Callus-induced explants selection of ‘Pyrus betulifolia’

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Abstract. Efficient and stable in vitro regeneration system is the basic condition and key factor for the genetic transformation of pear. In order to find the most suitable explants to induce callus, three different explants, including seeds, hypocotyls and young leaves, were selected to induce callus respectively, and the regeneration ability of the three explants was compared, and finally the best material was selected. The results showed that loose and plump callus could be induced by using hypocotyl as explant in a short time.

1. Introduction

Pear belongs to pear genus of apple subfamily of rosaceae, which is one of the important deciduous fruit trees in the world, distributed all over the country, with a long history of cultivation, a variety of varieties, strong adaptability, stable and high yield, and deeply loved by the masses. Pear is the most widely used rootstock in northern China. As an ideal rootstock for pear cultivation in production, ‘Pyrus betulifolia’ is vigorous in growth, saline-alkali resistant, cold-resistant, water-resistant and adaptable, and has a good affinity with ‘white pear’, ‘sand pear’, ‘western pear’ and other cultivated varieties [1].

At present, pear production in China is developing towards the direction of improved varieties, base, dense planting, dwarfing, and high quality, but there are still many urgent problems to be solved in the production, such as the control of diseases and insect pests, and the improvement of fruit quality. The childhoods of pears are longer, and the seedlings usually need 5 years to bear fruit [2]. It takes a long time to select and breed new varieties by using traditional breeding technology. With the continuous progress of plant tissue culture and genetic engineering technology, it is possible to use biotechnology for breeding [3]. Using plant tissue culture technology can not only overcome the harm of virus disease, but also can quickly reproduce high-quality seedlings. The rapid propagation of pear tissue culture has attracted people's attention [4], but there are still few reports [5], mainly because it is difficult to establish tissue culture clones of pear and other woody plants, and explants are easy to brown [6]. In recent years, many woody plants have been successfully studied in tissue culture, such as peach tree [7], apple [8], litchi [9] and other plants in vitro regeneration technology has achieved important research results, but there are still many problems in tissue culture research and tissue culture seedlings further industrialization of woody plants. Therefore, it is necessary to summarize and analyze studies on explants selection, key factors and prominent problems in woody plant tissue culture, which will be conducive to the further development of woody plant tissue research and provide theoretical reference.

Callus Browning is a common phenomenon in plant tissue culture [10]. Tang Shaohu et al. selected leaves, stem tips and bud segments of 'xueqing pear' as explants to induce callus respectively. In MS+2,4-D 2 mg/L + 6-BA 0.1 mg/L+ sucrose 30 g/L+ agar 8g/L, the dedifferentiation rate of stem tip was 100% in medium with pH=5.8 [11].
In this study, *Pyrus betulifolia* seeds, leaves, hypocotyls were used as explants respectively, and the laboratory existing *Pyrus betulifolia* callus induction medium (NN69+6-BA 0.5 mg/L+NAA 0.3 mg/L+2,4-D 0.2 mg/L+ sucrose 30 g/L+ agar 7.5 g/L, pH=5.8) was used as the basic culture medium. By comparing the effects of different explants on callus induction rate of pear, the optimal explants of callus that could be induced in the best state were found out under the condition of existing medium.

2. Materials and Methods

2.1. Experimental materials
In this experiment, healthy *Pyrus betulifolia* seedlings with vigorous growth and no diseases and insect pests and seeds of *Pyrus betulifolia* stored in sand were selected as materials. The young leaves and hypocotyls of the seedlings and seeds from dormancy were taken as explants respectively.

2.2. Experimental reagent
Medium: NN69+6-BA 0.5 mg/L+NAA 0.3 mg/L+2,4-D 0.2 mg/L+ sucrose 30 g/L+ agar 7.5 g/L, pH=5.8.
Disinfectant: 75% alcohol and 0.1% HgCl₂.

2.3. Experimental methods

2.3.1. Disinfection of explants. Place the young *Pyrus betulifolia* seedlings and peeled seeds in a beaker, adding suitable amount washing powder and washing under running water around 1 h.

2.3.2. Sterilization of explants. It was sterilized with 75% alcohol for 10 s, then rinsed 3 times with sterile water, and then sterilized with 0.1% HgCl₂ for 8 min, finally rinsed 5 times with sterile water.

2.3.3. Treatment of explants. Cut off the excess parts of *Pyrus betulifolia* seedlings, leaving hypocotyls and young leaves. Cut the hypocotyl into 3-5mm segments; on the back of the leaf, 3-5 scratches are made along the vein, causing wounds but not cutting the leaf. Use the scalpel to cut off the head and tail of the seed, one seed into two pieces.

2.3.4. Inoculation of explants. Drain the treated explants with filter paper. Ten 3-5mm long hypocotyl segments were placed in a bottle and inoculated 30 times for a total of 300 hypocotyl segments. The 6 leaves were placed in a bottle with the distal axis facing down and inoculated 30 times for a total of 180 leaves. The seeds were placed in bottles with the distal axis facing down. 10 pieces of seeds were inoculated into one bottle, 30 bottles in total. Observation was made after dark culture at 21°C for 20 days, and statistical data were obtained.

3. Results and Analysis
It can be seen from table 1 that different explants had a great influence on callus induction rate of *Pyrus betulifolia*. The results showed that the existing callus induction medium (NN69 + sucrose 30 g/L + agar 7.5 g/L +6-BA 0.5 mg/L+NAA 0.3 mg/L+2,4-D 0.2 mg/L, pH=5.8) in the laboratory was used for dark culture. After 2 weeks, the seed of *Pyrus betulifolia* as the explants of induced callus showed no change, and the induction rate was 0, so the callus induction effect was the worst. When the leaf was used for the explant of induced callus, the induction rate was 100%. After 3 days inoculation, callus grew from the wound. After 7 days, callus began to expand. After 2 weeks, a light and yellow callus appeared in the blade wound, which was slightly firm and in good condition (Figure 1). Compared with the callus induced by leaves, the induction rate of *Pyrus betulifolia* hypocotyls as explants was 97%. After 3 days inoculation, callus were grown at both ends of the hypocotyl, but only a small number of wounds at both ends of the hypocotyl showed browning, which made them impossible to induce callus. This was because the smaller the explants were, the larger the ratio of wound to volume, and the greater the degree of injury and browning. After 2 weeks, Callus was loose in structure and good in shape,
showing light yellow or white (Figure 2). In conclusion, although the induction rate of callus induced by the hypocotyls as explants was slightly lower than leaves, the callus induced by the hypocotyl were superior to leaves. Therefore, the hypocotyl was the best explant to induce callus of pear.

| Explants type | Inoculation quantity | Callus number | inductivity |
|---------------|----------------------|--------------|-------------|
| Seed          | 300                  | 0            | 0%          |
| hypocotyl     | 300                  | 291          | 97%         |
| Young leaves  | 180                  | 180          | 100%        |

4. Conclusion
In the construction of plant regeneration system, the acquisition of explants is the first step. The callus that induced by different explants were different. In this study, three different explants (‘Pyrus betulaefolia’ seed, leaf and hypocotyl) were selected to obtain two methods for rapid and efficient callus induction, namely hypocotyl and leaf. Among them, the hypocotyl materials had browning phenomenon due to the greater degree of youth and smaller explants, and the induction rate was 98%.

Hypocotyl-induced callus had better morphology and more loose structure. Therefore, ‘Pyrus betulaefolia’ hypocotyl was the best explant. Though the induction rate of callus induced by leaves as explants was 100%, the callus structure was dense. Therefore, the hypocotyl was more suitable as an explant to induce callus than the leaves.

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