Effects of Different Growth Regulating Substance on the 
*Actinidia deliciosa* Leaves Excised Direct Regeneration

Yong Zhang, Lingling Kong, Haoyue Zhang and Haoru Tang*

College of Horticulture, Sichuan Agricultural University, 611130 Chengdu, China

*Corresponding author e-mail: htang@sicau.edu.cn

Abstract. Using the leaves of test-tube seedlings of *Actinidia deliciosa* as explants, the effect of different growth regulating substance on the *Actinidia deliciosa* leaves excised regeneration system was researched, including the differentiation of adventitious buds, the regeneration and rooting of regenerated shoots. The results indicated that the maximum adventitious buds regeneration frequency achieved 76.6% on the medium of Murashige and Skoog medium (MS) containing 5.0 mg·L\(^{-1}\) 6-benzylaminopurine (6-BA) and 0.2 mg·L\(^{-1}\) naphthaleneacetic acid (NAA); the most suitable medium for shoot multiplication was MS with 2.0 mg·L\(^{-1}\) 6-BA, 0.2 mg·L\(^{-1}\) NAA and 0.1 mg·L\(^{-1}\) gibberellin acid (GA\(_3\)) and the multiplication coefficient was up to 4.76; 1/2MS with 0.7 mg·L\(^{-1}\) 1h-indolo-3-butanoic acid (IBA) was the most suitable for the rooting of regenerated shoots, and the rooting rate was 100%.

1. Introduction

*Actinidia deliciosa* is a perennial vine of the genus Actinidia of the kiwifruit family. Its flesh is mostly green or emerald green, fresh meat tender, high sugar content, taste delicious. The anti-cancer effect of its fruit has long been confirmed, so it has great production value. The rapid propagation of *Actinidia chinensis* by tissue culture technology at home and abroad has been paid much attention since 1975 by the tissue culture of the stem segments of Actinidia in Hirsch [1]. In kiwifruit tissue culture, stem segments, leaves, petioles and shoot tip were used as explants [2-7]. S.L. Ding [8] was inoculated on the same medium with the stem tip, leaf block and stem segment of *Actinidia deliciosa* respectively, and found that stem tips were the best explant materials for rapid propagation. Although leaf differentiation is slow, it can also be used in strict disinfection, but the stem segments are not suitable for rapid propagation. X.P. Zhao [9] used 'Hongyang' kiwifruit female young leaves as explants to induce adventitious bud directly, it was found that the induction rate of adventitious buds was 100% in MS+3.0 mg·L\(^{-1}\) 6-BA+1.0 mg·L\(^{-1}\) NAA medium. X.H. Wu et al. used 'Heywood' kiwifruit leaves to induce adventitious buds, and the most suitable medium was MS+3.0 mg·L\(^{-1}\) 6-BA+0.1 mg·L\(^{-1}\) NAA. In previous experiments, plantlets were used as explant donors and field plants were rarely used as explant donors. In this experiment, we used the *Actinidia deliciosa* kiwifruit field leaves as the experimental material, and the methods for the one step germination of the leaves were investigated by comparing the culture medium of different hormone levels.
2. Materials and methods

2.1. Materials.
On March 30th, 2016, several slices of *Actinidia deliciosa* kiwifruit leaves were collected at the Experimental Base of Sichuan Agricultural University. The leaves blades with a leaf age of about 30 days were selected. The leaves were hypertrophic, dark green in color, and veins were obvious. The leaves were well developed without pests. After collection, put it in water for cultivation.

2.2. Methods.
The collected young kiwi fruit 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 used was MS medium, containing agar 7 g·L⁻¹, sucrose 30 g·L⁻¹ and pH value of 5.8. Adventitious shoot induction medium was supplemented with different concentration of NAA (3, 4, 5 and 6 mg·L⁻¹) and 6-BA (0.1, 0.2, 0.5, and 1.0 mg·L⁻¹). The adventitious buds were induced to dark culture for 30 days and then transferred to light culture. After 10 days of light culture, the number of adventitious buds and adventitious bud induction rate were counted. Each medium was inoculated with 6 explants and repeated 5 times. 0.2 g·L⁻¹ NAA, 0.1 was added to the proliferation medium contained 0.2 g·L⁻¹ NAA, 0.1 g·L⁻¹ GA₃ and different concentration of 6-BA (2.0, 3.0, and 4.0 mg·L⁻¹). The adventitious buds were propagated for 30 days and the adventitious bud propagation coefficient and the growth status of the buds were counted. Each medium was inoculated with 6 explants and repeated 5 times. Different concentrations of IBA (0.3, 0.5, 0.7, and 0.9 mg·L⁻¹) were added to the rooting medium. After 30 days, the rooting rate, number and length of adventitious roots were counted. Each medium was inoculated with 6 adventitious buds and repeated 5 times *Actinidia deliciosa*.

