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GAMMA - RAYS INDUCTION OF MUTATION IN CONCHOCELIS OF *PORPHYRA YEZOENSIS*

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Abstract Free-living conchocelis of *Porphyra yezoensis* Ueda (Bangiales, Rhodophyta) were treated with ^{60}Co-γ rays of different doses (ranging from 100 Gy to 1000 Gy) to induce mutation. Most of the conchocelis maintained the capability of penetrating into shells, growing and forming colonies in shells, but their vitality was seriously impaired by the irradiation of γ-rays. A few conchocelis pigments were mutagenized directly into different color pigment mutants whose progeny-conchospores and foliose thalli had the same colors. However, some irradiated conchocelis did not show the change in color at the conchocelis stage. The pigment mutation could be observed only after the conchospores of these conchocelis had germinated into young foliose thalli. Irradiation of low dose (100 Gy) promoted the growth of thallus and many with altered morphology were observed. Conchospores of the irradiated conchocelis attached to the culture nets were cultured in the sea, and growth of these progenies was observed and measured.

Key words: *Porphyra yezoensis*, conchocelis, ^{60}Co-γ ray, pigment mutant

INTRODUCTION

It is known that there are several types of pigmentation mutants with different color as compared with the wild type in some species of *Porphyra* (Aruga, 1974; Miura et al., 1974; Miura, 1985). In *P. yezoensis*, the yellow type mutant was obtained in laboratory by crossing red and green type pigmentation mutants (Miura, 1978). Later, light red , light green and light yellow type pigmentation mutants were obtained by Miura (1985). Niwa et al. (1993) isolated violet and orange type pigmentation mutants in laboratory cultures from sectorially variegated chimeric thalli. Although the pigmentation mutant can be obtained in laboratory culture and field culture, some scientists tried to induce mutants by chemical mutagensis and other means. Katayama (1983, 1984) used several chemical mutagens to induce mutants formation in *Porphyra yezoensis* and *P. tenera*. Dai et al. (1990) and Yan (1992, 1993) mutagenized protoplasts and isolated somatic cells of *P. yezoensis* by means of colchicine and ultra violet light (UV), obtaining several kinds of morphology mutants and pigment mutants. Their studies focused on the mutations of *Porphyra* gametophytes, although the specifics of the mutant were not described in their reports. Mitman and Van der Meer. (1994) obtained several pigment mutants by chemical mutagenesis with N-methyl-N'-nitro-N-nitrosoguani-

dine (NG) in young germlings of chonchospores and blades of *P. purpurea*, but not in choncho-celis. Xu et al. (1994) and Xu (1996) used NG to effectively induce mutants of different morphology and pigments in the different stages of three species of *Porphyra*. Yan and Aruga (1997) obtained 7 kinds of different color oliose thalli from *P. yezoensis* monospore germlings treated with NG. In Rhodophyta, a color phenotype can be used as a mark for specific biological events in life history after genetic analyses(Van der Meer, 1977; Van der Meer and Todd, 1977, 1980; Niwa et al., 1993; Xu, 1996). It can also be used in breeding new cultivars of *P. yezoensis* by cross-breeding (Miura and Shin, 1989; Shin, 1992). The pigmentation mutants of *P. yezoensis* are therefore interesting and attractive experimental material in various physiological and biotechnological studies. Utilization of ionizing irradiation such as γ-rays for mutagenesis in *Porphyra* has not yet been reported. It had been utilized mainly in higher plants, and was found effective for inducing mutants in them (Cheng et al., 1991; Gu et al., 1991; Zheng et al., 1991). This paper describes how *P. yezoensis* conchocelis mutants were obtained by γ-rays, a type of ionizing radiation.

MATERIALS AND METHODS

Materials

Thalli of *P. yezoensis* were collected from the sea area of Rudong, Jiangsu Province, China, then desiccated and transported to laboratory. The thalli were rinsed in seawater and brushed clean. The thalli's mature parts were cut down into small pieces and cultured with seawater in flasks until colonies of conchocelis appeared. The colonies of free-living conchocelis were picked out and cultured in a fertilized flask with PES culture medium.

