Plant Regeneration from Suspension Cell-derived Protoplasts using Agarose-solidified media and Nurse Culture in Rice

Hyun-Soon Kim* and Francisco J. Zapata**

Abstract: Fertile plants have been regenerated from suspension cell-derived protoplasts using agarose-solidified media and nurse culture in three Korean japonica rice varieties. Protoplast plating efficiency were significantly 2.4 - 5.4% higher in R2 medium than in KPR in all three varieties. Nurse cell was necessary for cell division in protoplast culture and plating efficiency was the highest in Oryza sativa cell(Oc) line, followed by Suwon 366 and Anjungbyeo. Out of 69 protoplast-derived plants(Ro) into soil, only 49 plants were fertile with a frequency of 71% which showed a uniform morphology.

Key words: Rice, Protoplasts, Agarose-solidified media, Nurse cell, Protoplast-derived plants.

Protoplasts provide the useful material for genetic modification via somatic variation and transformation of rice plants. The successful regeneration of fertile plants from protoplasts or cells is one of the essential prerequisites. But regeneration and transformation from rice protoplasts are genotype-dependent and only limited number of genotypes successfully regenerated (Lee et al., 1989, Ghosh & Zapata, 1990, Yin et al., 1993). In addition, regeneration efficiency of rice plants remains still quite low.

Therefore, an efficient system for

* 농촌진흥청 호남농업시험장(National Honam Agricultural Experiment Station, Rural Development Administration, Iksan 570-080, Republic of Korea)
** 오스트리아 국제원자력연구소(Plant Breeding Unit, International Atomic Energy Agency(IAEA), A-2444, Seibersdorf, Austria)
production of fertile plants needs to be well established. Hence, this study was undertaken to increase regenerated plants from suspension cell-derived protoplasts using agarose-solidified media and nurse cell on the different genotypes.

MATERNALS AND METHODS

Material maintenance
Calli induced from mature seeds of three Korean japonica rice varieties, Anjungbyeo, Mangeumbyeo and Suwon 366, on N6 medium (Chu et al., 1975) containing 2 mg/l of 2,4-D. Four-week-old primary embryogenic calli were used for suspension initiation. The cell suspension were maintained in AA medium (Muller & Grape, 1978) with 2 mg/l of 2,4-D. The liquid medium was replaced at 3 to 5 days intervals for 1 month. When the cell suspensions were established within 5 months, the liquid medium was replenished with fresh medium at weekly intervals.

Protoplast isolation and culture
Protoplasts were isolated from suspension cells with 20 ml of filter sterilized enzyme mixture containing 2% (w/v) Cellulase RS (Yakult Honsha Co. Ltd., Japan), 0.1% (w/v) Pectolyase Y23 (Seishin Pharmaceutical Co. Ltd., Japan) dissolved in CPW salts (Freason et al., 1973) with 9% (w/v) mannitol (CPW9M). The cell-enzyme mixture was incubated on rotary platform shakers (40 rev/min) for 3-4 hours at 27°C, and then passed through a series of two layers of nylon mesh each of 45, 30 and 25μm diameter to remove undigested cell clumps. Protoplasts were pelleted by centrifugation at 800 rpm for 5 min and then washed 3 times with CPW9M. The protoplasts were cultured using an agarose-bead method (Shillito et al., 1983) and mixed nurse culture method (Kyozuka et al., 1987) with the following modifications. One ml of double-strength protoplast culture medium was mixed with an equal volume of 2.4% Sea Plaque agarose. The purified protoplasts (3.0 and 5.0 x 10^5/ml) were gently mixed with warm protoplast medium at 40°C and then transferred into petri dishes (60 x 15mm). The medium for protoplasts was with KPR (Kao & Michayluk, 1975) and R2 medium containing 2 mg of 2,4-D/l and 137 g/l of sucrose. After solidified medium, 100 mg of active growing nurse cells were suspended in 5 ml of protoplast culture liquid medium and added to each petri dish containing the agarose block. For the nurse cell effect on protoplast culture of Anjungbyeo, cells of Anjungbyeo, Suweon 366 and Oryza sativa cell (Oc) line were used as nurse cells.

The plates were rotated on a shaker (Teher SH-R2) at 40 rpm in the dark at 25±1°C for 8 to 10 days and then the nurse cells were removed and replaced with the same fresh protoplast culture medium. The plating efficiency of protoplast was determined at this time. After that, the protoplast-derived colonies were cultured for