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Genetic Improvement of *Porphyra* through Cell Culture Techniques: Present Status and Future Prospects

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Abstract In recent years the application of non-conventional breeding techniques has greatly contributed to the genetic improvement of crop plants. The available protoplast isolation and regeneration methods in *Porphyra*, offer similar benefits in genetic improvement of the cultivated strains through somatic hybridization and cell culture techniques.

We have analyzed the intrageneric and intergeneric fusion product thalli for their biological, biochemical characteristics and tolerance levels to the *Pythium* and *Olpidiopsis* disease. The fusion product thalli were more similar to the parent *P. yezoensis* in their biological characters. While, the pigment and amino acid contents of the intrageneric fusion products revealed the hybrid nature. No apparent differences were found among the intrageneric fusion products in tolerance against *Pythium* disease. However they showed relatively high tolerance against *Olpidiopsis* disease. While, the intergeneric fusion product thalli have shown high tolerance both against *Pythium* and *Olpidiopsis* disease.

This paper also considers the present status of the somatic hybridization and cell culture techniques and envisages the future prospects of the application of these techniques for genetic improvement of *Porphyra*.

Key words: Cell selection, electrofusion, disease resistance, *Olpidiopsis*, *Porphyra*, protoplasts, *Pythium*, somatic hybridization.

The *Porphyra* farming industry in Japan has made a rapid increase in the production of 'Hosi-nori' in the recent years. Much of this has been ascribed to the increasing demand and introduction of highly productive strains through conventional breeding and cultivation techniques. To date, these improved strains were selected and have been bred by directional selection of specific lines among the progeny of the large and vigorous fronds isolated from natural populations (Miura, 1984). Successful intraspecific crosses (Miura and Shin, 1992) and improved mutant lines (Imada and Abe, 1992) were also reported through conventional breeding techniques. Plant improvement by the conventional breeding techniques is hampered by the rather limited gene pool owing to sexual incompatibility even between related species, and the time scale of most breeding programs. While, non-conventional techniques provide a novel means of rapid identification and characterization of useful traits to complement and supplement the very successful methods of conventional breeding.

In the recent years, the cell culture techniques proved useful in improvement of crop species, are being applied to seaweeds for similar benefits. Protoplasts provide an opportunity to combine novel genomes and a medium for introduction of foreign genes. They also offer an easy and less complex way for selection at the cellular level. There are more than 23 genera of seaweeds from which protoplasts have been successfully isolated. Even in *Porphyra* the techniques for successful isolation and subsequent regeneration of the isolated protoplasts were reported for large number of species (Reddy *et al.*, 1994).

The purpose of the present report is to review the progress made and discuss the future prospects for the application of these techniques to the genetic improvement of

Table 1. Present status of cell fusion and plant regeneration in *Porphyra*.

Fusion species	Results	References
P. yezoensis (×) *P. yezoensis* (green)	Callus and thallus	Fujita and Migita (1987)
P. yezoensis (×) *P. pseudolinearis* ♀	Callus and thallus	Fujita and Saito (1990)
P. yezoensis (×) *P. haitanensis*	Callus	Dai *et al.* (1993)
P. yezoensis (×) *P. tenera* (green)	Callus and thallus	Araki and Morishita (1990)
P. yezoensis (green) (×) *P. suborbiculata*	Callus and thallus	Mizukami *et al.* (1995)
P. yezoensis (×) *P. vietnamensis*	Callus and thallus	Matsumoto *et al.* (1995)
P. tenera (×) *P. suborbiculata*	Callus and thallus	Matsumoto *et al.* (1992)
P. linearis (×) *P. miniata*	Callus and thallus	Chen *et al.* (1995)
P. suborbiculata (×) *P. tenuipedalis*	Callus and thallus	Achiha and Nakashima (1995)
P. yezoensis (×) *Bangia atropurpurea*	Callus and thallus	Fujita (1993)
P. pseudolinearis (×) *B. atropurpurea*	Callus and thallus	Fujita (1993)
P. yezoensis (×) *Monostroma nitidum*	Thallus	Kito *et al.* (1996)

Porphyra. No effort is made to provide an exhaustive listing of the literature on the subject, but rather to highlight the significant developments. The application of cell culture techniques for strain improvement through somatic hybridization and cell line selection of *Porphyra* are discussed.

Somatic Hybridization

Somatic hybridization gives a chance to combine asexually two heterozygous genomes without meiotic recombination. This is very important where there is a need to pool resistance or qualities that are polygenic and inherited quantitatively. Successful somatic hybridization involves, 1. Isolation and regeneration of protoplasts, 2. Selection of somatic hybrid cells or thalli, 3. Regeneration of plants from selected somatic hybrid colonies, 4. Conformation of the somatic hybrid nature.