Culture conditions

Free-living conchocelis were cultured at 18-20℃ with a 12L:12D photoperiod (30-50 μmol/(m^2·s)). Conchocelis in shell were cultured at 25-26℃ with a 12L:12D photoperiod (50-60 μmol/(m^2·s)) at developing stage and at 23-24℃ with a 10L:14D photoperiod (50 μmol/(m^2·s)) at conchosporangia-branch developed stage. When the conchocelis in shell had matured, they were cultured in 17-18℃ seawater agitated by a pump. After 7-8 days, they began to release conchospores.

Conchospores cleaved and germinated into sporelings at 18-20℃ with a 12L:12D photoperiod (70-80 μmol/(m^2·s)). After sporelings of the conchospores were 5 mm in length, they were cultured at 12-15℃ with a 12L:12D photoperiod (80 μmol/(m^2·s)).

Irradiation by γ-rays

Ten mL of free-living conchocelis was placed at approximate density of 0.02 g/mL (wet wt) into each of five 15 mL tubes. The conchocelis were evenly distributed by shaking them during γ-rays irradiation from a ^{60}Co source.

The irradiated conchocelis were cultured in the dark the first day and in renewed seawater everyday for the first three days. On the 4th day the conchocelis were cut into about 100-200 μm long fragments and spread at approximately 1000 fragments /cm^2 on shells.

Irradiation of a large number of conchocelis by γ-rays

About 4 g (wet wt) free-living conchocelis in 200 mL seawater in a 500 mL flask were irradiated with a dose of 500 Gy for 74 min at a distance of 0.95 m from a ^{60}Co-source. The 3rd day after irradiation the conchocelis were cut into fragments and spread at approximately 1000 fragments/cm^2 on shells in a 18 m^2 shallow pond. After 5 months, the conchocelis in shells began to release conchospores when temperature was about 18-20℃. Conchospores became attached at approximately 61 conchospores per cm rope to the culture nets. Sixty nets of conchospores were transported the next day and cultured in the sea (Plate I:6).

RESULTS AND DISCUSSION

Conchocelis irradiated by γ-rays

Free-living conchocelis irradiated by γ-rays maintained the capability of penetrating into, growing and forming colonies in shells. However, the vitality of the conchocelis was seriously impaired by γ-rays irradiation. Table 1 shows that the survival rate of conchocelis decreased as the irradiation dose increased; almost no conchocelis survived when the irradiation dose was over 1000 Gy.

Some pigment mutants of various colors were found in shells with irradiated conchocelis (Plate I:1). Although the mutation frequency of conchocelis pigments increased with increase of irradiation dose, the number of the mutant colonies did not increase due to the large decrease in survival rate of conchocelis. In this study, 100-250 Gy was found to be optimal dose for effectively inducing pigment mutants.

Table 1 Effect of ^{60}Co-γ ray irradiation on survival of conchocelis of *Porphyra yezoensis*

Group	1	2	3	4	5
Dose(Gy)	1000	500	250	100	0
Dosage(Gy/min)	32.07	16.03	8.02	4.01	0
No. of colonies	7	44	126	522	1534
No. of pigment mutants	1	4	8	10	0
Survival rate(%)	0.77	3.16	6.48	21.45	100
Mutation frequency(%)	14.29	9.09	6.35	1.90	0

Progenies of conchocelis irradiated by γ-rays

Under optimal culture conditions the irradiated conchocelis in shells released conchospores as effectively as the normal conchocelis. Most of the conchospores released by irradiated conchocelis had normal pigments, and only a few conchospores had pigments of various colors. These mutant spores germinated into thalli with the same color as the conchospores. While most normal conchospores developed into normal gametophytic thalli, a few became chimeras having both normal and mutant pigmentations (Plate I:2-4).

Tables 2 and 3 show that some offsprings of the groups irradiated with a dose of over 100 Gy underwent pigment mutations. The larger the irradiation dose, the higher was the proportion of pigment mutants. Most of the pigment mutants had only a single color (red, green, yellow or purple,

etc.), but a few were chimeras of normal and mutant thalli showing 2 or 3 different colors (Plate I: 2-4). These results were similar to those in Xu's report (1996). In the conchocelis stage, cytoplasmic mutations occurred directly within the conchocelis and were evident in both conchospores and gametophytic offsprings. Recessive nuclear mutations did not show until the germination of conchospores, when they formed a mosaic chimera of normal and mutant types.

