Anti-browning in Tissue Culture of 'Donghong' Kiwifruit

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Abstract. The bud stem segment of 'Donghong' kiwifruit was selected to study the anti-browning problem in kiwifruit tissue culture. The effects of different antioxidants and adsorbents added to the culture medium and cold treatment on the browning of explants in vitro were studied. The results showed that except for cold treatment at 4°C for 24 h, other treatments could effectively reduce the browning rate of explants. Among them, the addition of citric acid (CA) to the medium had the best anti-browning effect, and the browning phenomenon did not occur in the treatment of 0.5 mg/L of CA, but the axillary bud germination rate of the stem segment was low. Polyvinylpyrrolidone (PVP) and activated carbon (AC) were added to the medium to have a high germination rate. When the concentration of PVP was 300 mg/L, the germination rate was the highest, reaching 50.00%, and the browning rate was 30.00%. In general, the addition of 300 mg/L of PVP to the culture medium was the optimal treatment in the tissue culture of 'Donghong' kiwifruit.

1. Introduction
‘Donghong’ kiwifruit is a new red-fleshed kiwifruit variety selected from the “Hongyang” live-generation offspring. It was approved by the National Forest Species Certification Committee in 2012. It is extremely resistant to soft rot, more resistant to ulcer disease, heat and drought, and has high yield and stability. The fruit is premature and storable, and has a long shelf life. Therefore, ‘Donghong’ kiwifruit is a variety with great economic benefits [1].

Explant browning is a common phenomenon in plant tissue culture and has been reported in various fruit trees, such as peach [2], pear [3], blueberry [4], cherry [5] and walnut [6, 7]. The browning of explants is mainly caused by polyphenols in plants, which form brown anthraquinones under the action of polyphenol oxidase, which results in the death of explants. Common anti-browning measures include adding an antioxidant or a phenolic adsorbent to the culture medium, pretreating the explant, and changing the disinfection method. Commonly used antioxidants include Vc, CA, AgNO3, etc. Commonly used adsorbents are AC and PVP. Ac is a physical adsorption process, lacking adsorption selectivity. It can not only absorb toxic substances in the medium to reduce browning, but also absorb nutrients in the medium to cause competitive inhibition of plant growth [8].

In the previous experiments, the author found that during the tissue culture of 'Donghong' kiwifruit, the stem segment was browned seriously. Therefore, in order to select the best anti-browning treatment in tissue culture of 'Donghong' kiwifruit stem segments and lay a foundation for rapid propagation technology of kiwifruit, this experiment used 'Donghong' kiwifruit stem segments with buds as materials for planting.
to study the effects of different browning inhibitors and cold treatment on the browning of kiwifruit stem segments

2. Materials and Methods

2.1. Materials
In this experiment, the young stem segments of 'Donghong' kiwifruit were used as explants and planted in Chengdu Academy of Agriculture and Forestry. Choose robust, disease-free, robust plants that are cut on sunny days (9:00-10:00).

2.2. Experimental reagents
Medium: MS + 2.0 mg/L 6-BA + 0.2 mg/L NAA + 30 g/L sucrose + 7 g/L agar, pH adjusted to 5.8.
Anti-browning treatment: Vc(100mg/L, 200mg/L, 300mg/L), PVP (100mg/L, 200mg/L, 300mg/L), AC(100mg/L, 200mg/L, 300 mg/L), CA (0.5 mg/L, 1 mg/L, 2 mg/L), cold treatment at 4°C for 24 h. The medium and shoots which were not subjected to browning treatment were used as controls.
Disinfectant: 75% ethanol, 0.1% HgCl₂.

2.3. Methods
Take the stem section of 'Donghong' kiwifruit, remove the leaves, place in a beaker, add appropriate amount of washing powder and rinse for about 2 h under running water. It was sterilized with 75% ethanol for 30 s, then sterilized with 0.1% HgCl₂ for 7 min, and then rinsed 5 times with sterile water. The treated stem segments were blotted with filter paper, cut into small pieces of buds of about 2 cm, and inoculated. 6 bottles were inoculated per treatment, and 5 bottles per bottle were inoculated. 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 30 days, the browning and germination of each treated stem segment were observed and counted.

3. Results and Analysis

3.1. Effects of different antioxidants on browning of 'Donghong' bud stem segments
It can be seen from Table 1 that in the case of only looking at the browning rate, the addition of Vc and CA in the medium can effectively reduce the browning rate in the tissue culture of 'Donghong' kiwifruit compared with the control group. And the anti-browning effect of the AC group was better than that of the ascorbic acid group. The anti-browning effect of each concentration in the Vc group was not much different. On the contrary, in the CA group, there was a significant change, and the concentration of CA showed a negative correlation with the browning rate. As the concentration of CA increased, the browning rate increased. Among them, browning did not occur in the treatment of 0.5 mg/L of CA. It was indicated that CA was more suitable as an anti-browning agent in the tissue culture of 'Donghong' kiwifruit than Vc. However, the addition of Vc and CA to the medium did not significantly increase the germination rate.