The first successful interspecific somatic hybridization and subsequent thallus regeneration between two color types of *Porphyra yezoensis* was reported by Fujita and Migita (1987). Since then, many attempts were made on fusion and subsequent regeneration of the fused products through intrageneric and intergeneric somatic hybridization, using available suitable protoplast methods (Table 1). Dai *et al.* (1993) demonstrated protoplast fusion between *P. yezoensis* and *P. haitanensis*. The hybrid cells divided and formed callus-like cell masses, but no blade differentiation was achieved. Chen *et al.* (1995) attempted electrofusion between *Porphyra linearis* and *P. miniata*, with the aim of obtaining a hybrid with superior taste and texture and with large frond size. The putative hybrid cells divided and produced plantlets with few cells which later died due to contamination.

Araki and Morishita (1990) reported successful intergeneric somatic hybridization and subsequent regeneration of brown, green and sectorially variegated chimeric thalli from *Porphyra yezoensis* and *P. tenera* (green). Mizukami *et al.* (1995) described hybrid-like thalli form the monospores released from the fusion product thalli of *Porphyra yezoensis* (green) and *P. suborbiculata* (wild type) protoplasts. Matsumoto *et al.* (1995) inactivated the *P. vietnamensis* protoplasts by treatment with 5 mM iodoacetamide and fused them with *P. yezoensis* protoplasts in an attempt to develop high temperature resistant somatic hybrids. Monospores released from fused product thalli were cultured, and the regenerated fusion product thalli showed good growth at 23℃. The loss of this trait in the next progeny was observed. Achiha and Nakashima (1995) described the use of the resistant nature of the *P. suborbiculata* to refrigeration (at −75℃ for 6 days) for selective elimination of regenerated sporelings and self fused products of non-resistant *P. tenuipedalis* cells from the fusion product population of *P. suborbiculata* and *P. tenuipedalis*. The presumptive hybrid thalli showed spinulate processes in thallus edge and formed monospores like *P. suborbiculata* while retaining the broad linear-lanceolate shape of *P. tenuipedalis*. Matsumoto *et al.* (1992) described the protoplast fusion and the development of

