Browning Treatment in Tissue Culture of ‘Miliang 1’ Delicious Kiwifruit

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Abstract. Explant browning is a common problem in tissue culture and has become a limiting factor in the development of kiwifruit tissue culture. The effects of different sampling time and disinfection conditions on tissue culture browning of ‘Miliang 1’ delicious kiwifruit stem segments were studied in this study. Finally, the best months of harvest and the best disinfection conditions were screened out. The results showed that the browning rate of ‘Miliang 1’ taken in April was significantly lower than other time periods, and the browning rate gradually increased with time, reaching a maximum of 33.3% in September, significantly higher than other times. There was no significant difference in the browning rate among May, June, July and August. When the disinfection treatment was 75% alcohol 10s + 0.1% mercury liter for 2 min, the number of pollution was much higher than other treatments, the disinfection treatment time was obviously insufficient. Whatmore, the longer the disinfection time, the less the number of pollution, but the more the number of browning was increased. When the disinfection treatment was 75% alcohol 30s + 0.1% mercury 5min, the number of browning was significantly higher than other treatments. With the increase of alcohol and mercury treatment time, the number of explant contamination decreased, but the number of death increased significantly. Generally speaking, the final survival rate of callus induction in the treatment of 75% alcohol 20s + 0.1% mercury for 5min was significantly higher than other treatments, which was more suitable for the callus induction culture and disinfection of ‘Miliang 1’ kiwifruit.

1. Introduction

Kiwifruit is commonly known as ‘Yangtao’, ‘Tengli’ and ‘Kiwi’ in China. It is a deciduous berry vine tree[1]. Its important commercial value is that fruits are rich in nutrients, especially the high content of minerals, fibers and vitamin C that human body must have. Kiwifruit is known as the "king of fruits" in China, and is favored by people for its vitamin C content, which is several times or even tens of times higher than other fruits[1]. Kiwifruit is one of the most successful examples of plant cultivation and domestication from wild to commercial cultivation in the 20th century[2]. Kiwifruit is rich in varieties. Among them, ‘Miliang 1’ has good quality, strong adaptability and resistance to stress [4].

Kiwifruit is a functional dioecious plant[5]. It is easy to degenerate varieties by seed propagation and difficult to inherit the good traits of female parent to offspring[6]. However, due to the lack of natural resources of kiwifruit, traditional breeding methods such as sowing, grafting and cutting were limited by seasonal conditions, which limit the development and promotion of ‘Miliang 1’ kiwifruit varieties. Therefore, the rapid propagation of ‘Miliang 1’ kiwifruit by tissue culture technology has attracted more and more attention, and the research on tissue culture of ‘Miliang 1’ kiwifruit has also developed[7]. Browning is a common phenomenon in the process of tissue culture of various plants, especially woody
plants[7]. Browning has become a major obstacle to the rapid propagation of Kiwifruit in vitro. Few reports have been reported on the prevention of browning of ‘Miliang 1’. Therefore, it is of great practical significance to study the rapid propagation technology of ‘Miliang 1’ kiwifruit with local characteristics and resources to promote its development and promotion.

After the inoculation material was cut, the cells near the incision were damaged and the segregation effect between tissues disappeared, resulting in the release of phenolic compounds and phenoloxidase overflowing. Under suitable conditions, phenolic substances (substrates) and oxygen react with phenoloxidase to produce brown substances, which gradually diffuse to the culture medium, while inhibiting the activity of other enzymes, and water so that the tissue of explants is poisoned and the explants die[9,10]. Therefore, it is considered that effective control of browning is the key to the success of plant tissue culture[11]. Different collection time and disinfection methods of explants lead to different degrees of browning[12]. Explant sampling time and disinfection conditions were selected, and the optimum sampling time and disinfection conditions were selected.

Zhang Yujie[13] found that in the study of in vitro rapid propagation of kiwifruit in dogs and juveniles, when alcohol and mercury were used as disinfectants, the pollution rate decreased with the increase of disinfection time, but when the treatment time exceeded a certain limit, the survival rate and pollution rate were serious. Zheng[14] found that different sampling seasons had significant effects on the browning rate in vitro culture of stem and leaf plant regeneration of Actinidia arguta, and the browning rate of the stem segment of Actinidia arguta was the lowest in spring.

In this study, stem segments of ‘Miliang 1’ were used as explants, MS as basic medium, and materials were taken at different months and disinfected in different ways. The effects of different sampling time and disinfection methods on explant browning of ‘Miliang 1’ kiwifruit were compared, and the optimum conditions for preventing browning in tissue culture of ‘Miliang 1’ kiwifruit were found.

2. Materials and methods

2.1. Experimental materials

In this experiment, young stem segments of 'Miliang 1', which was provided by chongzhou farm of sichuan agricultural university, were used as explants.

Medium: MS+0.5 mg/L 6-BA+0.5 mg/L NAA+0.5 mg/L 2,4-D+7 g.L-1 agar, pH=5.8
Disinfectant: 75% alcohol and 0.1% mercury

2.2. Experimental methods

2.2.1. Screening of growth time of kiwifruit explants. The lignification degree of explants is very important for tissue culture. In this experiment, seven months old stem segments of Actinidia chinensis were selected for tissue culture experiments, including March, April, May, June, July, August and September. Stem segments were cut into about 1 cm length after routine disinfection and inoculated into culture medium. Each treatment was inoculated with 10 stem segments of Actinidia chinensis for three times, totaling 30 stem segments. After 20 days, the number of browning stems and the success rate of callus induction were observed and recorded, and the growth of callus was observed.