Growth of progenies of conchocelis irradiated by γ-rays

The growth of conchocelis progeny was also affected by γ-rays irradiation. Table 4 shows that the growth of gametophytic thalli derived from irradiated conchocelis is limited and inhibited by a dose of 500 Gy or more. The average lengths and widths of thalli of gametophytic progenies after irradiation by 250 Gy and 100 Gy doses were greater than those in the untreated groups. The ratio of length to width in the 100 Gy group was the highest.

Table 2 Expression of pigment mutation in haploid gametophytic progenies from conchocelis treated with γ-rays I

Dose (Gy)	1000	500	250	100	0
Dose/min(Gy/min)	32.07	16.03	8.02	4.01	0
Total color blocks	–[a)]	168	166	165	150
Blocks of mutant cells	–[a)]	51	38	31	0
Blocks of wild type cells	–[a)]	117	128	134	150
Frequency of mutant in thalli	–[a)]	0.3036	0.2289	0.1879	0

a): 1000 Gy group released too few conchospores to be counted

Table 3 Expression of pigment mutation in haploid gametophytic progenies from conchocelis treated with γ-rays II

Dose (Gy)	1000	500	250	100	0
Dose/min (Gy/min)	32.07	16.03	8.02	4.01	0
Tests	150	150	150	150	150
Wild type	—[a)]	106	113	126	150
Single color mutants	—[a)]	26	22	15	0
2 chimera blocks	—[a)]	17	14	9	0
3 chimera blocks	—[a)]	1	1	0	0

a): 1000 Gy group did not release conchospores or released too few to be counted.

Table 4 Effect of irradiation on growth of progenies of conchocelis treated with γ-rays

Dose (Gy)	500	250	100	0
Dose/min (Gy/min)	16.03	8.02	4.01	0
Average length of thalli(μm)	122.9	151.5	167.3	140.4
Average width of thalli(μm)	26.6	30.2	30.8	29.6
Length/width	4.63	5.02	5.43	4.83

Results of irradiation of a large amount of conchocelis

Colonies formed by several kinds of pigment mutants were observed in shells on which irradiated conchocelis grew. The frequency of pigment mutations was about 9.46%. When the temperature was lower than 20℃ in autumn, mature conchocelis in shells began to release conchospores, some of which were pigment mutants. The conchospores attached to culture nets were grown in the sea (Plate I:6).

Because young thalli released large numbers of monospores during the first 2 months, the quantity of young thalli on culture nets increased significantly. After 50 days culture, the number of

young thalli on nets was three times greater than that after 20 days culture, but the number and proportion of pigment mutants decreased gradually (Table 5). The length and width of most pigment mutants cultured in the sea were less than those of normal individuals (Plate I:5), because most pigment mutants grew more slowly and released fewer monospores than normal individuals. There were still a few pigment mutants growing on the culture nets after 3 months.

Table 5 Growth of gametophytic thalli derived from γ-ray irradiated conchocelis cultured in the sea

Culture time (d)	No. of thalli on net[a)]	No. of pigment mutants	Occurrence frequency of pigment mutants
20	263	107	0.4068
35	508	57	0.1122
50	811	54	0.0666

a): No. of thalli including pigment mutants per cm strand of culture net.

CONCLUSION

Based on the experimental results mentioned above, it can be deduced that γ-rays irradiation remarkably affects the growth and mutation rate of conchocelis of *P. yezoensis* and its progeny.

After γ-rays irradiation, some conchocelis showed pigment mutants with various colors (Plate I:1). The progeny-conchospores and gametophytic thalli derived from these pigment mutants showed identical colors. However, some irradiated conchocelis did not show the change in color at the conchocelis stage. The mutation in pigments could be observed only after conchospores of these conchocelis had germinated into young gametophytic thalli.

Irradiation of low doses could promote the growth of thallus of irradiated conchocelis. Many individuals having altered morphology were observed. Further studies could be undertaken to investigate whether the diversity has a genetic basis.

When cultured in the sea (Plate I:6), most of the gametophytes with pigment mutants grew slowly and released few monospores. After a period of culture, the normal thalli grew vigorously, while the occurrence frequency of pigment mutants gradually decreased until only a few pigment mutants remained which grew very fast.

In summary, γ-ray irradiation can effectively induce pigment mutants in the conchocelis of *P. yezoensis*. With the further development of individual screening technology, γ-ray irradiation will be utilized as a new breeding method.

