

Plant Regeneration from Protoplasts of Indica Rice

Sung-Ho Lee*, Young Goel Shon**, Soo In Lee**, Zhoo Hyeon Kim**
and Moo Je Cho*

Indica 벼의 원형질체들로부터 식물체 재분화

이성호* · 손영걸** · 이수인* · 김주현** · 조무제*

ABSTRACT : An efficient protocol for plant regeneration from protoplasts of the indica rice variety IR43 has been developed. The procedure involved plating of embryogenic suspension-derived protoplasts on the surface of a filter membrane overlaying agarose-embedded feeder cells. *Lolium multiflorum* cell suspensions were preferable to these of *Oryza ridleyi* as feeder cells and *Lolium* suspensions supported colony formation from up to 0.68% of the protoplasts, depending on the age of cell suspensions. Plant regeneration frequency was significantly improved by using maltose alone or in a 1:1(w/w) combination with sucrose as carbohydrate source and a simple dehydration treatment using a high concentration of agarose in the regeneration medium. Medium containing maltose or maltose mixed with sucrose increased the plant regeneration frequency compared with medium containing sucrose alone. The plant regeneration frequency was increased to 30.7 to 70.7% following dehydration treatment, while the non-treated controls showed a regeneration frequency of 3.1 to 30.6%. Protoplast-derived plants were transferred to the glasshouse, flowered with morphologically normal.

Key words : Carbohydrate, Dehydration, *Oryza sativa* L., Protoplast-derived plants.

Indica rice varieties are the most widely cultivated in the tropical regions of the world, and are also the most important as a major food source for half of the world's population. Hence, much attention has been focused on the improvement of these varieties using biotechnology, including reproducible protoplast-to-plant regeneration systems. In general, protoplasts of indica were more difficult to culture and regenerate as

compared to japonica rices. However, since Kyo-zuka et al.(1988) established a protocol for regeneration of indica rices using nurse culture methods, a number of researchers have reported plant regeneration from protoplasts of indica rices(Lee et al., 1989; Su et al., 1992; Datta et al, 1992; Torrizo & Zapata, 1992; Ghosh Biswas & Zapata, 1993; Jain et al., 1995).

Feeder cells for nurse culture systems have

* 경상대학교 식물분자생물학 및 유전자 조작 연구센터(PMBBRC, Gyeongsang National Univ., Chinju 660-701, Korea)

** 경상대학교 농과대학 원예학과(Dept. of Horticulture, Gyeongsang National Univ., Chinju 660-701, Korea)

〈'97. 7. 10 接受〉

been used extensively for protoplast culture in cereals (Rhodes et al., 1988; Shillito et al., 1989; Prioli & Söndahl, 1989; Funatsuki et al. 1992). Feeder cells from several species have been used to support cell division from protoplasts in indica. Two different protoplast culture procedures have been developed using feeder cell system. Protoplasts were embedded in agarose (Abdullah et al., 1986) and the latter placed in liquid protoplast culture medium containing feeder cells (Kyo-zuka et al., 1988; Torrizo & Zapata, 1992). In the other system, protoplasts were cultured on the surface of a filter membrane positioned on top of agarose-solidified protoplast culture medium in which the feeder cells were embedded (Lee et al., 1989; Su et al., 1992; Jain et al., 1995).

The indica rice variety IR43 was developed at IRRI. It belongs to varietal Group I. Rice varieties belonging to Group I, as classified on the basis of isozyme polymorphism, are lowland rices found throughout tropical Asia and are characterized by the 'C' morphological type (Glaszmann, 1987). Varieties of Group 1 rice have proven to be less responsive to either tissue or protoplast culture compared to other indica or japonica varieties belonging to Groups II-VI (Torrizo & Zapata, 1992). Plant regeneration from protoplasts has been reported in several varieties of Group I indica rices (Lee et al., 1989; Su et al., 1992; Torrizo & Zapata, 1992; Su et al., 1992; Datta et al., 1992; Jain et al., 1995.)

The aim of the work described in this study was to develop an efficient protoplast culture system using the filter membrane procedure with feeder cells for the Group I true indica rice variety IR 43 and to maximise plant regeneration from protoplast-derived calli by dehydration treatment using a

high concentration of agarose in the shoot regeneration medium.

