxidase released from ruptured lysosomes after cutting. Considering all factors, the optimal sterilization protocol for 'Yangshi Jinhong 50' kiwifruit stem segments with axillary buds was 75% ethanol for 30 seconds + 15% Ca(ClO) for 5 minutes + 0.1% mercuric chloride for 8 minutes.

2.2 Axillary Bud Induction in 'Yangshi Jinhong 50' Kiwifruit

Following inoculation, axillary buds began germinating after approximately 7 days, with distinct light green shoots visible by day 13. As culture continued, buds gradually turned dark green, reaching a maximum germination rate of 91.67% after 35 days (Table 2), with shoot heights ranging from 2–5 cm. Analysis of Table 2 revealed that when 6-BA concentration ranged from 3–5 mg · L^{1}, 0.1 mg · L^{1} NAA was optimal for axillary bud germination, with germination rates 75% across three media. Medium 2 achieved the highest germination rate of 91.67%, significantly exceeding the other eight media, producing robust buds

with well-developed stems and vigorous growth (Figure 1 [Figure 1: see original paper]A). Medium 6 yielded a 79.17% germination rate but produced weak, slender shoots with good leaf development but stunted stem elongation. Overall, increasing NAA concentration from 0.1 mg · L ^{1} to 0.3 mg · L ^{1} inhibited axillary bud germination. Therefore, the optimal medium for axillary bud germination of 'Yangshi Jinhong 50' kiwifruit stem segments was MS + 4.0 mg · L ^{1} 6-BA + 0.1 mg · L ^{1} NAA.

2.3 Adventitious Bud Differentiation from Callus at Stem Segment Base

All nine media induced substantial callus formation at the base of 'Yangshi Jinhong 50' kiwifruit stem segments (Figure 1 [Figure 1: see original paper]B), with callus induction rates exceeding 80%. However, callus redifferentiation into adventitious buds proceeded slowly: explant bases showed obvious swelling within 7 days, callus formed between days 7-15, and bud differentiation required approximately one month, with adventitious buds emerging gradually over 1-3 months. Medium 7 (3 mg · L ^{1} 6-BA + 0.3 mg · L ^{1} NAA) produced the fastest and highest bud emergence, with a germination rate of 83.33% within the first two months, generating robust, well-growing shoots (Figure 1 [Figure 1: see original paper]C). Medium 1 (3 mg · L ^{1} 6-BA + 0.1 mg · L ^{1} NAA) also achieved a high germination rate of 75.00%, but bud emergence occurred primarily during months 2-3. The other seven media showed significantly different ($P<0.05$) germination rates. Table 3 demonstrated that 6-BA and NAA concentration ratios significantly affected callus induction and redifferentiation. At constant NAA concentration, increasing 6-BA concentration decreased adventitious bud induction from callus. At 5 mg · L ^{1} 6-BA, callus induction was high but bud germination was extremely low; Medium 6 showed limited bud differentiation with some adventitious root formation, while Medium 9 yielded 0% bud germination. Consequently, the optimal medium for callus induction and adventitious bud formation in 'Yangshi Jinhong 50' kiwifruit was MS + 3 mg · L ^{1} 6-BA + 0.3 mg · L ^{1} NAA.

2.4 Cluster Bud Proliferation in 'Yangshi Jinhong 50' Kiwifruit

During cluster bud culture, small amounts of callus formed at the shoot base after approximately 8 days, with bud primordia appearing on the callus and cluster buds observable by days 13-17. Table 4 showed that Medium 5 produced the best proliferation effect, with an average proliferation coefficient of 6.14 (Figure 1 [Figure 1: see original paper]D), significantly differing from other media ($P<0.01$). Medium 8 achieved an average coefficient of 5.10, slightly inferior to Medium 5, while Medium 7 performed poorest with only limited bud proliferation. At constant NAA concentration, the proliferation coefficient exhibited a "low-high-low" pattern with increasing 6-BA concentration, indicating that 4.0 mg · L ^{1} 6-BA was optimal for 'Yangshi Jinhong 50' cluster bud culture, with 0.4 mg · L ^{1} NAA as the most effective counterpart. All media induced

callus formation at plant bases, with larger callus masses in Media 3, 6, and 9. Media 3 and 6 produced three vitrified buds each, while buds proliferated in Medium 1 showed yellowish coloration and abnormal growth. Therefore, the optimal medium for cluster bud culture of 'Yangshi Jinhong 50' was MS + 4 mg · L ^{1} 6-BA + 0.4 mg · L ^{1} NAA.