Table 1. Effects of different antioxidants on browning of stems of 'Donghong' kiwifruit

| Antioxidants | concentration (mg/L) | Number of vaccinations | Number of browning | Browning rate | Number of germination | Germination rate |
|--------------|----------------------|------------------------|--------------------|---------------|-----------------------|-----------------|
| Vc           | 100                  | 30                     | 9                  | 30.00%        | 8                     | 26.67%          |
|              | 200                  | 30                     | 12                 | 40.00%        | 6                     | 20.00%          |
|              | 300                  | 30                     | 10                 | 33.33%        | 7                     | 23.33%          |
| CA           | 0.5                  | 30                     | 0                  | 0.00%         | 8                     | 26.67%          |
|              | 1                    | 30                     | 2                  | 6.67%         | 5                     | 16.67%          |
|              | 2                    | 30                     | 6                  | 20.00%        | 4                     | 13.33%          |
| CK           | 0                    | 30                     | 20                 | 66.67%        | 5                     | 16.67%          |
3.2. Effects of different adsorbents on the browning of stems of 'Donghong' kiwifruit

It could be seen from Table 2 that both PVP and AC could effectively reduce the browning rate. There was no difference in the effect between the two, and they showed the same regularity. As the concentration increased, the browning rate decreased first and then increased. Among them, the treatment with the lowest browning rate was 200 mg/L of PVP and 100 mg/L of AC, and the browning rates were 6.67% and 3.33%, respectively. The addition of PVP and AC to the medium could effectively increase the germination rate of stem segments, but the germination rate of the PVP group was higher than that of the AC group. However, the germination time of the AC group was earlier than other treatments. The germinated buds were distinct, the leaves were large and the color was dark green, and the growth was robust (Figure 1, a). When the concentration of PVP was 300 mg/L, the germination rate was the highest, reaching 50.00%, which was three times that of the control group.

Table 2. Effects of different adsorbents on the browning of stems of 'Donghong' kiwifruit

| Adsorbent | concentration (mg/L) | Number of vaccinations | Number of browning | Browning rate | Number of germination | Germination rate |
|-----------|----------------------|------------------------|--------------------|---------------|-----------------------|------------------|
| PVP       | 100                  | 30                     | 9                  | 30.00%        | 10                    | 33.33%           |
|           | 200                  | 30                     | 2                  | 6.67%         | 6                     | 20.00%           |
|           | 300                  | 30                     | 9                  | 30.00%        | 15                    | 50.00%           |
| AC        | 50                   | 30                     | 6                  | 20.00%        | 10                    | 33.33%           |
|           | 100                  | 30                     | 1                  | 3.33%         | 8                     | 26.67%           |
|           | 200                  | 30                     | 11                 | 36.67%        | 11                    | 36.67%           |
| CK        | 0                    | 30                     | 20                 | 66.67%        | 5                     | 16.67%           |

3.3. Effect of Cold Treatment on Browning of Stem Segment of 'Donghong' Kiwifruit

From Table 3, it could be seen that the browning rate and germination rate of explants treated with cold for 24 hours at 4 ℃ were not different from those of the control, and the browning rate of explants was serious. There were two reasons to cause it. First, it may be the explants in the 4 ℃ group were younger. Second, the processing time was not appropriate. When the explants were severely browned, some stem segments began to brown 2 hours after inoculation, after 24 hours, the medium was polluted to brown and the stem segments were completely browned to death (Figure 1, b).

Table 3. Effect of Cold Treatment on Browning of Stem Segment of 'Donghong' Kiwifruit

| Temperature | Time/h | Number of vaccinations | Number of browning | Browning rate | Number of germination | Germination rate |
|-------------|--------|------------------------|--------------------|---------------|-----------------------|------------------|
| 4 ℃         | 24     | 30                     | 15                 | 50.00%        | 5                     | 16.67%           |
| CK          | -      | 30                     | 20                 | 66.67%        | 5                     | 16.67%           |
4. Conclusion and Discussion
In previous studies, it can be found that browning in plant tissue culture was related to various factors, such as explant disinfection time, disinfection method, season of harvesting, and maturity of explants. The season of sampling and maturity of explants were closely related to the content of phenolic substances in plants. Phenolic substances were the main factors causing explant browning [9]. In this experiment, it was found that the degree of browning of explants taken in April and May was lighter than explants taken in June, which was consistent with the results of Yu [10]. It may be that the temperature in June was higher than that in April and May, and the content of phenolic substances in the explants was higher, resulting in serious browning. Moreover, in this experiment, it was found that the younger the stem segment, the more severe the browning situation, which was contrary to the previous research results [11], the previous studies found that the younger the explant, which contained the lower the content of phenolic substances, the lighter the degree of browning. We speculated that it might be related too long time of disinfection and the serious injury to explants. In this experiment, it was found that the semi-lignified stem segment was the best explant, its browning degree was light, and had a good germination rate, which was similar to the research conclusions of Li [12].

In the present experiment, it was found that 0.5 mg/L of CA had the best effect for preventing browning in the tissue culture of 'Donghong' kiwifruit, but in this treatment, the axillary bud germination rate was low, which was not optimal treatment. The addition of PVP and AC to the medium could effectively reduce the browning rate, and the axillary bud germination rate was high. The adsorption of AC is not selective and will adsorb nutrients and plant growth regulators in the culture medium. Therefore, considering 200mg/L PVP was the most suitable anti-browning additive in tissue culture of 'Donghong' kiwifruit. This was consistent with the conclusions of Liu [13] in the study on the prevention of browning of soft jujube kiwifruit tissue culture.

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