MATERIALS AND METHODS

1. Callus initiation and maintenance

Mature seeds of the indica rice variety IR43 (*Oryza sativa* L.) were gently dehusked and surface sterilized by soaking in 30% (v/v) 'Domestos' bleach for 30min with rotary agitation and rinsed five times with sterile tap water. Seeds of IR43 were inoculated onto the surface of Murashige & Skoog (1962) medium semi-solidified with 8.0g l⁻¹ agar (BBL, Microbiological Systems, Maryland, USA), and supplemented with 30.0g l⁻¹ sucrose, 2.0mg l⁻¹ 2,4-D and 1.0 g l⁻¹ casein hydrolysate for callus initiation and maintenance. The plates were incubated in the dark at 27°C. Embryogenic calli were selectively transferred to fresh same medium after 4 weeks of initiation. Subsequently, embryogenic calli were subcultured at monthly intervals.

2. Initiation and maintenance of cell suspension cultures

Cell suspension cultures were initiated by transferring approximately 1.5g fresh weight of embryogenic callus to 20ml of liquid medium in 100ml Erlenmeyer flasks. R2 medium (Ohira et al., 1973), modified by the addition of 560mg l⁻¹ L-proline and 30g l⁻¹ maltose, was used for the initiation as well as maintenance of cell suspensions. During the first four weeks of initiation of cell suspension cultures, fresh R2 liquid medium was replaced twice weekly, without reducing the cell density, by removing all the conditioned (spent) medium. After six weeks, pipetting

of fine cell suspension cultures was commenced with transfer of 1ml pcv of cells to fresh medium. Fine cell suspensions were maintained by weekly subculture to fresh medium. The cultures were maintained on a rotary shaker at 120rpm in the dark at 27°C

3. Protoplast isolation

Protoplasts were isolated from suitable established cell suspension cultures, in exponential growth phase, between 4~5 days after subculture by enzymatic digestion, through overnight incubation. The rest of the isolation procedure followed as described by Abdullah et al.(1986).

4. Preparation of feeder cell plate

Cell suspensions of *L. multiflorum* used as feeder cells in this study were kindly provided by Dr. E. Guiderdoni(IRAT-CIRAD, Montpellier, France) and maintained in N6 liquid medium (Chu et al., 1975) containing 2.0mg l⁻¹ 2,4-D. *O. ridleyi* cell suspensions, also used as feeder cells, were initiated using calli initiated from mature-seed scutella on MS-based medium (Murashige & Skoog, 1962) supplemented with 1.0g l⁻¹ casein hydrolysate and maintained in MS liquid medium containing 2.0mg l⁻¹ 2,4-D. Both cell suspensions were incubated on a rotary shaker(120rpm) in the dark at 27°C with weekly subculture. To prepare the feeder cell plates, cells were harvested from cell suspensions 3~5 d after subculture. Five ml pcv from the suspension of either *L. multiflorum* or *O. ridleyi*, or a 1:1 (v/v) mixture of both cells, were suspended in 100ml of molten KPR medium containing 0.8% (w/v) Sea Plaque agarose(FMC Corp., Rockland, ME, USA) at 40°C. Five ml aliquots were

dispensed into 5.5cm diameter Petri dishes. The plates were sealed with Nescofilm and then cultured in the dark at 27°C. These feeder plates were prepared 1d prior to protoplast isolation.

5. Protoplast culture

One Whatmann cellulose nitrate filter membrane(47 mm diameter and 0.2µm pore size) was used for each Petri dish. The intact membrane was laid on the surface of the medium with or without embedded feeder cells. Aliquots of 200µl containing 5×10⁵ml⁻¹ protoplasts was dropped onto the surface of the intact membrane and spread uniformly over the membrane surface using a sterilized plastic bacterial inoculation loop. The cultures were incubated in the dark at 27°C. After 21~28d, membranes with protoplast culture were transferred to the surface of agarose-solidified Linsmaier and Skoog (1965) medium supplemented with 2.5mg l⁻¹ 2,4-D (LS 2.5) without feeder cells and incubated for 14d. The plating efficiency of these cultures was determined on the percentage of protoplasts that had undergone division when cultured in medium semi-solidified with 0.4% (w/v) Sigma Type I agarose.