3. Results and analysis

3.1. Effects of Different Regulators on Adventitious Buds of Kiwifruit
The *Actinidia deliciosa* leaves were inoculated on the basic medium containing different concentrations of NAA and 6-BA, and after two weeks of dark culture, the tin foils on them were uncovered to calculate the bud rate, browning rate and mortality rate (Table 1), and noticeable changes have been observed in the leaves. The leaves appeared to expand and upwards. The leaves color became lighter, and wrinkles appeared on the surface of leaves. The edge of the leaves, especially at the veins, appeared budding and start to elongate. The visual height was 0~1 cm. Table 2 showed that the concentration of NAA in the medium had a significant effect on the induction of adventitious buds. When the NAA concentration was higher than 0.5 mg·L⁻¹, the budding rate of adventitious buds was low, while when the NAA concentration was 0.2 mg·L⁻¹, the budding effect was the best. When the concentration of 6-BA was higher than 5 mg·L⁻¹, the rate of adventitious buds was low. The results of comprehensive data analysis and direct observation showed that the effect of A₅, which not only had high budding rate, but also had many sprouting spots, green color and good growth, was the best. Compared with A₃ medium, it not only had low the rate of budding, but also had few buds leave, and only a few leaves appeared to expand, most of the leaves remained the original state. Therefore, the optimum medium for the direct regeneration of adventitious buds from the leaves of *Actinidia deliciosa* was A₅, containing MS+5.0 mg·L⁻¹ 6-BA+0.2 mg·L⁻¹ NAA.
Table 1. Statistics of pollution rate after 14 days

| Medium number | Pollution rate (%) | Browning mortality (%) | Survival rate |
|---------------|--------------------|------------------------|---------------|
| A1            | 6.7                | 30                     | 63.3          |
| A2            | 10                 | 30                     | 60            |
| A3            | 6.7                | 23.3                   | 70            |
| A4            | 6.7                | 26.7                   | 66.6          |
| A5            | 0                  | 20                     | 80            |
| A6            | 6.7                | 23.3                   | 70            |

Table 2. Effects of 6-BA and NAA on adventitious buds regeneration from leaves of *Actinidia deliciosa*

| Medium number | 6-BA (mg·L⁻¹) | NAA (mg·L⁻¹) | Number of explants inoculated | Regenerated shoot explants | Blasting rate (%) |
|---------------|---------------|--------------|------------------------------|----------------------------|-------------------|
| A1            | 3.0           | 0.1          | 30                           | 11                         | 36.37±1.87        |
| A2            | 3.0           | 0.5          | 30                           | 7                          | 23.33±1.63        |
| A3            | 3.0           | 1.0          | 30                           | 5                          | 16.67±3.32        |
| A4            | 4.0           | 0.2          | 30                           | 16                         | 53.33±2.88        |
| A5            | 5.0           | 0.2          | 30                           | 23                         | 76.67±1.73        |
| A6            | 6.0           | 0.2          | 30                           | 18                         | 60.00±2.47        |

Fig. 1 Plant regeneration from leaves of *Actinidia deliciosa*

A: Leaves expanded 14 days after culture; B: Adventitious shoots appearing after 30 days of leaf culture; C: Adventitious shoots after 10 days of light culture; D: Clump shoots formed after 30 days of culture on a growth medium.

3.2. Effects of Different Regulators on Adventitious Bud Propagation in *Actinidia deliciosa*

The adventitious buds with good growth status were inoculated onto the proliferation medium containing different regulators. After 1 week of culture, small sprouts around the individual adventitious buds were clearly observed. After four weeks, adventitious buds formed a cluster of buds, and the color turned from green to dark. Adventitious bud proliferation results were shown in Table 3, medium B₁ had the best proliferation effect, at the same time, its growth state is the best, and seedling height is generally above 1.5 cm. By comparison, B₅ is the worst. When the concentration of 6-BA was higher than 3.0 mg·L⁻¹, with the increase of concentration, the proliferation coefficient decreased. Therefore, the optimal medium for the proliferation of adventitious buds of *Actinidia deliciosa* was B₁, containing MS+2.0 mg·L⁻¹ 6-BA+0.2 mg·L⁻¹ NAA+0.1 mg·L⁻¹ GA₃.
Table 3. Effects of 6-BA on adventitious buds proliferation of *Actinidia deliciosa*