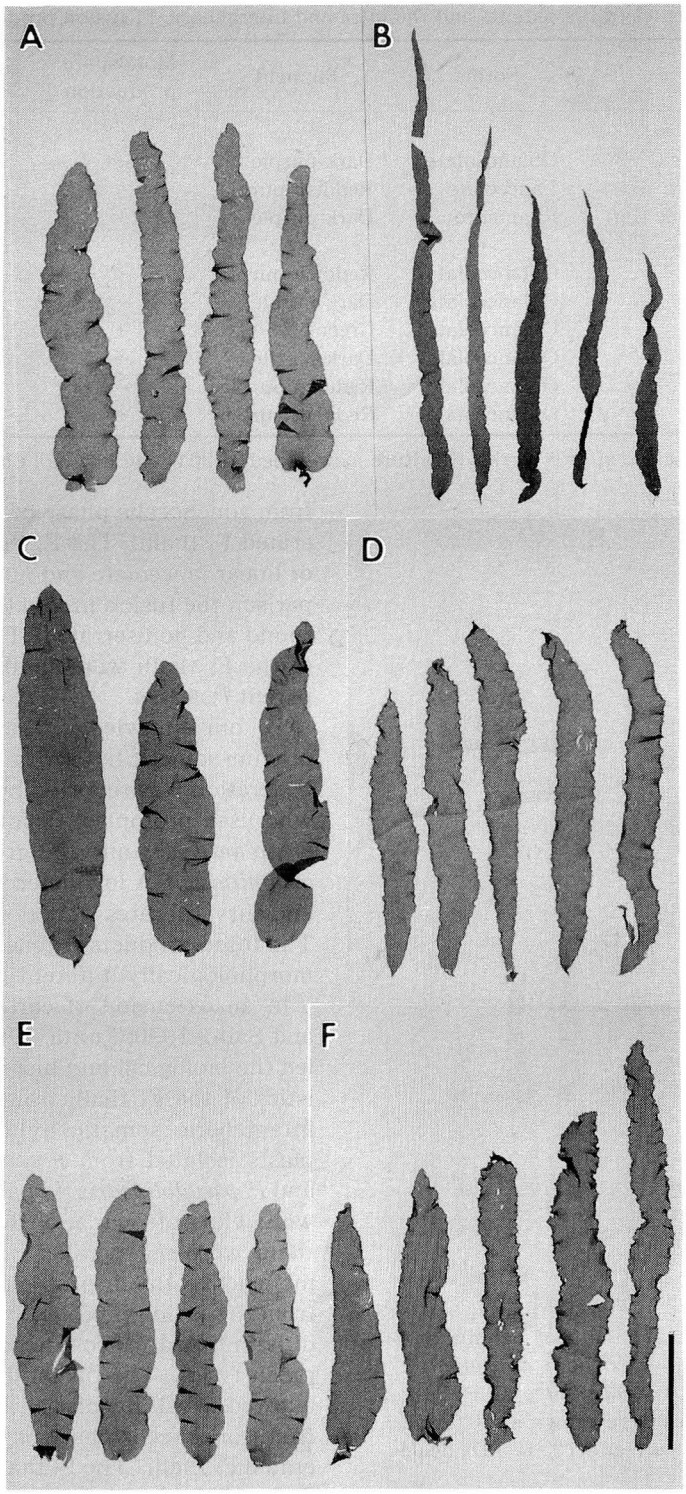

Fig. 1. Six week old thalli of parents and F_1 progeny of *P. yezoensis* and *P. pseudolinearis*. A, *P. yezoensis*; B, *P. pseudolinearis*; C, PyPp-1; D, PyPp-2; E, PyPp-2G; F, PyPp-3. (Cultures were grown at 20℃ and 80 μmol photon $m^{-2}s^{-1}$ (12 : 12 LD)), Scale bar = 2 cm.

Table 2. Characteristics of the parents and the intra and intergeneric F_1 fusion product progeny.

Species	Form	Pigment	Monospore production	Fertility	Chr. No. (n)
Parents					
Porphyra yezoensis (Py)	Oblanceolate	Dark purple	+	+	3
P. pseudolinearis ♀ (Pp)	Lanceolate	Reddish purple	−	−	4
Bangia atropurpurea (Bg)	Filamentous	Dark purple	+	+	3
Progeny					
PyPp-1	Oblanceolate	Reddish purple	+	+	3
PyPp-2	Oblanceolate	Dark purple	+	+	3
PyPp-2G	Oblanceolate	Green	+	+	3
PyPp-3	Oblanceolate	Dark purple	+	+	3
PyBg-1	Oblanceolate	Reddish purple	+	+	3
PyBg-2	Oblanceolate	Reddish purple	+	+	3

+; positive, −; negative after 6 weeks of culture maintained at 20℃ and 80 μmol photon m^{-2} s^{-1}.

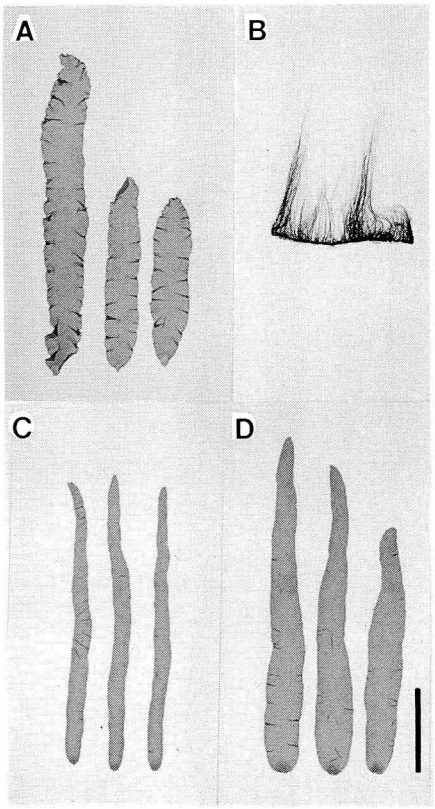

Fig. 2. Five week old thalli of parents and F_1 progeny of *P. yezoensis* and *B. atropurpurea*. A, *P. yezoensis*; B, *B. atropurpurea*; C, PyBg-1; D, PyBg-2, (Cultures were grown at 20℃ and 80 μmol photon $m^{-2}s^{-1}$ and 12 : 12 LD), Scale bar = 2cm.

F_1 thalli of *Porphyra tenera* and *P. suborbiculata*. The fusion product thalli were allowed to self fertilise, and the conchospores from conchocelis phase of these thalli regenerated F_1 thalli. The F_1 thalli were elliptical or linear lanceolate and non-serrated in comparison the fusion product thalli which were round and non-serrated. The protein profiles of the F_1 thalli were similar to those of the parent *P. tenera*.

To our knowledge, the first time, interphylum somatic hybridization and thallus regeneration was reported by Kito *et al.* (1996) who used protoplast fusion products of *Porphyra yezoensis* and the green alga, *Monostroma nitidum*. A low heterokaryon frequency and survival rates were observed in cultures. The fused products regenerated into various morphologically different thalli.

