Establishment of an Efficient Leaf Regeneration System for ‘Jinyan’ Kiwifruit

Establishment of an Efficient Leaf Regeneration System for 'Jinyan' Kiwifruit

Qinglin Song[a], Lingling Kong[b], Yutong Xie[c], Rui Wang[d] and Yong Zhang[*]

College of Horticulture, Sichuan Agricultural University, Chengdu, China

*Corresponding author e-mail: zhyong@sicau.edu.cn, [a]1002472068@qq.com, [b]2925195205@qq.com, [c]1158071272@qq.com, [d]1294299474@qq.com

Abstract. In this experiment, 'Jinyan' kiwifruit field leaves were used as explants for tissue culture to study the effects of different plant growth regulators on the regeneration of leaves of 'Jinyan' kiwifruit, including the induction of adventitious shoots, the proliferation of adventitious shoots and the rooting of adventitious buds. Establish a rapid propagation system for 'Jinyan' kiwifruit. The results showed that the optimal medium for the regeneration of adventitious shoots of 'Jinyan' kiwifruit leaves was MS + 6-BA 4.0 mg·L^{-1}+NAA 0.1 mg·L^{-1}, the budding rate was 76.67%; and the best medium for adventitious shoots proliferation was MS+6-BA 2.0 mg·L^{-1}+NAA 0.4 mg·L^{-1}+GA_3 0.1 mg·L^{-1}, the average proliferation coefficient was 3.33; the optimal rooting medium was 1/2MS +IBA 0.6 mg·L^{-1}, the rooting rate was 86.67%, and the average root length was 2.30 cm.

1. Introduction

Actinidia chinensis Planch is a perennial vine of *Actinidia* genus, and 'Jinyan' is one of the three fine yellow kiwifruit varieties in the world, which had higher yield than other varieties, fruity sweetness and storability [1]. It had the same storability as ‘Haywater’, with a long shelf life and promising market prospects, was far superior to other domestic kiwifruit main varieties and was one of the featured agricultural optimization industries [2].

Sichuan had a complex terrain and diverse ecological conditions. It provided a large number of suitable areas for the growth and development of kiwifruit and had strong market competitiveness. Therefore, kiwifruit was an important industry in Sichuan Province. However, the hybridization of kiwifruit was difficult, and the conventional breeding method was adopted, which was difficult to breed a large number of seedlings in a short period of time, which cannot meet the needs of production development. However, the plants obtained by the tissue culture method not only maintained the genetic traits of the improved varieties, but also had a high reproductive speed and large-scale reproduction, which was an important breeding method for good varieties [3].

Hirsch [4] first used kiwi stem segments as explant materials for in vitro culture in 1975. After that, kiwifruit in vitro culture research had made great progress. The kiwifruit tissue culture in China began in Gui [5]. So far, although plant regeneration of several kiwifruits had been reported, including internodes, roots, leaves and immature seeds, even, studies had shown that immature seeds had greater potential as explants that induced callus with high regenerative capacity [6]. However, there are few reports on the in vitro regeneration system of 'Jinyan' kiwifruit. Shang et al [7] cultured the leaves of

'Zhonghua' kiwifruit, and in the medium of ZT 1.0 mg·L^{-1} and NAA 0.3 mg·L^{-1}, the adventitious bud differentiation rate of callus was the highest. In the study of tissue culture and rapid propagation techniques of kiwifruit 'Huate' and 'Hongyang' kiwifruit leaves, Long [8-9] believed that the leaves were easily induced to form callus, and the resulting callus was easy to differentiate into seedlings. Shi et al [10] also proposed that the induction rate of leaf callus was higher, and the different states produced depend on the type of auxin added.

In the previous experiments, tissue culture seedlings were used as explant donors to avoid direct explants from the field, but the mature plants in the field were more familiar with the cultivation conditions. In this experiment, the leaves of 'Jinyan' kiwifruit field plants were used as explants for callus induction and adventitious shoot differentiation, subculture and rooting culture. The in vitro regeneration of plants was determined by setting different hormone levels of the medium, and the most technical parameter combinations were selected to establish a set of efficient and stable 'Jinyan' kiwifruit leaf regeneration system.

2. Material and Method

2.1. Material

In the Pujiang County of Sichuan Province, the branches of the 'Jinyan' kiwifruit field seedlings were well-developed and placed in water to grow new leaves.

2.2. Method

The collected young kiwifruit leaves were rinsed under running water for 2 hours, and after soaked in 75% (v/v) alcohol for 10 seconds, then rinsed once with sterile water. Finally, it was sterilized with 0.1% (m/v) mercury for 10 minutes, then rinsed with sterile water 4~5 times. The leaf blade tip and blade tip were subdivided on a sterile table and cut it into 0.5 cm × 0.5 cm leaf disks, then inoculated into the medium with the surface facing up. The basic medium was MS medium, and the rooting medium was 1/2 MS medium.

