Browning Treatment in Tissue Culture of ‘Hongyang’ Kiwifruit

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Abstract. Explant browning is a frequent problem in kiwi tissue culture and has become a limiting factor in the development of kiwi tissue culture. Taking the stem segments of ‘Hongyang’ kiwifruit as test materials, the effects of different concentrations of these three browning inhibitors on the browning of tissue culture were studied. Finally, a type of browning inhibitor which can control the browning of explants was selected. And concentration, so as to better solve the problem of browning in the tissue culture of Hongyang kiwifruit. According to the experimental results: ascorbic acid(Vc), citric acid, polyvinylpyrrolidone(PVP) three kinds of reagents treated the stem segments of Hongyang kiwifruit, all have anti-browning effect; polyvinylpyrrolidone PVP treatment has the best anti-browning effect, followed by Vc, and poor lemon acid. The results showed that the addition of 0.3 g·L⁻¹ PVP can effectively inhibit browning.

1. Introduction
Kiwifruit, a perennial berry deciduous vine, one of the first four wild fruit trees that was successfully domesticated and cultivated in the 20th century [1]. ‘Hongyang’ (Actinidia chinensis cv. Hongyang) is a Chinese kiwifruit variety. It is a unique kiwifruit variety in China. It was discovered by the Sichuan Kiwifruit Resources Research Institute and the Cangxi County Agricultural Bureau of Sichuan Province in 1986 from the seedlings of Chinese kiwifruit. After germination and continuous grafting, breeding and domestication in the past 10 years, the traits were stabilized and verified by the Sichuan Crop Variety Approval Committee in 1997 and named as 'Hongyang' kiwifruit [2]. At the Wuhan International Kiwifruit Symposium in 2002, it was listed as the third-generation kiwifruit of the world, and was officially promoted in 2006 [3].

Based on the fact that most kiwifruits are dioecious [4], the long seed breeding cycle, the difficulty of identifying the sex of young plants and obtaining a large number of female seedlings. Seed seedlings are prone to high separation and are prone to species degradation [5]. But, with the traditional grafting method and cutting method, it is difficult to breed a large number of seedlings in a short period of time due to the limited number of branches. However, the rapid propagation of ‘Hongyang’ kiwifruit by tissue culture method is not only fast, but also easy to maintain the excellent traits of ‘Hongyang’ kiwifruit. It can also lay the foundation for technologies such as genetic improvement of varieties and recombinant DNA genetic transformation. Since the 1970s, the rapid propagation of kiwifruit by tissue culture techniques has become an important breeding method, and...
there are more and more researches on the establishment of kiwifruit tissue culture and rapid propagation system [6-10]. However, in the process of in vitro rapid propagation, browning of explants in primary culture often occurs [11], and it has become a major obstacle to the rapid propagation of kiwifruit in vitro, especially for the browning treatment of kiwifruit in red kiwifruit. There are few reports on prevention. Therefore, it is of great practical significance to carry out research on the rapid propagation technology of ‘Hongyang’ kiwifruit, which has local characteristics and resources, which is helpful to promote its development and promotion.

The browning in plant tissue culture refers to the release of brown material from the surface to the medium during the initial differentiation or subdivision of the explant, so that the medium gradually turns brown, and the explants also the phenomenon of further browning and death [12]. Browning is ubiquitous in plant tissue culture. This phenomenon is related to fungal contamination and excessive hydrosis (vitrification) were called the three major problems of plant tissue culture. However, controlling browning is more difficult than controlling fungal contamination and excessive watering. Therefore, it is considered that the effective control of browning is the key to the success of plant tissue culture [13]. In recent years, plant tissue culture technology has developed rapidly, and research on browning has become more and more in-depth.