In an extension of earlier studies (Fujita and Saito, 1990; Fujita, 1993), we have studied the biological and biochemical characteristics of the F_1 thalli from intrageneric and intergeneric somatic hybridization. Protoplasts isolated from *P. yezoensis* (Fig. 1, A) and *P. pseudolinearis* (female) thalli (Fig. 1, B) were electrofused and the fusion product thalli were regenerated. For obtaining homogeneous thalli, protoplasts were isolated from the fusion product thalli and they were differentiated microscopically into wild and green types. The fusion product thalli were allowed to self fertilise and the conchospores from conchocelis phase of these thalli, regenerated F_1 thalli. The F_1 thalli PyPp-1, PyPp-2, PyPp-2G, PyPp-3 were analyzed for their biological and biochemical characteristics (Fig. 1, C–F). Non-variegated thalli with reddish purple (PyPp-1 and PyPp-2), dark purple

Table 3. Comparison photosynthetic pigment content and composition of the thalli of the parents and the intra and intergeneric F_1 fusion product progeny.

Species	Pigments* (mg per g. dry wt.)					
	Chl	Car	PE	PC	Total	PE/PC
Parent						
P. yezoensis (Py)	7.20	2.00	48.5	24.2	81.9	2.00
P. pseudolinearis ♀ (Pp)	4.60	1.10	42.5	22.7	70.9	1.87**
B. atropurpurea (Bg)	8.10	2.40	49.4	31.5	91.4	1.57**
Progeny						
PyPp-1	7.60	2.15	54.4	29.3	93.4	1.81
PyPp-2	7.18	1.70	42.1	23.7	74.6	1.78
PyPp-2G	6.80	1.70	30.3	28.1	66.9	1.08
PyPp-3	6.90	1.70	43.7	23.0	75.3	1.90
PyBg-1	7.00	1.90	46.0	23.5	78.4	1.96

* Chl: Chlorophyll, Car: Carotenoid, PE: Phycoerythrin, PC: Phycocynin.
** Laboratory cultured, all the remaining are field cultivated thalli.

(PyPp-3) or green (PyPp-2G) colored thalli were regenerated. The F_1 thalli produced monospores (unlike the parent *P. pseudolinearis*), were fertile and retained the chromosome number (n=3), similar to the parent *P. yezoensis* (Table 2).

The intergeneric somatic hybridization of *P. yezoensis* (Fig. 2, A) and *Bangia atropurpurea* (Fig. 2, B) protoplasts and subsequent regeneration of F_1 fusion products were studied. The fusion product thalli PyBg-1 and PyBg-2 (Fig. 2, C-D) were analyzed for their characteristics. They produced monospores and retained the chromosome number (n=3) and were similar in thallus shape to the parent *P. yezoensis* (Table. 2).

The F_1 fusion products were analyzed for chlorophyll, carotenoids (Strickland and Parsons, 1972), and phycocynin and phycoerythrin (Tsuchiya *et al.*, 1961) and were compared with parents (Table 3). We observed the chlorophyll-*a* and carotenoid content of PyPp-1 were different from those of their parents, while those of PyPp-2 and PyPp-3 were intermediate. The phycoerythrin/phycocyanin (PE/PC) ratios of PyPp-2 and PyPp-3 were similar to those of *P. pseudolinearis*, and the thalli appeared reddish purple. The PE/PC ratio of PyPp-3 was intermediate and the thalli were deep purple in color. A very low PE/PC ratio was observed in PyPp-2G corresponding to its green color. The pigment composition of the intergeneric fusion product PyBg-1 was similar to the parent *P. yezoensis*.

Contents of free amino acids of the F_1 progeny were analyzed (Noda *et al.*, 1975) using amino acid analyzer (Japan Electric Comp.) and compared with those of the parents. Major free amino acids such as alanine, glutamic acid and aspartic acid of PyPp-1, PyPp-2 and PyPp-3 (except for aspartic acid) were intermediate in content to those of their parents. The rest of the amino acids were similar to the parent *P. yezoensis*. While, PyPp-2G contained low amino acid contents (except for alanine and glutamic acid) in comparison to the parents. The intergeneric fusion product PyBg-1 also showed alanine, glutamic acid and aspartic acid contents intermediate between those of their parents (Table 4). Although an increase in some free amino acid content over the parents was observed, this may be ascribed to the slow growth of the F_1 thalli (data not shown).