6. Dehydration treatment and plant regeneration

Protoplast-derived colonies, 1~2mm in diameter, were transferred to 25 well plastic dishes with one colony in each well containing 2ml 1.0% agarose-solidified MS plant regeneration medium supplemented with 2.0mg l⁻¹ kinetin and 0.5mg l⁻¹ NAA(MSKN) with either 3.0%(w/v) sucrose or 3.0%(w/v) maltose or combination of 1.5%(w/v) sucrose and 1.5%(w/v) maltose. The cultures were incubated in the dark at 27°C for 2

weeks for dehydration treatment. After 2 weeks, dehydrated calli cultured on 1% (w/v) agarose-containing medium were transferred to the corresponding 0.4% (w/v) agarose medium and incubated in the light ($55\mu\text{mol m}^{-2} \text{sec}^{-1}$, daylight fluorescent tubes, 16h photoperiod at 27°C). The colonies were also cultured onto the same regeneration medium, semi-solidified with 0.4% (w/v) agarose as control. Plant regeneration frequency was recorded after 28d as the percentage of protoplast-derived colonies forming plants. Plantlets regenerated from protoplasts were transferred to 0.4% (w/v) agarose-solidified MS micropropagation medium containing 2mg l^{-1} BAP and 5% (w/v) sucrose (MSBP) and incubated at 27°C in the light for 28d. Shoots were then individually transferred to agarose (0.4% w/v)-solidified MS medium containing 1.5mg l^{-1} NAA and incubated at 27°C in the light for root differentiation. Young plants regenerated were transferred to pots and kept in the glasshouse.

RESULTS

1. Initiation and maintenance of cell suspension cultures

Calli were gradually initiated at the scutella surface of cultured dehusked mature seeds within 4 weeks of culture on MS medium. The fine friable and globular embryogenic calli were selected and transferred into the liquid culture media for initiation of cell suspensions. A fine cell suspension cultures were established rapidly in modified R2 medium after 6~7 weeks of initiation of cell suspension cultures.

2. Protoplast isolation and culture

Satisfactory yields of protoplasts ($2.1\sim 8.3\times 10^6$ g f.wt.⁻¹) were obtained by overnight incubation with enzyme mixture. Freshly isolated protoplasts were densely cytoplasmic (Fig. 1a). First and second cell divisions commenced within 5 days (Fig. 1b,c) and third divisions (8 cells stage) were observed within 1 week (Fig. 1d). The most suitable time for transfer to LS 2.5 medium was found to be 3 weeks of culture, depending on the growth of the microcolonies. Early transfer of culture membranes to LS 2.5 medium resulted in fewer microcolonies. Microcolonies of 1~2mm in diameter were obtained after 5 weeks of culture (Fig. 1e). At this stage, it was essential to transfer microcolonies to shoot regeneration medium. Protoplasts failed to divide when plated on the surface of filter membranes overlaying KPR medium without feeder cells. However, when protoplasts were cultured on the filter membrane employing agarose-embedded feeder cells of *L. multiflorum* or *O. ridleyi* or a 1:1 (v/v) mixture of these feeder cells, there was sustained division leading to colony formation. In the presence of *L. multiflorum* or *O. ridleyi* feeder cells, the protoplast plating efficiencies ranged from 0.06 to 0.68%, depending on the feeder cell type (Table 1). In the case of protoplasts isolated from 6-month-old cell suspensions, the plating efficiency was 3.5-fold higher when *L. multiflorum* suspensions were used as feeder cells compared with cells of *O. ridleyi*. A combination of the two types of feeder cells did not enhance the plating efficiency as reported for other Group 1 rice varieties (Jain et al., 1995); rather the plating efficiency seemed to decline. With increase in the age of cell suspensions, the plating ef-

Table 1. Effect of age of cell suspension cultures and feeder cells on protoplast plating efficiency in the indica rice variety IR43

Age of cell suspension (months)	Protoplast yield ($\times 10^6$ gf.wt. ⁻¹)	Feeder cell	Plating efficiency (%)
6	2.1~4.9	None	0.00 \pm 0.00
		<i>L. multiflorum</i>	0.23 \pm 0.02
		<i>O. ridleyi</i>	0.06 \pm 0.02
		<i>L. multiflorum</i> + <i>O. ridleyi</i>	0.18 \pm 0.01
8	5.7~7.2	<i>L. multiflorum</i>	0.44 \pm 0.04
10	8.3	<i>L. multiflorum</i>	0.68 \pm 0.15

Mean \pm S.E. of 3 replicates. Plating efficiency was expressed as the percentage of protoplasts forming visible colonies after 5 weeks of culture.

efficiency progressively increased from 0.23% to 0.68% in the presence of *L. multiflorum* feeder cells (Table 1).

