Plant Regeneration from Protoplasts Derived from Callus of *Phalaenopsis* Alliance

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Plant regeneration from protoplasts of orchids was very difficult and only succeeded when callus of *Phalaenopsis* was used. On a previous occasion, we reported the efficient isolation and refinement of protoplasts derived from callus, here we examine the cultural requirements.

MATERIALS AND METHODS

Callus of *Phalaenopsis* 'Hanaboushi' × *P. equestris* 'Ilocos' cultured on new phalaenopsis medium (NP) supplemented with coconut water (CW) and sucrose for 4 to 8 weeks was used. Callus which was precultured on NP medium (without CW and sucrose) for 3 weeks was soaked in an enzyme solution and shaken for 1.5 h at 25C on a reciprocal shaker (77 strokes min^{-1}) under reduced light. The enzyme-protoplast mixture, to which was added a washing solution, was filtered through a stainless sieve of 66-mm pore size to remove undigested cell clumps and centrifuged at 100 × g for 5 min. Then the sediment, to which was added fresh washing solution was mixed with ficoll solution and overlayed with washing solution. Next they were centrifuged at 230 × g for 30 min. After centrifuging, the layer of protoplasts in the washing solution was withdrawn with a pipette and washed in washing solution. After washing, the culture medium was added to the protoplast solution and centrifuged at 100 × g for 5 min. Finally, the protoplast solution, with fresh medium added to the sediment, was transferred into a 35-mm plastic dish and cultured at 25C in the dark. The medium was supplemented with 0.3 M sucrose, sorbitol, and maltose in order to examine the effect of osmoticum on the rate of survival of the protoplasts and their rate of division.

RESULTS AND DISCUSSION

The rate of survival was highest when supplemented with sorbitol and the survival rate was 80% at 1 week and 60% at 4 weeks. The next highest survival rates were those with the maltose. Cell division was observed in all supplemented media after 1 week of culture. The rate of division after 4 weeks of culture was highest in the sucrose-supplemented medium, but it did not lead to colony formation. In the sorbitol- and maltose-supplemented media, colony formation and plant regeneration occurred. The rate of division with maltose was higher than that with sorbitol, however, the quantity of protoplasts was higher with sorbitol.

The above result showed that sorbitol was suitable for the culture of protoplasts as osmoticum.