The thallus morphology of all the fusion products were very similar to that of *P. yezoensis*, while the pigment and amino acid analysis revealed the hybrid nature of the F_1 thalli. While the pigment and amino acid content may reflect the genetic constituency, their composition and quantity may depend on the culture conditions (temperature, salinity etc.). To further confirm the hybrid/cybrid nature, the isolated nuclear and chloroplast DNA of the parents and the F_1 progeny were analyzed using random amplified polymorphic DNA (RAPD) patterns with spe-

Table 4. Amino acid analysis of the thalli of parents and the intra and intergeneric F_1 progeny (mg per gram dry weight).

Amino acid	Py	Pp	Bg	PyPp-1	PyPp-2	PyPy-2G	PyPp-3	PyBg-1
Ala	21.18	8.54	4.01	15.97	16.47	10.87	12.78	17.76
Arg	0.15	0.02	0.27	0.10	0.06	0.05	0.10	0.08
Asp	3.63	0.82	2.02	2.24	1.78	0.69	4.09	2.62
Cys	0.21	0.09	0.09	0.15	0.19	0.15	0.24	0.11
Glu	10.83	5.13	12.23	8.39	6.00	6.19	4.91	6.89
Gly	0.28	0.53	0.32	0.21	0.22	0.06	0.24	0.23
His	0.20	0.04	0.14	0.09	0.09	0.03	0.17	0.12
Ile	0.11	0.14	0.13	0.06	0.22	0.06	0.46	0.29
Leu	0.06	0.07	0.12	0.06	0.24	0.06	0.55	0.35
Lys	0.06	0.03	0.14	0.05	0.09	0.06	0.21	0.20
Met	0.03	0.02	0.03	0.02	0.01	0.04	0.03	0.03
Phe	0.09	0.05	0.15	0.05	0.11	0.15	0.30	0.74
Pro	0.44	0.12	3.14	0.28	0.29	0.19	0.35	0.18
Ser	1.55	0.44	0.62	0.99	0.52	0.09	0.70	0.28
Tyr	0.12	0.05	0.64	0.09	0.16	0.09	0.31	0.17
Thr	0.74	0.39	0.26	0.56	0.76	0.53	1.10	0.13
Val	0.23	0.23	0.25	0.17	0.35	0.14	0.63	0.41
Taurine	32.11	19.17	20.02	28.32	32.30	4.07	32.36	22.12
Ammonia	0.08	0.08	0.04	0.05	0.05	0.01	0.04	0.06

Py; *Porphyra yezoensis*, Pp; *P. pseudolinearis*, Bg; *B. atropurpurea*, PyPp-1, PyPp-2, PyPp-2G, PyPp-3, PyBg-1; F_1 progeny.

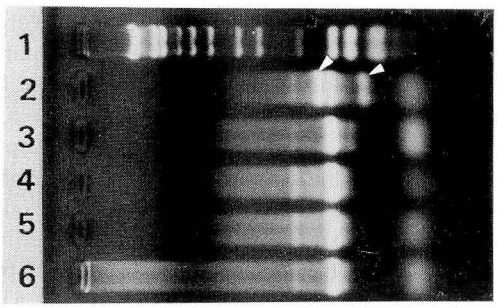

Fig. 3. RAPD banding patterns of nuclear DNA (CnCc primer) of parents and F_1 progeny of *P. yezoensis* and *P. pseudolinearis*. Lane 1, λDNA (*Sty*I)+pUC19(*Hae*III); Lane 2, *P. pseudolinearis*; Lane 3, *P. yezoensis*; Lane 4, PyPp-1; Lane 5, PyPp-2; Lane 6, PyPp-3.

cific primers (Kim, 1997). No variation was observed in RAPD banding patterns of the F_1 progeny nuclear DNA, and the banding patterns were similar to their parent, *P. yezoensis* (Fig. 3). Interestingly, the RAPD patterns of the chloroplast DNA revealed genetically heterogeneous nature of PyPp-1, PyPp-2 and PyPp-3 which differed from parental banding patterns, confirming the cybrid nature (Fig. 4) (Kim and Fujita, unpublished observations).

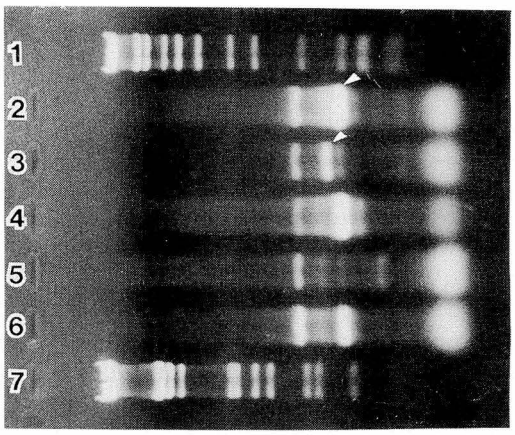

Fig. 4. RAPD banding patterns of chloroplast DNA (CnCc primer) of parents and F_1 progeny of *P. yezoensis* and *P. pseudolinearis*. Lane 1, λDNA (*Sty*I)+pUC19(*Hae*III); Lane 2, *P. pseudolinearis*; Lane 3, *P. yezoensis*; Lane 4, PyPp-1; Lane 5, PyPp-2; Lane 6, PyPp-3; Lane 7, λDNA (*Hind*III/*Eco*RI).