3. Effect of carbohydrate source on plant regeneration

To investigate the effect of carbohydrate source on plant regeneration, protoplast-derived colonies, originally grown on membranes with feeder cells of *L. multiflorum*, were transferred onto plant regeneration medium with either added sucrose or maltose, or a mixture of both, as carbohydrate source. Protoplast-derived colonies produced plantlets through somatic embryogenesis after 2~4 weeks of culture on regeneration medium semi-solidified with 0.4%(w/v) agarose (Sigma Type 1). Plant regeneration frequency varied considerably with carbohydrate source in the MSKN medium. Maltose supplemented medium was superior for regeneration of green plants from protoplast-derived colonies compared with medium containing only sucrose. The mean percentage of protoplast-derived colonies exhibiting plant regeneration on sucrose, maltose and a mixture of both of these carbohydrates were 3.1 \pm 1.8, 19.3 \pm 3.5 and 17.5 \pm 6.5, respectively, from 4 independent experiments with 3 replicates in each experiment (Table 2). Thus, maltose

alone or in combination with sucrose (1:1, w/w) as carbohydrate source in plant regeneration medium, increased the plant regeneration frequency 6-fold compared with sucrose alone. The results suggest that maltose as carbohydrate source increases somatic embryogenesis and plant regeneration from protoplast-derived colonies in indica rice.

4. Effect of dehydration on plant regeneration

Dehydration treatment or partial desiccation increased plant regeneration significantly from suspension-cultured cells and callus initiated from mature seeds in both indica and japonica rice (Tsukahara & Hirose, 1992; Rance et al., 1994). Protoplast-derived colonies were dehydrated using a high concentration of agarose to investigate the effect of dehydration on plant regeneration. As dehydration treatment, protoplast-derived colonies were precultured in the regeneration medium semi-solidified with 1%(w/v) agarose (Sigma Type 1) for 2 weeks in the dark at 27°C. The colonies were also cultured in the same regeneration medium semi-solidified with 0.4%(w/v) agarose as control. Dehydrated colonies in 1% agarose underwent little callus proliferation and no differentiation compared to controls grown in 0.4% agarose. However, dehydrated colonies be-

Table 2. Effect of dehydration and carbohydrate source on plant regeneration from protoplast-derived colonies of the indica rice variety IR43

Experiment number	Dehydration treatment	Percentage of protoplast-derived colonies exhibiting plant regeneration on:		
		Sucrose (3%, w/v)	Sucrose (1.5%, w/v) +maltose(1.5%, w/v)	Maltose (3%, w/v)
1	No	3.1 ± 1.8(ND)	21.9 ± 5.6 (ND)	30.6 ± 5.8(ND)
2	No	ND	10.7 ± 3.5(3.5)	12.0 ± 2.3(3.0)
	Yes	ND	38.7 ± 3.5(8.0)	44.0 ± 8.3(7.7)
3	No	ND	12.0 ± 6.1(4.0)	10.7 ± 1.3(2.0)
	Yes	ND	42.7 ± 10.7(11.7)	30.7 ± 8.7(8.1)
4	No	ND	25.3 ± 10.9(5.0)	24.0 ± 4.6(3.6)
	Yes	ND	70.7 ± 17.9(11.0)	41.3 ± 11.8(8.7)
Mean	No	3.1 ± 1.8(ND)	17.5 ± 6.5(4.2)	19.3 ± 3.5(2.9)
	Yes	ND	50.7 ± 10.7(10.2)	38.7 ± 9.6(8.2)