3. Results and analysis

3.1. Effects of different plant growth regulators on the induction of adventitious shoots in 'Jinyan' kiwifruit

After about 15 days, the leaves were observed to change significantly, grew faster and the surface began to expand and wrinkles appeared. The bulge was upward, the leaf color became lighter, and the compact light green callus visible to the naked eye appears. After inoculation for about 30 days, dark green buds were differentiated from the callus and began to elongate. The visual height was between 0 and 1 cm, and the volume of some callus was increased. At this time, the number of regenerated shoot explants, the budding rate, and the growth state of each treatment were counted. The results were shown in Tables 1 to 4.

Different types and concentrations of cytokinins and auxins had different effects on the induction of adventitious shoots of 'Jinyan' kiwifruit. In general, the combination of 6-BA/NAA and ZT/NAA hormones induced better adventitious shoots, and the combination of 6-BA/IBA and ZT/IBA hormones was less effective. It can be seen from Table 2 that the adventitious shoot differentiation rate of A15 medium was the highest, reaching 76.67%, and the difference was significant with other treatments. The budding effect was the best, not only the high rate of sprouting, but also the number of buds, the color was green, and the growth was good. From Table 3, ZT promotes the differentiation of adventitious shoots, as the concentration of ZT increased, the rate of adventitious shoot differentiation increased. Therefore, the optimum medium for adventitious shoots regeneration in 'Jinyan' kiwifruit leaves was A15 medium, which was MS + 6-BA 4.0 mg·L^{-1} + NAA 0.1 mg·L^{-1}.

Table 1. Comparison of 6-BA/IBA hormone combinations inducing adventitious shoots

Media number	6-BA ($mg \cdot L^{-1}$)	IBA ($mg \cdot L^{-1}$)	No. of explants inoculated	No. of regenerated shoot explants	Budding rate (%)
A_1	2.0	0.5	30	3	10.00 ± 0.58bc
A_2	2.0	1.0	30	2	6.67 ± 0.56c
A_3	2.0	1.5	30	3	10.00 ± 0.57bc
A_4	4.0	0.5	30	5	16.67 ± 1.23ab
A_5	4.0	1.0	30	3	10.00 ± 1.18bc
A_6	4.0	1.5	30	4	13.33 ± 1.13b
A_7	6.0	0.5	30	4	13.33 ± 1.90b
A_8	6.0	1.0	30	5	16.67 ± 1.82ab
A_9	6.0	1.5	30	6	20.00 ± 1.76a

Note: Note: Different lowercase letters in the same column data indicate significant difference ($P<0.05$), the table below is same.

Table 2. Comparison of 6-BA/NAA hormone combinations inducing adventitious shoots

Media number	6-BA ($mg \cdot L^{-1}$)	NAA ($mg \cdot L^{-1}$)	No. of explants inoculated	No. of regenerated shoot explants	Budding rate (%)
A_{10}	2.0	0.01	30	7	23.33 ± 0.61f
A_{11}	2.0	0.05	30	8	26.67 ± 0.62f
A_{12}	2.0	0.10	30	11	36.67 ± 0.61e
A_{13}	4.0	0.01	30	19	63.33 ± 1.24c
A_{14}	4.0	0.05	30	21	70.00 ± 1.21b
A_{15}	4.0	0.10	30	23	76.67 ± 1.21a
A_{16}	6.0	0.01	30	16	53.33 ± 1.92d
A_{17}	6.0	0.05	30	18	60.00 ± 1.90c
A_{18}	6.0	0.10	30	16	53.33 ± 1.90d

Table 3. Comparison of ZT/NAA hormone combinations inducing adventitious shoots

Media number	ZT ($mg \cdot L^{-1}$)	NAA ($mg \cdot L^{-1}$)	No. of explants inoculated	No. of regenerated shoot explants	Budding rate (%)
A_{19}	0.5	0.01	30	8	26.67 ± 0.14f
A_{20}	0.5	0.05	30	9	30.00 ± 0.13f
A_{21}	0.5	0.10	30	11	36.67 ± 0.12e
A_{22}	1.0	0.01	30	19	63.33 ± 0.29c
A_{23}	1.0	0.05	30	18	60.00 ± 0.28cd
A_{24}	1.0	0.10	30	21	70.00 ± 0.26b
A_{25}	1.5	0.01	30	17	56.67 ± 0.43d
A_{26}	1.5	0.05	30	19	63.33 ± 0.42c
A_{27}	1.5	0.10	30	22	73.33 ± 0.40a

Table 4. Comparison of the effects of ZT/IBA hormone combination on adventitious shoots induction