About thirteen diseases originating from *Porphyra* thalli have been reported (Nippon Suisan Gakkai, 1973; Fujita, 1990). The contaminated sheets lack lustre and are uneven and discolored in appearance. As these are the primary factors for fixing the quality and

Table 5. Comparison of *Pythium porphyrae* and *Olpidiopsis* sp. infection levels between thalli of the parents and F_1 fusion product progeny.

Species	Infection sites per cm^2 (relative levels)*	
	Pythium	*Olpidiopsis*
P. yezoensis (Py)	24.8 (1.00)	2.2×10^3 (1.00)
P. pseudolinearis ♀ (Pp)	9.4 (0.37)	2.3×10 (0.01)
B. atropurpurea (Bg)	+	+
PyPp-1	17.5 (0.71)	8.1×10^2 (0.39)
PyPp-2	25.4 (1.02)	3.8×10^3 (1.73)
PyPp-2G	20.8 (0.89)	2.3×10^3 (1.05)
PyPp-3	20.2 (0.83)	6.1×10^2 (0.28)
PyBg-1	9.7 (0.39)	22.0×10^2 (0.10)
PyBg-2	10.5 (0.42)	5.0×10^3 (2.27)

+; negligible, * infected thalli after 24 h (*Pythium*) and 48 h (*Olpidiopsis* sp.) in cultures at 20℃ 60 μmol photon m^{-2} s^{-1} (12 : 12 LD).

the market value (Noda and Iwata, 1983), we analyzed the F_1 fusion product thalli for their relative infection levels to *Pythium porphyrae* (red rot fungus) and *Olpidiopsis* sp. (chytrid blight fungus) with the parent *P. yezoensis* (Table 5) to look for hybrids with increased disease resistance. The zoospore suspension (1 m*l*) from the thalli infected with red rot or chytrid blight were inoculated into the conical flasks containing the thallus segments (1 cm^2) and were cultured for 28 h or 48 h, before the infection levels were calculated. The relative infection levels of the F_1 fusion products showed no marked decrease in red rot infection levels relative to the parents, but PyPp-1 and PyPp-3 showed relatively low infection levels to chytrid blight compared with the parent *P. yezoensis*. The intergeneric F_1 fusion products PyBg-1 and PyBg-2 showed very low infection levels to red rot and chytrid blight in comparison to *P. yezoensis*.

Cell Selection

The cell selection methods have been applied for generation of herbicide resistance, disease resistance, salt tolerance and amino acid overproducing crop plants (Loh, 1992). Using cell cultures, mutations can be induced and a large number of cells can be manipulated and selected using less complex selection methods. Since mutant cells can be selected and regenerated into plants, cell selection through cell and tissue cultures should give rise to commercially useful improved strains.

As the field of *Porphyra* cell culture techniques developed, similar approaches were envisioned using cell selection strategies. A review of the existing literature reveals some attempts to recover and regenerate mutant cells resistant to low salinity, higher temperatures, and with altered amino acid and amino acid analogue contents. Masuda *et al.* (1995) reported high temperature tolerance of regenerated thalli obtained through cell selection. The protoplasts selected at 24℃ showed, stable inheritance of high temperature resistance through three generations (gametophyte).

Iwabuchi (1995) developed thalli with increased growth at low salinity levels through cell selection methods. The isolated protoplasts were subjected to selection pressures at 14.4 ppt salinity and the thalli were regenerated from the selected cells. Protoplasts were reisolated from the regenerated thalli and selection was carried out at 17.2 ppt salinity. These selected cells resulted in cell lines with increased growth at lower salinity over the non-selected thalli. The selected lines were allowed to self fertilize and the conchospores from the conchocelis phase of these thalli regenerated F_1 thalli. Interestingly, the inheritance of the low salinity resistance trait was observed in these F_1 thalli.

Future Prospects and Conclusions

A review of the literature suggests that the application of cell culture techniques to *Por-*

phyra has had mixed success. Methods have been developed for successful isolation, fusion and regeneration of protoplasts and cell lines resistant to temperature stress and low salinity have been reported. Even though the techniques of protoplast isolation and culture are well established, there is a need to improve somatic hybrid cell culture techniques. An extensive review of the literature reveals the use visual identification of the somatic hybrid cells, leading to misidentification of the fusion products. Therefore it is very important to have a more effective and reliable selection system based on genetic complementation or biochemical markers.

