Establishment of Rapid Propagation System of ‘Xu Xiang’ Kiwifruit

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Abstract. Through tissue culture of the stem segments with bud of the ‘XuXiang’ kiwifruit, a rapid propagation system of ‘XuXiang’ kiwifruit was established by screening the formula of plant growth hormone concentration which was the best medium for its growth, multiplication medium and inducing-root medium. Results of tests are as follows: The optimal culture medium in the beginning culture phase was MS + 2.0 mg·L⁻¹ 6-BA + 0.2 mg·L⁻¹ NAA, the induction rate of adventitious buds with shoot stems by this formula can reach to 86.89%. When the multiplication medium was MS + 1.0 mg·L⁻¹ 6-BA + 0.1 mg·L⁻¹ NAA, the number of buds in the stem segment of kiwifruit was the largest and the multiplication coefficient was 5.02. The rooting medium was the 1/2MS medium with IBA concentration of 1.5 mg·L⁻¹, the rooting rate reached 93.56%, and the growth of root was good.

1. Introduction
Actinidia chinensis belongs to the perennial vine of the genus Actinidia, and has other names such as sheep peach and macaque pear [1]. Nowadays, there are 66 species of kiwifruit plants in the world, of which 62 species are planted in China. The production value is higher, such as chinensis, delicious, argute and so on. The main varieties of delicious kiwifruit are ‘Qinmei’, ‘Hayward’ and ‘Xuxiang’ [2]. ‘Xuxiang’ kiwifruit was selected from the delicious kiwifruit seedlings introduced from the Beijing Botanical Garden in Xuzhou City, Jiangsu Province in 1975, which had the characteristics of strong adaptability, early results, good quality and sweet and fragrant flavor, so, it was deeply loved by consumers. At present, the cultivation area of ‘Xuxiang’ is relatively high, and it has a good development prospect, and it is hopeful to become the main variety of Chinese kiwifruit [3].

Tissue culture seedlings were the main development direction of modern agricultural factory seedlings, which had the advantages of maintaining good traits, stable inheritance, rapid reproduction, no time and space restrictions, and can provide annual seedlings [4]. The rapid propagation of kiwifruit by tissue culture techniques at home and abroad had become an important breeding method. The research on kiwifruit tissue culture in China began in Gui [5], he used kiwifruit stem segments as explants to cultivate callus, induced shoot differentiation and grew into intact plants. In the research of kiwifruit tissue culture in China, many scholars had succeeded in the tissue culture of macaques such as 'Hongyang' and 'Hayward' [6-8]. However, there were genetic differences between different varieties of kiwifruit, the survival rate of tissue culture seedlings was different from that of tissue
culture seedlings in different explants, culture medium, plant hormone ratio, culture environment, and tissue culture had strong specificity, therefore, no medium was suitable for any one variety.

In the previous studies, the 'Xuxiang' kiwifruit was rarely cultured. In this study, the 'Xuxiang' kiwifruit bud stems were used as experimental materials to screen out the best medium for germination, proliferation and rooting of axillary buds, in order to provide a solid foundation for the preservation and rapid propagation of *Actinidia delicacies* germplasm resources.

2. Material and Method

2.1. Material

The kiwifruit variety was 'Xuxiang', and a new hydroponic shoot with a uniform growth state and uniformity (about 25 cm in length) was selected.

2.2. Method

The collected stems were rinsed under running water for 2 hours, and after soaked in 75% (v/v) alcohol for 15 seconds, then rinsed once with sterile water. Finally, it was sterilized with 0.1% (m/v) mercury for 7 minutes, then rinsed with sterile water 4~5 times. The stem section of the axillary shoot with about 1 cm was excised as an explant, and inoculated into shoot induction medium, 50 treatments per treatment. Statistics were obtained after 30 days, and the shoots were transferred to a proliferation medium containing 0.1 mg·L⁻¹ GA₃, 30 shoots per treatment, and statistics were obtained after 35 days. Finally, the shoots were transferred to rooting medium, and the rooting medium was 1/2MS.

