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Optimizing Culture System of Ri T-DNA Transformed Roots for *Citrus grandis* cv. Changshou Shatian You

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Abstract

Genetic transformation experiments of the different explants from *Citrus grandis* cv. Changshou Shatian You infected with *Agrobacterium rhizogenes* were carried out in darkness or in light. The optimizing culture system of Ri T-DNA transformed roots for *C. grandis* cv. Changshou Shatian You was constructed as follows: After the ventral wounded striations on the single activation cotyledon were inoculated by *A. rhizogenes* A4 (logarithmic period), they were cocultured at (25 ± 2)°C in darkness for 25-30 days; some transformed roots were generated from wounded striations of most cotyledons. The genetically transformed ratio is (83 ± 11)%. Axenic Ri T-DNA transformed roots (hairy roots) were harvested after five subcultures. Explants were activated on MT medium. The MS medium was used for subculture of transformed roots. Mass Ri T-DNA transformed roots in which the hormone was produced independently were harvested from this optimizing culture system. White, fresh Ri T-DNA transformed roots were (1.14 ± 0.07) cm long, (0.73 ± 0.04) mm wide, and the growth direction of transformed roots was negative geotropism.

Key words: *Citrus grandis* cv. Changshou Shatian You, *Agrobacterium rhizogenes*, Ri T-DNA transformed root, optimizing culture system

INTRODUCTION

The dual cultural system found in 80 years of Ri T-DNA carrot's (*Daucus carota* L.) transformed root organs with arbuscular mycorrhizal fungi (AM fungi) (Douds 2002; Fortin *et al.* 2002) is an effective method to study hyphal function of AM fungi. In this way, Ri T-DNA transformed carrot roots as a host stimulated the sporulation of some AM fungi. According to this principle and method, we tried to screen out feasible explants from different parts in citrus seedlings and study the Ri T-DNA genetic transformation of the arbor plants. The aim is to harvest enough citrus transformed roots which are used to screen highly compatible or highly effective AM fungi species (isolates) colonized in citrus root organs and study citrus mycorrhizal eco-physiology. Li *et al.* (1996) reported that he had harvested transformed (lower genetic ratio 25-36%) plants of sweet orange (*Citrus sinensis*) when cotyledon fragments mediated by *Agrobacterium rhizogenes* R1000, or A4 or 15834 were cocultured for 15 days in darkness. The construction of citrus mycorrhizae dual cultural system *in vitro*, however, demands that we must establish a more effective Ri T-DNA genetic transformation system for harvesting abundant citrus root organs. Changshou Shatian You (*Citrus grandis* cv. Changshou Shatian You) is the famous indigenous pomelo in Chongqing City of China, which was used in this experiment. The seed volume is larger and seed

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number greater in fruits of this pomelo than other citrus varieties. The wounded explants of embryos, epicotyls, hypocotyls, and the radicles from the seedlings cultured *in vitro* were infected by *Agrobacterium rhizogenes* by the ways such as dipping, daubing or dropping in the bacterial liquid. The construction of an optimizing culture system for Changshou Shatian You Ri T-DNA transformed roots would be a good preparation technique, but similar research is rare at present.

MATERIALS AND METHODS

Materials

The fruits of *Citrus grandis* Osbeck cv. Changshou Shatian You were sampled from mature orchards located at Changshou Lake Island in the Changshou County, Chongqing City, China. The strains A4 and R105 of *Agrobacterium rhizogenes* were purchased from the China Center of Agricultural Culture Collection.

Methods

Seed treatments The mature pomelo fruit surface was sanitized with water and then air-dried. After surface sterilization with 75% ethanol, the fruit was migrated in benchtop. The peel was removed and the mature and plump seeds were taken out, which were then dipped in 40°C warm water for 30 min and in 1% NaOH for 15 min. The seeds were rinsed with sterilized water repeatedly and the pectin on the surface of the seeds was discarded. After disinfecting in 75% ethanol for 1 min, the seeds were taken out and dipped in 0.2% HgCI for 10 min. The seeds were rinsed with axenic distilled water 10 times. The seed coats were peeled and small parts of cotyledons were cut away at the wider end of the embryos so as to create a wound for carrying *Agrobacterium rhizogenes* in the future experiments.

Preparations for explants We planted the narrow end of the embryos earthwards in 25 mL MT medium, 5 embryos per bottle. One hundred bottles were cultured in darkness at (25 ± 2)°C for 3 days. Twenty bottles of embryos were for embryo explants and 20 bottles were for cotyledon explants. Other bottles were cultured for 20 days and provided 14 h 1 600 lx of illumination per day. Then the axenic seedlings were harvested. The epicotyls, hypocotyls, and radicles were cut from the seedlings and used in genetic transformation, and experimented together with embryos and cotyledons after infection by *Agrobacterium rhizogenes*.

Activation of *Agrobacterium rhizogenes* The plate medium surface of YMB was inoculated with *Agrobacterium rhizogenes* A4 or R105 by drawing lines. The petri dishes were incubated at 25°C for 3 days. The bacteria picked from a clone were then drawn on a new solid YMB medium for activation again at 25°C for 2 days. Single clones of both strains from active dishes were picked out and used to inoculate the liquid YMB medium and culture in a shaker (135 r/min, 25°C) until the logarithmic growth period of *Agrobacterium rhizogenes* (SOD 0.6-0.8 under l=600 nm) was reached. These bacteria liquids would be used for future genetic transformation experiments.

Explant inoculation and transformation culture (1) The axenic embryos activated in solid MT medium for 3 days were picked out and dipped in activated bacterial liquid (0.2%, v/v) for 30 min. They were synchronously treated in a 42 Hz ultrasonic bath for 25 min. The embryos were picked out and put onto the autoclaved filter paper for extracting the unwanted bacterial liquid. Then the narrow ends of the embryos were inserted in solid MT medium, which had no hormone but 500 mg L^{-1} of cefotaxime, 5 embryos per petri dish, and 20 dishes per treatment. (2) The cotyledons were cut from the axenic embryos that had been activated for 3 days. The dorsums of the cotyledons were put on the filter paper and 5-6 lines were superficially cut on the ventral part of the cotyledons with an inoculator. The dorsum of the single cotyledon was laid downwards and the ventral upwards on the hormone-free and cefotaxime-free MT medium. The wounded line could not penetrate the cotyledons horizontally or vertically through. 0.2 mL activated liquid of *Agrobacterium rhizogenes* was dropped in line-shaped-wounds and no excess liquid outflow dropped to the surface of the medium from the cotyledon. Twenty dishes were cultured in darkness and 20 dishes in light. 5-9 single cotyledons were incubated in each petri dish. (3) Epicotyls and hypocotyls as well as radicles were cut from seedlings which had been cultured for 20 days. A cross shape was scored at the morphologically downward