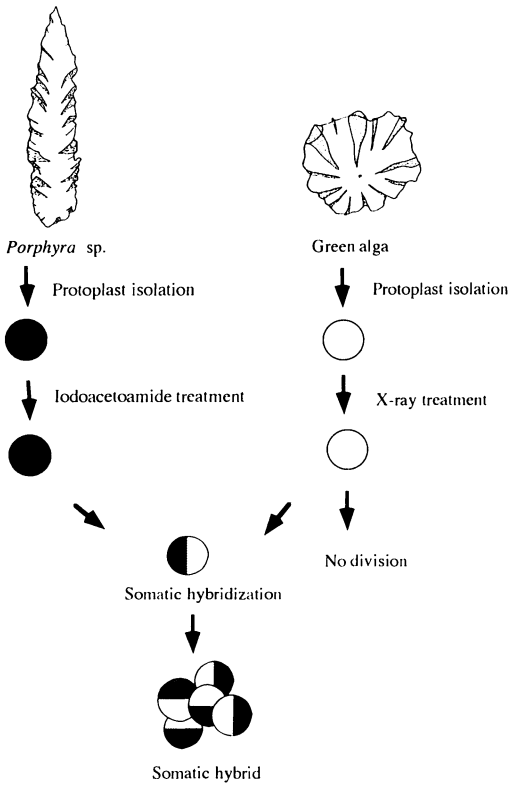

Fig. 5. A schematic representation for asymmetric hybrid production through somatic hybridization.

Kisaka *et al.* (1994) have demonstrated a technique for asymmetric hybridization between rice and tobacco through protoplast fusion. A similar approach holds great promise for transferring a few useful genes where mixing of the entire genomes is not desired. A scheme for asymmetric hybridization in *Porphyra* to generate desirable cybrids is explained in Fig. 5.

To improve disease resistance in *Porphyra*, one of the approaches is to select the parents with disease resistance and transfer the traits through somatic hybridization. Interestingly, wild populations of *Porphyra* spp. posses great variability in their infection levels to *Pythium porphyrae* and *Olpidiopsis* sp. (Table 6). Although the results we have presented are encouraging, the development of a disease resistant variety through somatic hybridization seems difficult. Rigorous field tests and quality assessment of the cultured thalli can only reveal the stability of these hybrid lines. A more detailed knowledge of the infection mechanism and the related biochemical and molecular changes will be useful in developing a variety of *Porphyra* resistant to red rot or chytrid blight.

As the induction and screening of mutants

Table 6. Comparison of *Pythium porphyrae* and *Olpidiopsis* sp. infection levels among *Porphyra* spp.

Species	Infection sites per cm^2 (relative levels)*	
	Pythium	*Olpidiopsis*
P. yezoensis	4.1×10^2 (1.00)	1.4×10^4 (1.00)
P. kinositae	1.7×10^1 (0.04)	4.1×10^3 (0.29)
P. lacerata	5.2×10^2 (1.26)	2.1×10^4 (1.50)
P. pseudolinearis	2.4×10^2 (0.58)	7.1×10 (0.01)
P. seriata	+	+
P. suborbiculata	1.7×10 (0.04)	+
P. suborbiculata f. *latifolia*	+	+
P. tenuipedalis	+	+

+; negligible, *infected thalli after 24 h (*Pythium*) and 48 h (*Olpidiopsis*) in cultures at 20℃, 60 μmol photon m^{-2} s^{-1} (12 : 12 LD).

Table 7. Amino acid composition and content of *Porphyra* spp. and *B. atropurpurea* (mg per gram dry weight).

Amino acid	Species*					
	Pyc	Pyw	Pt	Pp	Ps	Bg
Ala	12.06	15.53	8.44	8.54	5.99	4.01
Arg	0.13	0.04	0.07	0.02	0.06	0.27
Asp	2.05	1.91	1.29	0.82	0.98	2.02
Cys	0.13	0.08	0.08	0.09	0.08	0.09
Glu	11.40	5.83	4.60	5.13	5.37	12.23
Gly	0.30	tr	0.14	0.53	0.19	0.32
His	0.06	0.06	0.05	0.04	0.04	0.14
Ile	0.18	0.07	0.06	0.14	0.08	0.13
Leu	0.21	0.06	0.05	0.07	0.07	0.12
Lys	0.10	0.06	0.11	0.03	0.07	0.14
Met	0.03	0.01	0.02	0.02	0.03	0.03
Phe	0.13	0.26	0.03	0.05	0.05	0.15
Pro	0.13	0.11	0.05	0.12	0.11	3.14
Ser	0.27	0.20	0.58	0.44	0.14	0.62
Tyr	0.17	0.12	0.09	0.05	0.12	0.26
Thr	0.48	0.41	0.42	0.39	0.45	0.64
Val	0.34	0.14	0.15	0.23	0.16	0.25
Taurine	19.54	14.61	10.63	19.17	14.23	20.02
Ammonia	0.12	0.02	0.05	0.08	0.04	0.04