Media number	ZT ($mg \cdot L^{-1}$)	IBA ($mg \cdot L^{-1}$)	No. of explants inoculated	No. of regenerated shoot explants	Budding rate (%)
A_{28}	0.5	0.5	30	2	6.67 ± 0.14d
A_{29}	0.5	1.0	30	4	13.33 ± 0.25c
A_{30}	0.5	1.5	30	3	10.00 ± 0.42cd
A_{31}	1.0	0.5	30	6	20.00 ± 0.23b
A_{32}	1.0	1.0	30	5	16.67 ± 0.28bc
A_{33}	1.0	1.5	30	7	23.33 ± 0.37ab
A_{34}	1.5	0.5	30	8	26.67 ± 0.38a
A_{35}	1.5	1.0	30	6	20.00 ± 0.38b
A_{36}	1.5	1.5	30	7	23.33 ± 0.42ab

3.2. Effects of different plant growth regulators on the proliferation of adventitious shoots of 'Jinyan' kiwifruit

After 15 days of culture, small shoot spots appeared around the adventitious shoots. Then, a single adventitious bud was observed to form a shoot, and the color changed from green to dark.

The concentration combination of different regulators had significant effects on the proliferation of adventitious buds of ‘Jinyan’ kiwifruit. In terms of the average proliferation number, the proliferation effect of the medium B5 was the best, and the average proliferation coefficient was 3.33, which had a significant difference from other treatments, at the same time, the growth state was better, which had dark green color, strong growth, many shoots that was higher and is generally above 1.5 cm. In contrast, B1 processing was the worst. Therefore, the optimal medium for adventitious shoot proliferation of 'Jinyan' kiwifruit was B5.

Table 5. Effect of different hormone combinations on adventitious shoot induction

Media number	GA_3 ($mg \cdot L^{-1}$)	NAA ($mg \cdot L^{-1}$)	6-BA ($mg \cdot L^{-1}$)	Average proliferation coefficient
B_1	0.1	0.2	1	1.33 ± 0.37d
B_2	0.1	0.2	2	1.67 ± 0.52c
B_3	0.1	0.2	3	2.33 ± 0.73b
B_4	0.1	0.4	1	2.67 ± 0.50b
B_5	0.1	0.4	2	3.33 ± 0.75a
B_6	0.1	0.4	3	2.67 ± 0.72b

3.3. Effects of different medium hormone ratios on adventitious bud rooting of 'Jinyan' kiwifruit

A large number of literatures showed that the rooting medium used for kiwifruit tissue culture was best at 1/2MS. Rooting began after 7 to 9 days of culture. The results (Table 6) showed that different plant growth regulators had an effect on the rooting of adventitious shoots, and the rooting rate was significantly higher than the control group. The induction of roots by IBA was better than NAA and IAA, that the rooting rate was high and the length was also longer, both around 2 cm. The concentration of IBA in medium C3 was 0.6 $mg \cdot L^{-1}$ with the best effect, the rooting rate was as high as 86.67%, and the average root length was 2.30 cm, that the growth rate of adventitious buds was fast, and the roots were dense and dense. However, when the concentration of IBA was higher, the roots were inhibited. It was concluded that the best rooting culture of ‘Jinyan’ kiwifruit was 1/2MS + IBA 0.6 $mg \cdot L^{-1}$.

Table 6. Effect of different concentrations of regulators on adventitious shoot rooting

Media number	Medium	IBA (mg·L^{-1})	IAA (mg·L^{-1})	NAA (mg·L^{-1})	Rooting rate	Average root length
C_1	1/2MS				53.33 ± 0.27e	1.21 ± 0.61d
C_2	1/2MS	0.3			70.00 ± 0.35c	1.83 ± 0.77b
C_3	1/2MS	0.6			86.67 ± 0.43a	2.30 ± 0.85a
C_4	1/2MS	0.9			76.67 ± 0.38b	2.13 ± 0.62a
C_5	1/2MS		0.2		56.67 ± 0.28e	1.56 ± 0.68c
C_6	1/2MS		0.4		63.33 ± 0.31d	1.68 ± 0.64bc
C_7	1/2MS		0.6		66.67 ± 0.33cd	1.72 ± 0.56bc
C_8	1/2MS			0.2	53.33 ± 0.27e	1.55 ± 0.68c
C_9	1/2MS			0.4	70.00 ± 0.35c	1.65 ± 0.63bc
C_{10}	1/2MS			0.6	76.67 ± 0.38b	1.78 ± 0.59bc