There are many factors affecting browning, such as plant species and genotypes, explants and physiological status, explant damage, medium composition and culture conditions, and culture time. The addition of browning inhibitors can alleviate the toxicity of phenolic substances and achieve the purpose of protecting explants. Browning inhibitors mainly include antioxidants and PPO inhibitors (polyphenol oxidase inhibitors), the former including ascorbic acid Vc, cysteine, citric acid, PVP, et al., the latter including sulfur dioxide, sulfite, chlorine Sodium and so on.

Jiang [14] in tissue culture of large-seeded kiwi and Zhang [15] in callus tissue culture of *Actinidia macrosperma* of soft jujube found that in callus tissue culture stem explants were easy to callus. Studies have also shown that the different positions of shoots have a greater impact on browning, and the lower buds are stronger and less prone to browning. Yu [16] successfully established the stem culture of kiwifruit in Hayward by using MS + 1.0 mg·L⁻¹ 6-BA + 0.1 mg·L⁻¹ NAA medium. Long [17] also used this medium to achieve the stem culture of ‘Hongyang’. Studies also found that different concentrations of plant growth regulators had an effect on the growth and browning of kiwifruit, high concentrations of cytokinins stimulate the production of phenols, while auxin NAA and IAA can delay the synthesis of polyphenols and reduce browning. Liu [18] screened the most suitable anti-browning ingredients by adding antioxidants, inhibitors and adsorbents to soft jujube kiwifruit, and after inoculation for 10 days, it was found that activated carbon, PVP, citric acid and ascorbic acid Vc were added to the medium had anti-browning effect, which adding 3% PVP could inhibit browning effect without affecting plant growth, while, activated carbon is an adsorbent that had certain side effects while inhibiting browning, that is, when adsorbing toxic phenols, it also adsorbed growth regulators in the medium, which hindered the growth of plants.

In this study, the stem segments of ‘Hongyang’ kiwifruit were used as explants, MS was used as the basic medium, and a certain concentration gradient of ascorbic acid Vc, citric acid, PVP was added to the medium, and no browning inhibitor was added as a control. The effects of three browning inhibitors on the browning of ‘Hongyang’ kiwifruit explants were compared and the best conditions for preventing browning in the tissue culture of ‘Hongyang’ kiwifruit were found. Therefore, it was better to solve the browning problem in the tissue culture of ‘Hongyang’ kiwifruit.

2. Materials and methods

2.1. Experimental materials
In this experiment, young stem segments of ‘Hongyang’ were used as explants and planted in Chong Zhou base of Sichuan Agricultural University. The healthy plants with vigorous growth and no pests or diseases were selected, and cut them on sunny days (9:00-10:00).
2.2. Experimental reagent

Medium: MS + 1.0 mg·L⁻¹ 6-BA + 0.1 mg·L⁻¹ NAA + 30 g·L⁻¹ sucrose + 7 g·L⁻¹ agar, pH=5.8.

Anti-browning inhibitors: ascorbic acid (0.1 mg·L⁻¹, 0.2 mg·L⁻¹, 0.3 mg·L⁻¹), citric acid (0.1 mg·L⁻¹, 0.2 mg·L⁻¹, 0.3 mg·L⁻¹), PVP (0.3 g·L⁻¹, 0.4 g·L⁻¹, 0.5 g·L⁻¹).

Disinfectant: 75% ethanol and 0.1% HgCl₂.

2.3. Experimental methods

2.3.1. Disinfection of explants. Low temperature treatment: take the stem segment of ‘Hongyang’, remove the leaves, in a beaker, adding suitable amount washing powder and washing under running for water around 2h, and placed it in a refrigerator at about 5°C for 24h.

2.3.2. Sterilization of explants. It was sterilized with 75% ethanol for 30 s, then sterilized with 0.1% HgCl₂ for 8 min, and then rinsed 5 times with sterile water.

2.3.3. Inoculation of explants. The treated stem segments were blotted with filter paper, cut into small pieces of about 2cm, and inoculated. 6 bottles were inoculated for each treatment, and 5 bottles were inoculated per bottle for a total of 30. The culture temperature was 23 to 26°C, the illumination time was 16 h/d, and the light intensity was about 2000 Lx. After inoculation for 10 days, the browning and browning rate of the treated stem segments were observed and counted.