3. Results and Analysis

3.1. Effects of Different Plant Growth Regulators on the Germination of Axillary Shoot in the Stem Segment of 'Xuxiang' Kiwifruit

| 6-BA (mg·L⁻¹) | NAA (mg·L⁻¹) | No. of explants | No. of shoots | Germination rate(%) | Growth Status |
|---------------|-------------|----------------|--------------|---------------------|--------------|
| 0.5           | 0.1         | 50             | 26.50        | 53.00 ± 0.58e       | Yellow green and curly leaves, weak shoots |
| 0.5           | 0.2         | 50             | 27.40        | 54.79 ± 0.58e       | Yellow green and curly leaves, weak shoots |
| 0.5           | 0.5         | 50             | 26.82        | 53.64 ± 1.07e       | Yellow green and curly leaves, weak shoots |
| 1.0           | 0.1         | 50             | 26.18        | 52.36 ± 1.47e       | Yellow green and curly leaves, weak shoots |
| 1.0           | 0.2         | 50             | 34.00        | 67.99 ± 1.42c       | Green and flat leaves, thick shoots |
| 1.0           | 0.5         | 50             | 32.46        | 64.91 ± 0.57d       | Green and flat leaves, thick shoots |
| 2.0           | 0.1         | 50             | 33.34        | 66.68 ± 0.43c       | Green and flat leaves, thick shoots |
| 2.0           | 0.2         | 50             | 43.45        | 86.89 ± 1.20a       | Green and flat leaves, thick shoots |
| 2.0           | 0.5         | 50             | 35.98        | 71.95 ± 0.88b       | Green and flat leaves, thick shoots |

Note: Different lowercase letters in the same column data indicate significant difference (P<0.05), the table below is same.
On the 4th day after inoculation, the color of the explants in the primary culture gradually changed, the green began to deepen, and the axillary shoots began to expand. It can be concluded from Table 1, that the medium containing 2.0 mg·L⁻¹ 6-BA and 0.2 mg·L⁻¹ NAA was best suitable for axillary shoot germination, which the germination rate of axillary buds was up to 86.89%. Leaves were dark green, flat and healthy and shoots were thick in this medium. The medium had the least promoting effect on axillary shoot germination, and the germination rate was only 52.36%, which contained 1.0 mg·L⁻¹ 6-BA and 0.1 mg·L⁻¹ NAA, and the color of the leaves was yellow-green and curly, poor growth of axillary shoots. It can be concluded that in the concentration range of 0.5-2.0 mg·L⁻¹ 6-BA promoted the germination of axillary shoots, and with the increase of 6-BA concentration, the promoting effect was enhanced, so the concentration was 2.0 mg·L⁻¹ had the best effect. In the range of 0.1-0.5 mg·L⁻¹ NAA also promoted the germination of axillary shoots. When the concentration of NAA was 0.2 mg·L⁻¹, the promoting effect was the strongest. The concentration of NAA was too high or too low to affect the germination of axillary shoots. It showed that stem of kiwifruit was more suitable as an explant for in vitro culture.

3.2. Effects of Different Plant Growth Regulators on the Proliferation of 'Xuxiang' Kiwifruit Shoot

The shoots of 'Xuxiang' kiwifruit after germination were cut and placed on the medium for proliferation culture. After 8 days of inoculation, the callus was started at the base of the axillary shoot, and the sprouts began to germinate. After 20 days of inoculation, there were 1-2 leaflet extensions in the leaf axils, and the callus became larger, most of them were transparent green, a little yellowish, and the surface was rough and irregular. After 28 days of inoculation, the shoots began to grow length. From the average proliferation number in Table 2, the optimal combination of proliferation culture was MS + 1.0 mg·L⁻¹ 6-BA + 0.1 mg·L⁻¹ NAA + 0.1 mg·L⁻¹ GA₃, and the average proliferation number was up to 5.02. In the concentration range of 0.5-2.0 mg·L⁻¹, in the concentration range of 0.5-2.0 mg·L⁻¹, as the concentration of 6-BA increases, the leaves become flat, the leaves color become greener, and the shoots become thicker. The concentration of 6-BA was too high to inhibit the axillary shoots proliferation. When the 6-BA was 1.0 mg·L⁻¹, the average number of shoots and the average proliferation coefficient of the shoots were higher. In the range of 0.1-0.5 mg·L⁻¹, as the concentration of NAA increased, the proliferation of shoot was inhibited, the proliferation coefficient became smaller, the leaves were smaller and the color became yellowish, and the stem segments became thin.