4. Discussion and conclusion

Tissue culture technology had become a breeding method for rapid propagation of kiwifruit at home and abroad [11], but there were genotypic differences among different varieties of kiwifruit, and the different ratios of plant growth regulators in the medium were also the key factors affecting the success of kiwifruit tissue culture. [12]. In this study, the optimal medium was MS+6-BA 4.0 mg·L^{-1}+NAA 0.1 mg·L^{-1} in adventitious shoots induction, which was different from previous studies [13], because different genotypes of kiwifruit required different mass fractions and ratios of auxin and cytokinin for budding and proliferation in vitro or the different endogenous hormones in leaves of different varietiesthe genotypes of different varieties were different. In the past research on regeneration system, it was suggested that ZT was indispensable for the induction of adventitious buds. Zhang et al. [14] and Xie et al. [15] all suggested that ZT was beneficial to the proliferation and differentiation of callus. However, the results of this experiment showed that 6-BA was better than ZT and can replace expensive ZT, which was of great significance for reducing the cost of kiwifruit tissue culture. In the proliferation test of adventitious buds, it can be seen that both 6-BA and NAA have the effect of promoting proliferatio, but high concentration of NAA had the effect of inhibiting proliferation. In the rooting culture of adventitious shoots, the medium containing IBA 0.6 mg·L^{-1} was found to have a significant rooting effect, and the later roots were thick and numerous with fluff and robust growth.

In this experiment, a set of high-efficiency regeneration system of 'Jinyan' kiwifruit was established, and the optimal medium for adventitious shoots induction, proliferation and rooting of 'Jinyan' kiwifruit leaves was obtained: MS+6-BA 4.0 mg·L^{-1}+NAA 0.1 mg·L^{-1}, MS+6-BA 2.0 mg·L^{-1}+NAA 0.4 mg·L^{-1}+GA3 0.1 mg·L^{-1}, 1/2MS+IBA 0.6 mg·L^{-1}. This laid the foundation for further research on genetic transformation in the future.

References

[1] H Q Guan, J H Li, T Yuan, et al. High-yield and stable cultivation management of Jinyan kiwifruit [J]. Special Economics of Animals and Plants, 2017 (8): 44-45.
[2] X H Wu, Y L Zhang, Y Zhou, et al. Establishment of High Frequency Direct Regeneration System of 'Hayward' Kiwifruit Leaves [J]. Chinese Journal of Plant Physiology, 2013, 49 (8): 759-763.
[3] M. Davey. Regeneration of fruit and ornamental trees via cell and tissue culture [J]. Encyclopedia of Applied Plant Sciences, 2017 (2): 455–461.
[4] A M Hirsch. The culture of Actinidia chinensis fruit tissues and the metabolism of free amino acids in stem segments cultured in vitro [J]. comptes rendus Hebdomadaires des Seances de I'Academic des sciences D, 1975, 280 (11): 1369-1372.
[5] Y L Gui. Callus induction and plant regeneration of kiwifruit stem segments in vitro [J]. Plant Physiology Communications, 1979, 21 (4): 339-343.
[6] I Asakuraa, Y Hoshino. Evaluation of plant regenerationability of different explants and

establishment of an efficient regeneration system using immature seeds in *Actinidia kolomikta*, a cold-hardy kiwifruitrelative [J]. Scientia Horticulturae, 2017 (220): 275–282.
[7] Y L Shang, H Li, J P Zhang, et al. Establishment of genetic transformation receptor system of Chinese kiwifruit [J]. Jiangsu Agricultural Sciences, 2013, 41 (6): 46-48.
[8] Q J Long, M Xie. Research on Tissue Culture and Rapid Propagation of Kiwifruit [D]. Hangzhou: Zhejiang Normal University, 2010.
[9] Q J Long, Y J Wu, M Xie. Tissue Culture and Rapid Propagation Techniques of Leaves and Bud Segments of 'Hongyang' Kiwifruit [J]. Journal of Zhejiang Agricultural Sciences, 2010, 22 (4): 429-432.
[10] W Shi, L Zhang, Y S Liu. Establishment of Leaves Regeneration System of 'Hongyang' Kiwifruit [J]. Journal of Hefei University of Technology (Natural Science), 2015, 38 (4): 548-551.
[11] D H Qu, J Z Wu, X G Wu, et al. Seed germination and in vitro rapid propagation techniques of wild jujube kiwifruit [J]. West Forestry Science, 2017, 46 (6): 56-60.
[12] P F Wei. Establishment of kiwifruit leaves regeneration system and effect of transgenic peptide D gene on protective enzyme activity [D]. Zhengzhou: Henan Agricultural University, 2016.
[13] Y J Zhang. Study on in vitro rapid propagation of kiwifruit in dog jujube [D]. Changchun: Jilin Agricultural University, 2014.
[14] Y J Zhang, Y Q Qian. Callus Induction and Plant Regeneration of Test Tube Seedlings and Stem Segments of Soft Jujube [J]. Acta Botanica Sinica, 1996, 16 (2): 137-141.
[15] Z B Xie, X D Lu. Screening of Suitable Hormone Combinations in Kiwifruit Tissue Culture [J]. Northern Fruits, 2003, 15 (3): 7-8.