* Pyc: *Porphyra yezoensis* (cultivated), Pyw: *P. yezoensis* (wild), Pt: *P. tenera* (wild), Pp: *P. pseudolinearis*, Ps: *P. suborbiculata* (wild), Bg: *B. atropurpurea* (indoor cultured); tr:<0.01.

Table 8. Effects amino acid concentrations on the protoplast regeneration of *Porphyra yezoensis* (wild).

Amino acid	Relative regeneration rate (%)*		
	0.1 mM	1 mM	10 mM
Ala	88.1	89.3	90.4
Arg	81.8	112.8	53.9
Asp	93.0	94.3	81.7
Cys	30.7	0	0
Glu	119.5	120.0	92.7
Gly	65.6	97.5	130.0
His	81.3	90.8	1.8
Leu	66.6	85.8	57.9
Lys	106.5	98.2	73.7
Met	29.0	0	0
Phe	35.5	98.4	108.0
Pro	58.8	39.8	6.8
Ser	30.4	35.8	96.2
Tyr	65.0	31.3	19.3
Thr	57.9	53.6	34.1
Val	47.8	113.0	84.6
Taurine	133.9	132.8	56.7

* 63.0% regeneration on control f/2 medium is taken as 100.

at the whole thallus level of multicellular algae is difficult, totipotent single cells offer a quicker and less complex route for screening at the cellular level and thus finds an immediate application in the generation of improved strains of *Porphyra*. It would be, therefore, most desirable to intensify work on the generation of mutant cell lines that eventually may be applied in different areas of *Porphyra* breeding.

It has been assumed that the taste of the Hoshi-nori is influenced by the content of free amino acids and relative concentrations of these to inosine 5′-monophosphate and guanine 5′-monophosphate (Nisizawa and Oofusa, 1990). The wild populations show no desirable traits for amino acid composition and quantity useful for transfer through cell fusion techniques (Table 7). Alternatively, in crop plants, the cell lines resistant to antibiotics and amino acids analogues were isolated (Widholm, 1972; Harms *et al.*, 1982) and have been applied for amino acid overproducing cell lines (Hibberd *et al.* 1980). To envisage similar benefits, Yamashita and Fujita (1996) reported the preliminary re-

sults evaluating amino acid concentrations which inhibited growth of isolated protoplasts. Enhanced growth and development was observed following the addition of a wide range of amino acids at various concentrations in the culture medium, but methionine and cystine at 1 mM and 10 mM showed an inhibitory effect (Table 8). The thallus regeneration was achieved from cultured protoplasts which had been mutagenized with ethyl methane sulphonate (EMS), in the presence of inhibitory concentrations of methionine (data not shown). These results suggests the possibility of developing amino acid overproducing *Porphyra* cell lines through cell selection.

The application of genetic engineering to *Porphyra* is still in its infancy. Kubler *et al.* (1994) reported transient GUS expression in the electroporated protoplasts of *Porphyra miniata*. There is need for development of stable shuttle vector systems for successful introduction and expression of foreign genes in *Porphyra*. We feel that the future for the application of cell culture techniques for *Porphyra* improvement will be both exciting and intellectually challenging.

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細胞培養法によるアマノリ属の育種：現状と課題

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近年，栽培植物の品種改良においては，従来の育種技術とは別の育種技術の適用によって成果が挙がっている．アマノリ類ではプロトプラスト分離及びプロトプラストから再生体を得る方法がほぼ確立されており，体細胞雑種形成法及び細胞培養法による養殖品種の品種改良が可能であると考えられる．スサビノリの野生色型と緑色型のプロトプラストの融合細胞からの再生体が最初に報告され，その後，アマノリ属の種類を中心とする種間，属間及び門間の体細胞雑種形成法が報告されている．また，単離細胞は，温度，塩分あるいはアミノ酸・アミノ酸アナログなどに耐性の変異株を選抜する細胞選抜法にも有用であると考えられる．細胞選抜法によって高温耐性及び低塩分耐性の株が選抜作出されている．本論文では体細胞雑種形成法と細胞培養法によるアマノリ属の育種の現状と将来の課題について考察する．