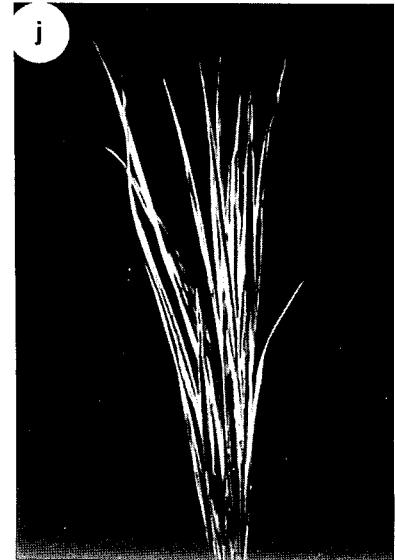
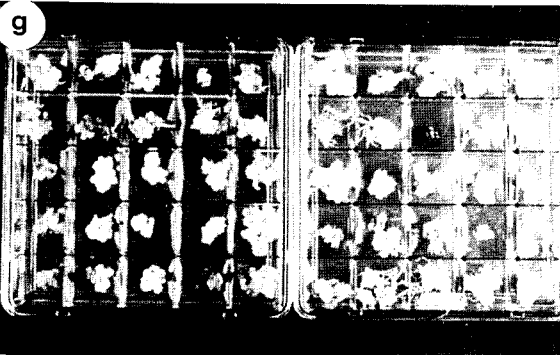
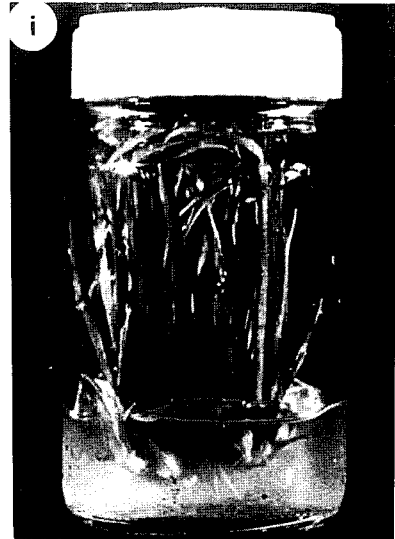
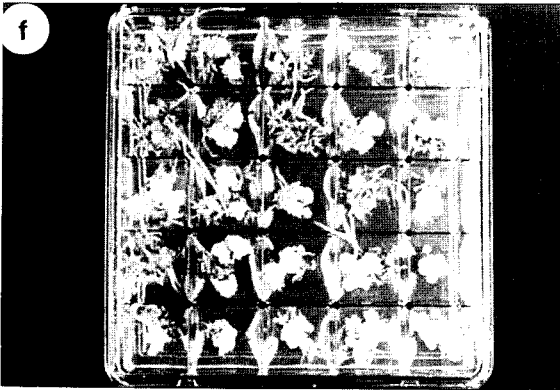
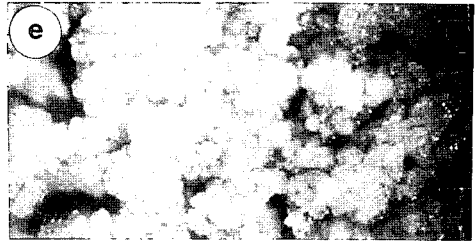
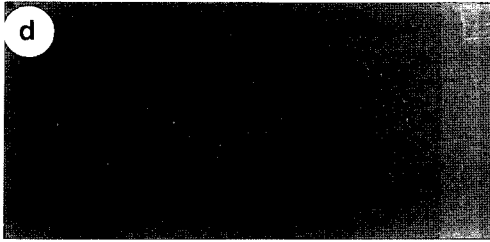
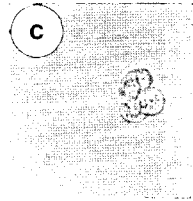
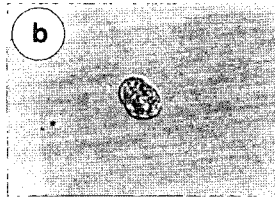
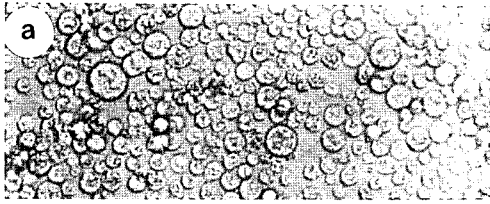
Mean ± S.E of 3 replicates, ND: not determined

Values in brackets are the average number of shoots per regenerating colony

came harder, drier and more compact than those of control cultures. After 2 weeks, when the dehydrated colonies were subcultured on the same regeneration medium semi-solidified with 0.4% agarose in the light at 27°C, plant regeneration was significantly increased compared to the control (Fig. 1g, h). Dehydrated protoplast-derived colonies regenerated at a frequency of 30.7 to 70.7%, whereas in the non-dehydrated control, only 3.1 to 30.6% of the tissues produced plants on the regeneration medium with added sucrose or maltose or a mixture of both car-

bohydrates, from 3 independent experiments (Table 2). In one square Petri dish, a plant regeneration frequency of 96% was obtained from dehydrated protoplast-derived colonies (Fig. 1f). Similarly, the average number of shoots per dehydrated colony was higher (Table 2). Thus, the results suggest that this simple dehydration treatment using a high concentration of agarose, enhances plant regeneration from protoplast-derived colonies in indica rice. This finding can be applied to plant transformation using the protoplast-to-plant system or to somatic hybridization in