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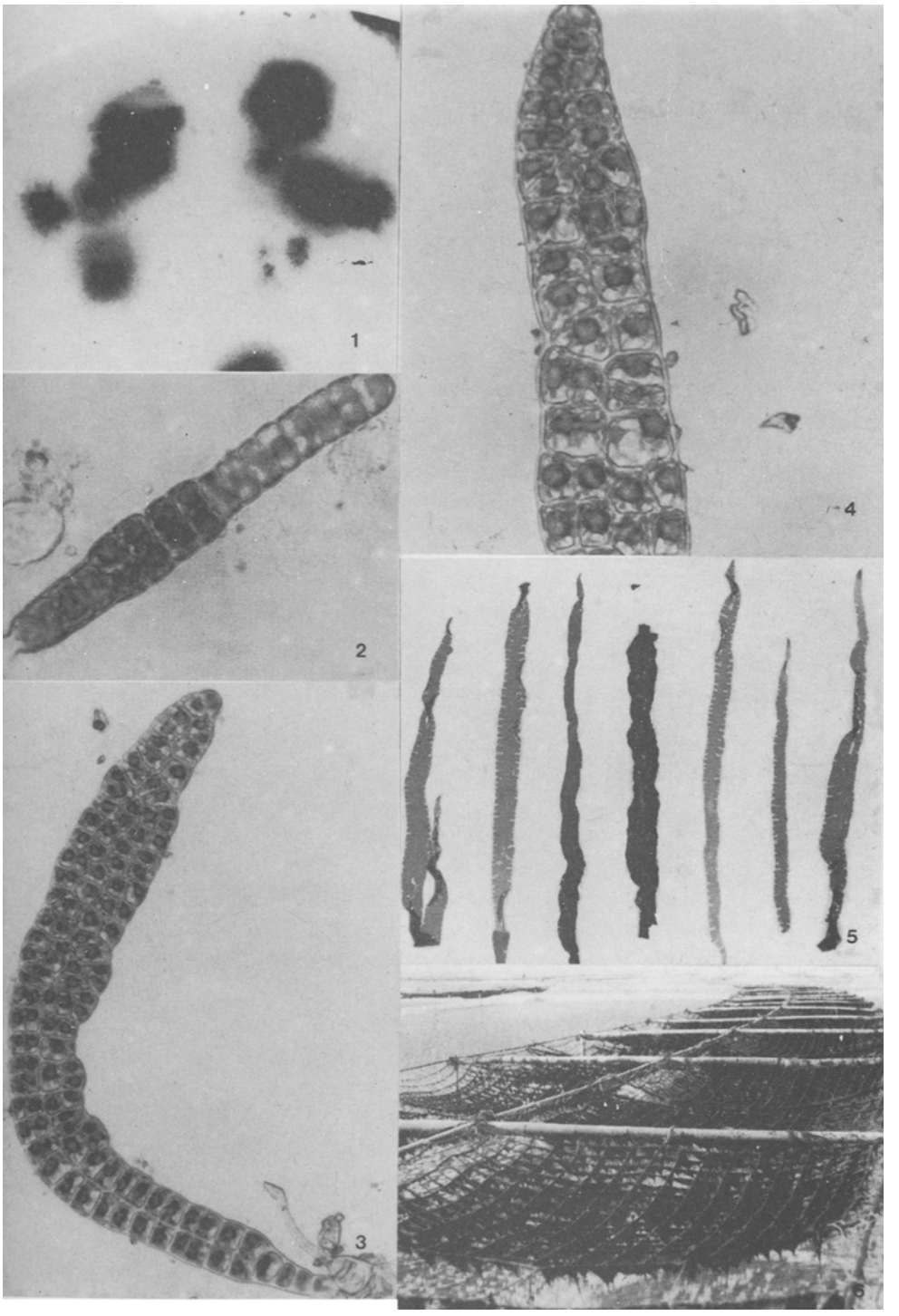

1.Pigment mutants of conchocelis colonies in shell. 2-4. Mosaic chimera of wild-type and mutants of gametophytic thallus derived from conchocelis irradiated by γ-rays. 5. Pigment mutants of various colors .6.Gametophytic sporeling from γ-ray irradiated conchocelis cultured in the sea.