| Medium number | 6-BA (mg·L⁻¹) | NAA (mg·L⁻¹) | GA₃ (mg·L⁻¹) | Proliferation coefficient | Cluster bud growth status                  |
|---------------|---------------|---------------|---------------|---------------------------|---------------------------------------------|
| B₁            | 2.0           | 0.2           | 0.1           | 4.76±0.36a                | dark green color, healthy growth, bushy buds |
| B₂            | 3.0           | 0.2           | 0.1           | 3.86±0.16ab               | green in color, healthy growth, cluster bud sparse  |
| B₃            | 4.0           | 0.2           | 0.1           | 3.23±0.16ab               | Lighter colors, less dwarf, uneven height    |

3.3. Effects of Different Concentrations of IBA on Rooting of Adventitious Buds

Rooting culture of strong test tube seedlings with similar growth potential and good growth condition was carried out. After 7 to 9 days of culture, rooting was initiated. The results showed that (Table 4), when the IBA concentration was lower than 0.7 mg·L⁻¹, the number of root increased, root length increased, and rooting was faster as IBA concentration increased. However, when the concentration of IBA was increased to 0.9 mg·L⁻¹, the root growth was worse than that of C₃, similar to C₁. From this, it was concluded that IBA concentration of 0.7 mg·L⁻¹ in the rooting medium is most beneficial for rooting adventitious shoots.

Table 4. Effects of IBA on adventitious buds rooting of *Actinidia deliciosa*

| Medium number | IBA (mg·L⁻¹) | Inoculation buds | Rooting buds | Rooting rate (%) | Number of root | Root length (cm) |
|---------------|--------------|------------------|--------------|------------------|----------------|-----------------|
| C₁            | 0.3          | 30               | 21           | 70.00±0.02abc    | 5.21±0.09b    | 1.22±0.03bc     |
| C₂            | 0.5          | 30               | 26           | 86.66±0.01ab     | 5.43±0.05ab   | 1.43±0.03ab     |
| C₃            | 0.7          | 30               | 30           | 100±0.03a        | 5.82±0.07a    | 1.57±0.05a      |
| C₄            | 0.9          | 30               | 22           | 73.33±0.02abc    | 5.36±0.09ab   | 1.44±0.14ab     |

4. Discussion

Plant tissue culture is a means to use plant cell totipotency to achieve plant regeneration in vitro, and is now widely used in asexual reproduction. In the tissue culture related experiments of kiwifruit leaves, the callus was first induced, then the callus was differentiated to form buds, and finally the roots were regenerated. In this experiment, NAA and 6-BA growth regulators were used to cross the callus stage to achieve direct regeneration of the leaves of *Actinidia deliciosa*. This method reduces the possibility of genetic variation in explants during culture and greatly reduces the time for the culture to bud.

The kiwifruit field leaves were used as explants. The sterilization effect was better with 75% alcohol and 0.1% mercuric, the pollution rate was 10%, and the browning rate was 30%. Y.J. Zhang [10] mentioned that in the disinfection of dog jujube kiwifruit leaves with alcohol for 30s, the survival rate is up to 40%, while the survival rate in this experiment reached 70%, which showed that 75% alcohol can be used as the disinfection of leaves. In this experiment, the optimum medium was MS+5.0 mg·L⁻¹ 6-BA+0.2 mg·L⁻¹NAA. However, the best buds of 'Hayward' obtained by the predecessors were MS+3.0 mg·L⁻¹ 6-BA+0.1 mg·L⁻¹ NAA. The difference is due to the different genotypes of different varieties, which requirement for the quality score and ratio of auxin and cytokinin is different from the bud and proliferation in vitro. What’s more, it may be due to the different endogenous hormones in different varieties of leaves. Z.B. Xie [11] et al. proposed that GA³ has the effect of promoting proliferation, and IBA also has the effect of promoting proliferation, but the effect is not as obvious as that of GA³. However, high concentrations of NAA have the effect of inhibiting proliferation, which is consistent with the experimental results. Y.L. Gui [12] et al. proposed that the rooting medium was better when immersed in 500ppm IBA solution for rooting induction of plantlets, but the effect of directly adding IBA to the rooting medium was not effective. Obviously, but
in this experiment, it was found that the medium containing 0.7 mg·L⁻¹ IBA had a significant rooting effect. Rooting began at 7 to 9 days, and the later roots were thick, villous, and robust. This experiment has been further explored in the exploration of the predecessors, with excellent and excellent selection, to find a suitable method for budding kiwifruit in one step, and to lay a foundation for tissue culture of delicious kiwifruit leaves.

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