Fig. 1. Protoplast culture and plant regeneration from protoplast-derived callus of the indica rice variety IR 43. a) Freshly isolated cell suspension-derived protoplasts. b,c) First and second divisions of protoplasts after 5 days of culture. d) Third division of protoplasts after 1 week of culture. e) Protoplast-derived colonies growing on the surface of a filter membrane after 5 weeks of culture. f) A square petri dish showing 96% plant regeneration frequency from dehydrated protoplast-derived colonies in MSKN medium with 1.5%(w/v) sucrose and 1.5%(w/v) maltose. g) Effect of dehydration on plant regeneration frequency in MSKN medium; Left: no dehydration treatment in MSKN medium with 3.0%(w/v) maltose; Right: dehydration treatment in MSKN medium with 3.0% (w/v) maltose. h) Effect of dehydration on plant regeneration frequency in MSKN medium; Left: no dehydration treatment in MSKN medium with 1.5% (w/v) sucrose and 1.5% (w/v) maltose; Right: dehydration treatment in MSKN medium with 1.5%(w/v) sucrose and 1.5% (w/v) maltose. i) Development of young plants from protoplast-derived callus after transfer to MSBP medium. j) Flowering plant regenerated from protoplasts after transfer to the glasshouse.



rice. Plantlets regenerated from protoplasts produced multiple shoots in MS micropropagation medium containing 2mg l⁻¹ BAP and 5%(w/v) sucrose (Fig. 1i). Individual shoots transferred to MS medium containing 1.5mg l⁻¹ NAA rooted readily and flowered with morphologically normal in the glass-house (Fig. 1j)

DISCUSSION

Embryogenic callus derived from scutella of mature seeds has been used frequently to initiate cell suspension cultures and the subsequent regeneration of plants from cell suspension-derived protoplasts in indica rice (Kyojuka et al., 1988; Lee et al., 1989; Su et al., 1992). The results presented in the present study also confirmed that mature seeds were suitable for the initiation of embryogenic callus and the establishment of fine embryogenic cell suspension cultures in indica rice variety IR43. Embryogenic cell suspension cultures have also been used as source material for the isolation of large number of totipotent protoplasts (Torrizo & Zapata, 1992; Datta et al., 1992; Jain et al., 1995). In present study, protoplast yield depended on the age of cell suspension cultures. In general, the older and finer suspensions gave a higher protoplast yield than younger suspension cultures. It might be that younger suspension cultures were normally composed of large cell clumps, consisting of irregular and elongated cells. Similar observations were reported by Abdullah et al. (1986).

Two different nurse culture systems using feeder cells have been used for protoplast culture of indica rice. Protoplasts were cul-

tured using agarose-embedding procedure (Abdullah et al., 1986) and placed in liquid protoplast culture medium containing feeder cells (Kyojuka et al., 1988; Torriza and Zapata, 1992). In the other system, protoplasts were cultured using filter membrane procedure with feeder cells (Lee et al., 1989; Su et al., 1992; Jain et al., 1995). The latter procedure was adopted for protoplast culture of the indica rice variety IR43 in present study and feeder cells were found to be essential for protoplast culture.

Carbohydrate source is one of the critical components in plant tissue culture media for successful somatic embryo initiation and plant regeneration. Sucrose has generally been used as the major carbohydrate source in the culture medium, because it is the main transport sugar in most plant species.

However, a number of reports have suggested that sucrose may not be the optimal sugar for all plant species. The replacement of sucrose with maltose (an α 1-4 linked glucose disaccharide) in shoot regeneration medium as the carbohydrate source was greatly beneficial for the induction of somatic embryogenesis and plant regeneration in alfalfa (Strickland et al., 1987), potato (Batty & Dunwell, 1989), petunia (Raquin, 1983) and wheat (Last & Brettell, 1990; Otani & Shimada, 1993; Navarro-Alvarez et al., 1994). In most cases of rice, sucrose has been used as carbohydrate source in shoot regeneration medium for the induction of somatic embryogenesis and plant regeneration. Recently, however, the plant regeneration frequency from cultured protoplasts in rice has been reported to be increased by replacing sucrose by maltose in the regeneration medium (Torrizo & Zapata, 1992; Ghosh Biswas & Zapata, 1993; Ghosh Biswas et al., 1994; Jain

et al., 1995).