2.2.2. Screening of Different Disinfection Conditions for Kiwifruit Explants. Rinse the stem segments of kiwifruit with the same growth, then soak them in 75% alcohol and disinfect them. Then rinse them with sterile water immediately for three times, pay attention to full shock washing, sterilize them with 0.1% HgCl2, and finally rinse them with sterile water for five times. Among them, alcohol soaking time and mercury soaking time were taken as variables. 10 kiwifruit stem segments were inoculated in each treatment, and 30 stem segments were repeated for three times. After 20 days, the browning of explants and the contamination of explants and media were observed and recorded.
Table 1. Selection of different disinfection conditions for kiwifruit explants

| Treatment | Disinfection way                  |
|-----------|----------------------------------|
| 1         | 75% alcohol 10s+0.1% mercury 2 min |
| 2         | 75% alcohol 10s+0.1% mercury 5 min |
| 3         | 75% alcohol 20s+0.1% mercury 5 min |
| 4         | 75% alcohol 20s+0.1% mercury 8 min |
| 5         | 75% alcohol 30s+0.1% mercury 5 min |

3. Results and analysis

3.1. Screening of time for extracting explants from Actinidia chinensis

It can be seen from table 2 that different sampling time had a great influence on the callus induction rate of delicious kiwi. The results showed that the browning rate of 'Miliang 1' kiwifruit stems taken in April was significantly lower than other periods. The browning rate increased gradually with time, reaching the maximum of 33.3% in September, which was significantly higher than other times. There was no significant difference in the browning rate among May, June, July and August. The callus induction rate of stems taken in April was significantly higher than other months. Except for browning number, the main manifestation was that the number of polluted media was reduced, which indicated that it selected in April was easy to disinfect.

Table 2. The screening of growth time of kiwifruit explants

| Stem segment sampling time | Number of processing | Browning number | Induced Number | Browning rate | Induction rate |
|---------------------------|----------------------|-----------------|----------------|---------------|---------------|
| March                     | 30                   | 5               | 17             | 16.67% ab     | 56.67% ab     |
| April                     | 30                   | 2               | 20             | 6.67% b       | 66.67% a      |
| May                       | 30                   | 3               | 17             | 10.00% ab     | 56.67% ab     |
| June                      | 30                   | 5               | 18             | 16.67% ab     | 60.00% ab     |
| July                      | 30                   | 5               | 14             | 16.67% ab     | 46.67% b      |
| August                    | 30                   | 7               | 15             | 23.33% ab     | 50.00% ab     |
| September                 | 30                   | 10              | 13             | 33.33% a      | 43.33% b      |

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

3.2. Screening of Different Disinfection Conditions for Kiwifruit Explants

According to table 3 of the experiment results, the different treatment time of alcohol and mercury had a great influence on the explant tissue culture of 'Miliang 1' kiwifruit. When 75% alcohol was 10s + 0.1% mercury for 2 min, the pollution number was much higher than other treatments, and the disinfection time was obviously insufficient. The longer the disinfection time, the less the pollution, but the more the browning. When the disinfection treatment was 75% alcohol 30s + 0.1% mercury for 5 min, the browning number was significantly higher than other treatments, reaching 7 strains. With the prolongation of alcohol and mercury treatment time, the number of explant death increased significantly, but the number of pollution decreased at the same time. Generally speaking, the final survival rate of callus induction was significantly higher when the disinfection time was 75% alcohol 20s + 0.1% mercury for 5 min, which was more suitable for the disinfection of 'Miliang 1' kiwifruit stem callus induction culture.
Table 3. Selection of different disinfection conditions for kiwifruit explants

| Disinfection way                  | Number of processing | Pollution Number | Browning number | Number of deaths | Survival rate |
|-----------------------------------|----------------------|------------------|------------------|------------------|---------------|
| 75% alcohol 10s+0.1% mercury 2min | 30                   | 8 a              | 2 bc             | 0                | 66.67% c      |
| 75% alcohol 10s+0.1% mercury 5min | 30                   | 2 b              | 1 bc             | 2                | 83.33% ab     |
| 75% alcohol 20s+0.1% mercury 5min | 30                   | 1 b              | 0 c              | 1                | 93.33% a      |
| 75% alcohol 20s+0.1% mercury 8min | 30                   | 0 b              | 4 ab             | 3                | 76.67% bc     |
| 75% alcohol 30s+0.1% mercury 5min | 30                   | 0 b              | 7 a              | 9                | 46.67% d      |

4. Conclusion
Different explant harvesting time had different effects on the browning rate of 'Miliang 1' delicious kiwifruit callus. The main reason was that the newly grown stem segments had less phenolic substances than other stem segments and were not easy to produce enzymatic browning. The young part contains a small amount of bacteria, which made disinfection easier and had better antibacterial effect. The shoot tip and middle branch were ideal explant materials, while the base branch was opposite. If the base branch was selected, the success rate of induction decreased and the browning rate increased. In this experiment, the current-year branches of kiwifruit from March to September were selected, the results showed that the month in which the stem segment was selected was April.

The experiment also proved the effect of different disinfection treatments on the success rate of callus induction in kiwifruit. The results showed that 75% alcohol disinfection for 20s + 0.1% mercury disinfection for 5 min was suitable for callus induction and culture of 'Miliang 1' delicious kiwifruit. In addition, the explant length of delicious kiwifruit should be kept between 1.5 and 2.5 cm.

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