Development of Flower Pigment Transgenic Chrysanthemum by Meristem-tip Culture and Agrobacterium Transformation

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ABSTRACT

Since meristem-tip culture has the fully ability to differentiate into plants without changing the original inherency and can produce a large amount of explants in a short time, it has been widely used for plant micropropagation. In this study, meristem-tip culture were used to obtain chrysanthemum explants for gene transformation. In order to observe the influence on the expression of flower color, two pigment genes, CHI and DFR, were used for transformation of chrysanthemum explants. Chrysanthemum plants from field were first sterilized and meristem-tips were cut and cultured in appropriate media for fifteen days to two months. Since the size of the cutting affect the survival rate, only 39% in 410 meristem-tip cultures were obtained and used for mass production. In addition, chrysanthemum from the field and meristem-tip culture were detected by RT-PCR using primers specific to Chrysanthemum stunt viroid, it was found that none of them was viroid-free. In the regeneration experiments, two chrysanthemum varieties, Huang-shin-Fang and Bai-Dong-Yang, showed that the best regeneration rate was in the MS medium containing 1 mg/l BA and 0.5 mg/l NAA. For Huang-shin-Fang, all the explants had the ability to regenerate into adventitious shoots, and the average regeneration number of each explant was 6.3. For Bai-Dong-Yang, thirty six out of forty eight explants were able to regenerate and each explant produced about 2.7 adventitious shoots. Hygromycin was used for the selection of transformed plants. From the hygromycin sensitivity test on non-transgenic chrysanthemum, it was found that 10 mg/l of hygromycin was high enough to stop the regeneration of chrysanthemum. Therefore, the transformation condition was performed by using MS medium with 1 mg/l BA, 0.5 mg/l NAA, and 10 mg/l of hygromycin. The suitable co-culture time for chrysanthemum and Agrobacterium is four days. There were 25 putative CHI transgenic chrysanthemum obtained from 109 explants. When the putative CHI transgenic plants were further analyzed by PCR amplification coupled with Southern blotting, nine of them showed positive reaction. Similarly, thirteen out of eighty three explants were able to regenerate from selection medium after DFR gene transformation, and 9 of them were further verified by PCR amplification coupled with Southern blotting. To further study the insertion number of the DFR transgenic plants, genomic Southern blotting were carried out. It was found that the DFR transgenic chrysanthemum all contained one transgene insertion. The transgenic lines are now kept in greenhouse for the observation of the flower pigment expression.

Keywords : meristem-tip culture, Chrysanthemum stunt viroid, RT-PCR, Agrobacterium transformation, co-culture, Southern blot.

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