### Table 2. Effects of different plant growth regulators on the proliferation of 'Xuxiang' kiwifruit shoot

| 6-BA (mg·L⁻¹) | NAA (mg·L⁻¹) | No. of explants | No. of shoots after proliferation | Proliferation coefficient | Growth Status                          |
|--------------|--------------|----------------|-----------------------------------|--------------------------|----------------------------------------|
| 0.5          | 0.1          | 30             | 133.5                             | 4.29 ± 0.16b             | Big and dark leaves green, thick stems |
| 0.5          | 0.2          | 30             | 117.0                             | 3.93 ± 0.15cd            | Small and green leaves, thin stems     |
| 0.5          | 0.5          | 30             | 109.9                             | 3.74 ± 0.04def           | Small and green leaves, thin stems     |
| 1.0          | 0.1          | 30             | 150.6                             | 4.98 ± 0.15a             | Big and dark green leaves, thick stems |
| 1.0          | 0.2          | 30             | 131.1                             | 4.35 ± 0.06b             | Big and dark green leaves, thick stems |
| 1.0          | 0.5          | 30             | 121.5                             | 4.06 ± 0.05bc            | Big and dark green leaves, thick stems |
| 2.0          | 0.1          | 30             | 117.9                             | 3.86 ± 0.05cde           | Small and green leaves, thick stems    |
| 2.0          | 0.2          | 30             | 108.6                             | 3.62 ± 0.02ef            | Small and green leaves, thin stems     |
| 2.0          | 0.5          | 30             | 99.9                              | 3.53 ± 0.07f             | Small and green leaves, thin stems     |
3.3.  Effects of Different Concentrations of IBA on Rooting of ‘Xuxiang’ Kiwifruit Bud

After 7 days of inoculation, roots were started, and after 12 days of culture, 50% of the seedlings rooted. It can be concluded from Table 3 that the rooting rate and the average number supplemented with IBA increased, and the growth status was better, indicating that IBA can promote the root formation of ‘Xuxiang’ kiwifruit. In the concentration range of 0-1.5 mg·L⁻¹, with the increase of IBA concentration, the rooting rate increased gradually, the roots were developed, the number increased, and the roots became thicker. When the medium was 1/2MS + 1.5 mg·L⁻¹ IBA, the rooting rate was as high as 93.56%, at the same time, the roots were white, the number were significantly larger than the blank control, and it was significantly thicker than the other two mediums.

Table 3. Effects of different concentrations of IBA on rooting of ‘Xuxiang’ kiwifruit shoot

| IBA (mg·L⁻¹) | No. of explants | Rooting rate (%) | Average no. of roots | Growth Status          |
|--------------|-----------------|------------------|----------------------|------------------------|
| 0            | 30              | 72.08 ± 0.88c    | 4.61                 | Less and thin roots    |
| 0.7          | 30              | 85.16 ± 0.19b    | 6.33                 | More and thick roots   |
| 1.5          | 30              | 93.56 ± 0.47a    | 7.82                 | More and thick roots   |

4. Conclusion

Plant growth regulators were essential in the growth and development of kiwifruit, and the suitable plant growth regulator concentration was the key factor for the germination and proliferation of kiwifruit in vitro. In this experiment, the shoot stem segments of ‘Xuxiang’ kiwifruit were used as explants for tissue culture, and different concentrations of plant growth regulators were added to the medium, and then the best primary medium was selected as MS + 2.0 mg·L⁻¹ 6-BA + 0.2 mg·L⁻¹ NAA, the axillary shoot induction rate reached 86.89%, which was close to the initial culture induction rate of kiwifruit shoot stems studied by Long [9], which indicated that the stem segments of kiwifruit had higher axillary shoot induction rate, when cultured in vitro. The optimal subculture medium was MS+1.0 mg·L⁻¹ 6-BA+0.1 mg·L⁻¹ NAA, the concentration of 6-BA was positively correlated with the proliferation coefficient in the test concentration range; the NAA concentration was negatively correlated with the proliferation coefficient. The best rooting medium was 1/2MS+1.5 mg·L⁻¹ IBA, which was inconsistent with the results of Huang [10] and Wu [11], and may be due to different varieties. The seedlings cultivated by the medium with the optimal formula were thick, the leaves were dark green, large and flat, and the root systems were developed, the proliferation coefficient was large, thus, a large number of seedlings can be obtained in a short time. Therefore, the formula provides technical support for the realization of delicious kiwifruit planting.

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