2.5 Rooting of Adventitious Buds

Adventitious buds developed root primordia at the shoot base after approximately 12 days in rooting medium, with root initiation beginning at day 18 and widespread rooting by day 22. Subsequent root elongation produced well-developed root systems by approximately 40 days. Table 5 demonstrated significant differences in rooting response with increasing IBA concentration. Except for Medium 3, rooting rate, average root number, and average root length generally increased with IBA concentration, peaking at 0.9 mg · L ^{1} IBA, while plant height decreased with increasing IBA concentration. The 0.9 mg · L ^{1} IBA treatment achieved the highest rooting rate (72.64%), greatest average root number (25.67 roots), and longest average root length (2.67 cm) (Figure 1 [Figure 1: see original paper]E). The 1.1 mg · L ^{1} IBA treatment ranked second, while 0.3 and 0.7 mg · L ^{1} IBA treatments produced rooting rates below 40% with poor root number and length. Thus, the optimal rooting medium for 'Yangshi Jinhong 50' adventitious buds was 1/2 MS + 0.9 mg · L ^{1} IBA.

2.6 Transplanting of Rooted Plantlets

After one week of acclimatization, rooted plantlets were transplanted to the prepared substrate. Following 40 days of observation, the survival rate exceeded 96%, with well-developed root systems suitable for field planting (Figure 1 [Figure 1: see original paper]F).

Discussion

Hormone ratios play a decisive role in tissue culture, with different types and concentrations exerting distinct effects on explant growth and differentiation (Huang AD et al., 2017; Yan HX et al., 2017). Previous studies on axillary bud induction have reported varying optimal combinations. For other Actinidia chinensis cultivars, 'Hongyang' achieved 83.33% germination with 1.0 mg · L ^{1} 6-BA + 0.1 mg · L ^{1} NAA (Long QJ et al., 2010); 'Qihong' reached 82.64% with 2.0 mg · L ^{1} 6-BA + 0.1 mg · L ^{1} NAA (Wang LQ, 2017); and 'Hort 16A' attained 85.15% with 3.0 mg · L ^{1} 6-BA + 0.1 mg · L ^{1} NAA (Wang LQ, 2017). This study achieved 91.67% germination using higher 6-BA concentration combined with lower NAA, likely due to genotype-specific hormone requirements. Additionally, stem segment bases readily formed callus that redifferentiated into adventitious buds, with 3.0 mg · L ^{1} 6-BA + 0.3 mg · L ^{1} NAA producing 83.33% germination without requiring medium changes. This approach was more time-efficient than the conventional method using 2,4-D for callus induction followed by 6-BA or

ZT with NAA for bud induction (Zhang TK et al., 2017), possibly reflecting the vigorous growth characteristics of 'Yangshi Jinhong 50'. Regarding cluster bud proliferation, Liu Z et al. (2013) summarized that high 6-BA concentrations promote adventitious bud multiplication but inhibit stem elongation in kiwifruit. This study identified 4.0 mg·L ^{1} 6-BA as optimal for cluster bud culture, which indeed produced high proliferation coefficients but excessive leaf growth with slow stem development, potentially hindering subsequent rooting. This phenomenon warrants further investigation. For adventitious bud rooting, Wu XH et al. (2013) demonstrated IBA superiority over NAA for 'Hayward' kiwifruit, while Zhao XP et al. (2013) achieved excellent results using IBA alone for 'Hongyang' kiwifruit. This study confirmed that appropriate IBA concentrations effectively promoted rooting in 'Yangshi Jinhong 50', though the effect was limited to a specific concentration range.

This study employed two pathways to obtain sterile plantlets of 'Yangshi Jinhong 50': direct axillary bud induction from bud-bearing stem segments, and callus formation at the stem base followed by adventitious bud production. Previous research successfully established both pathways for Actinidia chinensis (Tan XM, 2002), Actinidia arguta (Lin MM et al., 2016; Niu XL, 2012), and Actinidia kolomikta (Zhang YJ, 2014). Actinidia polygama (Wang YY, 2016) established a rapid propagation system through axillary bud induction alone, while Actinidia macrosperma (Jiang WM & Li FY, 2003) exhibited difficulty in callus differentiation. These studies consistently reported that axillary bud induction was more time-efficient and effective than adventitious bud induction via callus. This study required approximately 35 days for axillary bud induction compared to 2–3 months for callus-mediated adventitious bud formation. Hu KD (1991) noted that axillary bud induction maintains greater genetic stability and preserves maternal traits, while callus differentiation carries higher mutation risk. Therefore, direct axillary bud induction represents the most effective rapid propagation pathway for 'Yangshi Jinhong 50', while callus-derived adventitious buds provide a basis for genetic transformation research.

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