In the present study, to investigate the effect of carbohydrate source on plant regeneration, protoplast-derived colonies of the indica rice variety IR43 was transferred onto plant regeneration medium (MSKN) with either sucrose or maltose, or a mixture of both, as carbohydrate source. Maltose-supplemented medium was superior for regeneration of green plants from protoplast-derived colonies compared with medium containing only sucrose. The results suggest that maltose as carbohydrate source increases somatic embryogenesis and plant regeneration from protoplast-derived colonies in rice. Similarly, Ghosh Biswas & Zapata (1993) reported that the regeneration frequency was 8 to 12-fold higher in maltose-containing R2 medium than in sucrose-containing R2 medium for protoplasts of the indica rice. Torrizo & Zapata (1992) compared maltose with other standard carbon sources for plant regeneration from protoplast-derived colonies of the indica rice with nearly 60% of the calli developing into green shoots or plants in maltose-containing medium. Maltose-containing shoot regeneration media were also successfully used for plant regeneration from protoplast-derived colonies of two commercially cultivated Indian indica rice varieties Pusa Basmati 1 and Jaya (Jain et al., 1995)

Water stress treatment using mannitol and dehydration treatment or partial dessication for reducing water content in plant cells have been reported to significantly improve either protoplast plating efficiency in bentgrass (Asano & Ugaki, 1994) or the frequency of somatic embryogenesis and plant regeneration in rice (Lai & Liu, 1986; Tsukahara & Hirosawa, 1992; Rancé et al., 1994). In order

to investigate the effect of dehydration treatment on plant regeneration frequency, in the present study, protoplast-derived colonies of the indica rice variety IR43 were dehydrated in plant regeneration medium using high concentration of agarose. plant regeneration frequency was significantly increased compared to the control. The results suggest that this simple dehydration treatment enhances plant regeneration from protoplast-derived colonies in indica rice. Recently, as same results in present study, dehydration treatment using a dry filter paper and partial desiccation has been reported to increase plant regeneration in rice (Tsukahara & Hirosawa, 1992; Rancé et al., 1994). It is not clear that how dehydration treatment improves plant regeneration. However, it may change in translatable mRNAs and induce protein modification during protein synthesis.

Indica rices are the most widely cultivated in the tropical regions of the world. In order to utilize protoplast-to-plant regeneration systems as a means for rice improvement, simple, efficient and reproducible protocols for rice protoplast culture and regeneration of indica rice varieties need to be established. Hence, this filter membrane culture system with feeder cells for protoplast culture and plant regeneration system using maltose as carbohydrate source and dehydration treatment will suit these purposes.

적 요

Indica 벼의 원형질체들로부터 효과적인 식물체 재분화 방법을 개발하였다. 이 방법은 배형성 진탕 세포 배양체로부터 나출된 원형질체들을 feeder cell들이 agarose에 embedded된 배지 표

면에 놓인 filter membrane 위에 배양하는 것이다. Feeder cell로서 *Lolium multiflorum* 세포 배양체를 사용했을 때가 *Oryza ridleyi*를 사용했을 때보다 효과적이었고, 원형질체 평탄효율은 세포 배양체 age에 따라 달랐지만 최고로 0.68% 까지 증가되었다.

Carbohydrate source로서 maltose를 사용하거나 maltose와 sucrose를 1:1로 조합했을 때가 sucrose 단독으로 사용했을 때보다 식물체 재분화율이 증가되었고, 고농도의 agarose를 이용하여 원형질체로부터 유도된 캘러스를 dehydration 시켰을 때 또한 재분화율이 괄목하게 증가되었다. 식물체 재분화율은 control 캘러스로부터 3.1~30.6% 였지만 dehydration 처리한 캘러스로부터는 30.7~70.7%까지 증가되었다. 원형질체로부터 유도된 식물체들은 형태적으로 정상이며 개화했다.

