Regeneration of Rice Plants from Protoplasts

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Although several successful cultures of rice protoplast were reported,¹⁾ no complete plants regenerated had so far been found. In these cases, special cell lines accompanied by an easy release of protoplasts were used. However, these cell lines had already lost the capacity for regeneration.

We recently isolated cell lines with high regeneration capacity from which protoplasts were readily isolated. The protoplasts isolated from these cell lines divided with high frequency and formed many small cell clusters. A number of plantlets were regenerated from these calli.

Friable calli initiated from scutellums of rice seeds (*Oryza sativa*, cv. 'Nihonbare') and immature embryo (cv. 'Sasanishiki') were transferred to the liquid medium containing R2 inorganic salts,²⁾ B5 vitamins,³⁾ 0.3% casein hydrolysate, 1 ppm 2, 4-D and 3% sucrose. Cells were cultured in this medium at 25°C under 500 lux continuous fluorescence light on a gyrotary shaker at 60 rpm and subcultured every week in fresh suspension culture medium.

The cells cultured for 5 days in a subculture were used for the isolation of protoplast. About 1 g of the cells were mixed with 10 ml of an enzyme solution containing 1% Macerozyme R10 (Kinki Yakult Co. Ltd), 4% Cellulase RS (Kinki Yakult Co. Ltd), 0.5% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.5% potassium dextran sulfate and 0.4 M mannitol.

The pH of the enzyme solution was adjusted to 5.5. The enzyme-cell mixture was incubated at 27°C for the first 3 hr on a gyrotary shaker (40 rpm) and thereafter for 3 hr without shaking. The enzyme-protoplasts mixture was passed through a 40 μm nylon screen to remove debris. The protoplasts were resuspended and washed four times by centrifugation ($50 \times g$ for 5 min) with washing medium containing R2 inorganic salts and 0.4 M glucose. Finally the protoplasts were resuspended in culture medium containing R2 inorganic salts, 2 ppm 2, 4-D, B5 vitamins and 0.4 M sucrose. The density of protoplasts was adjusted to $10^6/\text{ml}$ and they were cultured in the dark at 25°C.

The first cell division was observed after 2 days of culture and the frequency of the cell division after 10 days was 10% at maximum. The calli of about 500 μm diameter were transferred to N6 medium⁴⁾ supplemented with 2 ppm 2, 4-D, 0.5 ppm benzyladenine and 3% sucrose. These protoplast-derived calli grew vigorously on this medium. The calli with more than 2 mm in diameter were transferred to N6 hormone free medium for differentiation and cultured under continuous fluorescence light (3,000 lux). From 5-20% of these calli depending on experiments, plantlets were regenerated and the plantlets developed to about 10 cm in height in vitro were transferred to pots in green house after acclimatization. More than 50 plants are grown in a green house so far and some of them are now at a stage of forming panicles.

Fig. 1. Small calli derived from rice protoplasts.

Fig. 2. Plants regenerated from rice protoplasts.

Since protoplasts isolated from 15 independent cell lines of two cultivars were successfully cultured and differentiated to plants, the method described here is supposed to be reproducible. Taking into consideration of this reproducibility and relatively high efficiency of regeneration into plants, this technique could be used for rice protoplasts fusion and foreign gene transfer into rice protoplasts.

References

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《和文要約》

イネプロトプラストからの植物体の再生

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イネ懸濁培養細胞から単離したプロトプラストを培養した。プロトプラストの約10%は分裂し、カルスを形成した。カルスを植物再生用培地に移し培養すると植物体が再生し、さらに温室内のポットに移し培養した。植物体は正常に成育し、幼穂形成期の植物まで生育した。