3. Results and analysis

The browning rate of each treatment after 10 days of inoculation was counted, and the effect of inhibiting browning by each treatment and the appropriate concentration were compared and analyzed. According to the experimental results, if no anti-browning measures were taken, the browning rate of explants in the tissue culture of ‘Hongyang’ kiwifruit was very high, indicating that the explants had low anti-browning ability. The selected three reagents of Vc, citric acid and PVP were used to treat the explants, and all of them had anti-browning effect. Among them, PVP treatment had the lowest browning rate, followed by Vc treatment, and the highest browning rate of citric acid treatment. The results showed that in the tissue culture of ‘Hongyang’ kiwifruit, PVP treatment had the best anti-browning effect.

In addition, in the lowest concentration treatment of PVP, the browning rate of explants was about 36.7% lower than that of the control group, and the effect was ideal. With the increase of PVP concentration, the anti-browning ability of explants gradually decreased. Among the treatment of Vc, the effect of 0.2 mg·L⁻¹ was better than the other two concentrations, which was 26.7% lower than that of the control group. Overall, the citric acid treatment inhibited the browning effect without the other two reagents, and the concentration between the two groups was brown. The difference in the effect was not obvious, and the browning rate dropped by about 16.7%.

| Processing | Concentration | Number of inoculation | Number of browning | Browning rate (%) |
|------------|---------------|-----------------------|--------------------|-------------------|
| Vc         | 0.1 mg·L⁻¹    | 30                    | 17                 | 56.7              |
|            | 0.2 mg·L⁻¹    | 30                    | 15                 | 50.0              |
|            | 0.3 mg·L⁻¹    | 30                    | 17                 | 56.7              |
| Citric acid| 0.1 mg·L⁻¹    | 30                    | 19                 | 63.3              |
|            | 0.2 mg·L⁻¹    | 30                    | 18                 | 60.0              |
|            | 0.3 mg·L⁻¹    | 30                    | 18                 | 60.0              |
| PVP        | 0.3 g·L⁻¹     | 30                    | 12                 | 40.0              |
|            | 0.4 g·L⁻¹     | 30                    | 14                 | 46.7              |
|            | 0.5 g·L⁻¹     | 30                    | 15                 | 50.0              |
| Control    | 0             | 30                    | 23                 | -                 |

Table 1. Effects of different browning inhibitors on browning of explants.
4. Conclusion
According to the experimental observation, the Browning in tissue culture of ‘Hongyang’ was very serious, it began in a short time, and explants were difficult to culture. Browning rate is high, and survival rate was low in control group. Slight browning began on the third day of culture, and light brown substances began to appear on the surface of the explant, and the color gradually deepened. The Browning time was similar in the experimental group, and the Browning degree was slightly lighter from the fifth day. The difference in late culture was increased, and the Browning time was light in the PVP group and heavy in the citric acid group.

The experiment was carried out on the browning of the ‘Hongyang’ tissue culture, and the most suitable inhibitors and their concentrations were screened by adding different concentrations of three inhibitors. Adding a browning inhibitor to the medium can change the redox potential around the explant, thereby inhibiting phenol oxidation and reducing browning. Different browning inhibitors are added, and the anti-browning effect is also different; the same browning inhibitor is used at different concentrations, and the anti-browning effect is also different, but the effect is different. PVP treatment added concentration of 0.3 g·L\(^{-1}\) is best, browning rate is 40%. Vc treatment added concentration of 0.2 mg·L\(^{-1}\) is best, browning rate is 50%; citric acid treatment effect is slightly worse, the concentration is 0.2 mg·L\(^{-1}\)With 0.3 mg·L\(^{-1}\), the browning rate was 60%, and the difference was small with the 0.1 mg·L\(^{-1}\) concentration control.

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