LITERATURE CITED

1. Abdullah R, Cocking E.C and Thompson J.A. 1986. Efficient plant regeneration from rice protoplasts through somatic embryogenesis. *Bio/Technol.* 4:1087-1090.
2. Asano Y and Ugaki M. 1994. Transgenic plants of *Agrostis alba* obtained by electroporation-mediated direct gene transfer into protoplasts. *Plant Cell Rep.* 13:243-246.
3. Batty N and Dunwell J. 1989. Effect of maltose on the response of potato anthers in culture. *Plant Cell Tiss. Org. Cult.* 18:221-226.
4. Chu C.C, Wang C.S, Sun C.S, Hsu C, Yin K.C, Chu C.Y and Bi F.Y. 1975. Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. *Sci. Sin.* 18:659-668.
5. Datta K, Potrykus I and Datta S.K. 1992. Efficient fertile plant regeneration from protoplasts of the Indica rice breeding line IR 72 (*Oryza sativa* L.). *Plant Cell Rep.* 11:229-233.
6. Funatsuki H, Lörz H and Lazzeri P.A. 1992. Use of feeder cells to improve barley protoplast culture and regeneration. *Plant Sci.* 85:179-187.
7. Ghosh Biswas G.C and Zapata F.J. 1993. High-frequency plant regeneration from protoplasts of indica rice (*Oryza sativa* L.) using maltose. *J. Plant Physiol.* 141:470-475.
8. _____, Burkhardt P.K, Wunn J, Klott A and I Potrykus. 1994. Fertile indica rice plants regenerated from protoplasts isolated from scutella tissue of immature embryos. *Plant Cell Rep.* 13:528-532.
9. Glaszmann J.C. 1987. Isozymes and classification of Asian rice varieties. *Theor. Appl. Genet.* 74:21-30.
10. Jain R.K, Khehra G.S, Lee S.-H, Blackhall N.W, Marchant R, Davey M.R, Power J.B, Cocking E.C and Gosal S.S. 1995. An improved procedure for plant regeneration from indica and japonica rice protoplasts. *Plant Cell Rep.* 14:515-519.
11. Kyojuka J, Otoo E and Shimamoto K. 1988. Plant regeneration from protoplasts of indica rice: genotypic differences in culture response. *Theor. Appl. Genet.* 76:887-890.
12. Lai K.-L and Liu L.-F. 1986. Further studies on the variability of plant regeneration from young embryo callus cultures of rice plants (*Oryza sativa* L.) Japan. *J. Crop Sci.* 55:41-46.
13. Last D.I and Brettell R.I.S. 1990. Embryo yield in wheat anther culture is influenced by the choice of sugar in the cul-

- ture medium. *Plant Cell Rep.* 9:14-16.
14. Lee L, Schroll R.E, Grimes H.D and Hodges T.K. 1989. Plant regeneration from indica rice (*Oryza sativa* L.) protoplasts. *Planta* 178:325-333.
 15. Linsmaier E.M and Skoog F. 1965. Organic growth factor requirements of tobacco tissue culture. *Physiol. Plant.* 18:100-127.
 16. Murashige T and Skoog F. 1962. A revised medium for rapid growth requirements of tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
 17. Navarro-Alvarez W, Baenziger P.S, Eskridge K.M, Shelton D.R, Gustafson V.D and Hugo M. 1994. Effect of sugars in wheat anther culture media. *Plant Breed.* 112:53-62.
 18. Ohira K, Ojima K and Fujiwara A. 1973. Studies on the nutrition of rice cell culture. I. A simple, defined medium for rapid growth in suspension culture. *Plant Cell Physiol.* 14:1113-1121.
 19. Otani M and Shimada T. 1993. High frequency of pollen embryo formation in *Triticum aestivum* L. on maltose containing medium. *Cereal Research Communications* 21:11-15.
 20. Prioli L.M and Söndahl. 1989. Plant regeneration and recovery of fertile plants from protoplasts of maize (*Zea mays* L.). *Bio/Technol.* 7:589-594.
 21. Rancé I.M, Tian W, Mathews H, De Kochko A, Beachy R.N and Fauquet C. 1994. Partial desiccation of mature embryo-derived calli, a simple treatment that dramatically enhances the regeneration ability of indica rice. *Plant Cell Rep.* 13:647-651.
 22. Raquin C. 1983. Utilization of different sugars as carbon sources for *in vitro* cultures of *Petunia*. *Z. Pflanzenphysiol.* 111:453-457.
 23. Rhodes C.A, Pierce D.A, Mettler I.J, Mascarenhas D and Detmer J.J. 1988. Genetically transformed maize plants from protoplasts. *Science* 240:204-207.
 24. Shillito R.D, Carswell G.K, Johnson C.M, Di Maio JJ and Harms D.T. 1989. Regeneration of fertile plants from protoplasts of elite inbred maize. *Bio/Technol.* 7: 581-587.
 25. Strickland S.G, Nichol J.W, McCall C.M and Stuart D.A. 1987. Effect of carbohydrate source on alfalfa somatic embryogenesis. *Plant Sci.* 48:113-121.
 26. Su R.-C, Rudert M.L and Hodges T.K. 1992. Fertile indica and japonica rice plants regenerated from protoplasts isolated from embryogenic haploid suspension cultures. *Plant Cell Rep.* 12: 45-49.
 27. Torrizo L.B and Zapata F.J. 1992. High efficiency plant regeneration from protoplasts of the indica cultivar IR58. *J. Exp. Bot.* 43:1633-1637.
 28. Tsukahara M and Hirose T. 1992. Simple dehydration treatment promotes plantlet regeneration of rice (*Oryza sativa* L.) callus. *Plant Cell Rep.* 11:550-553.