Short Communication

Variations in Visible Characters of Diploid-like Plants Derived from Gamma-irradiated Tetraploids in Rice (*Oryza sativa* L.)

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Populations of artificial autotetraploids of rice (*Oryza sativa* L. cvs. Nipponbare and Fukunishiki) were repeatedly irradiated with gamma-rays through several generations. Plants which did not differ in appearance from the original diploid plants occurred occasionally in the populations. These plants were designated as diploid-like plants. Eight diploid-like plants were obtained so far, and their generations were advanced without irradiation in order to examine the mode of segregation of visible characters in their progeny. The results indicated that plants showing several kinds of mutant characters could be obtained and were fully fertile. Usefulness of the tetraploid-irradiation technique for developing fertile rice plants which exhibit gross phenotypic changes is discussed.

KEY WORDS : *Oryza sativa* L., chromosome rearrangement, diploid-like plant, Gamma-rays, tetraploid.

Introduction

Research on the induction of mutations has mainly dealt with the induction of gene mutations (micromutations) for many years. With the advances of molecular genetics and DNA techniques, however, a more fundamental reorganization of genes, chromosomes and genomes (macromutations) has become the center of interest.

In the present experiments, artificial autotetraploids of rice plants were exposed to gamma-rays in order to obtain fully fertile rice plants exhibiting gross phenotypic changes induced through the reorganization of reduplicated chromosomes after irradiation.

Materials and Methods

Tetraploids

Seeds of two rice cultivars, Fukunishiki and Nipponbare, were treated with a 0.05 percent solution of colchicine for 24 hours on the 3rd day after the onset of soaking and were washed in running water overnight. About 10 percent of the seeds germinated normally and were raised to maturity in a paddy field. Tetraploids of Fukunishiki and Nipponbare were developed in 1978 and 1981, respectively.

Irradiation

The recurrent chronic irradiation of tetraploid rice (Fukunishiki) was started in 1979. Forty-day-old seedlings were transplanted to pots and three weeks later the pots were transferred to the Gamma Field (Institute of Radiation Breeding, NIAR, Japan). The plants were grown in the Gamma Field for two months. During this period, the plants developed from the ear primordium formation stage to the ripening stage. Doses ranging

from 210 R/day to 45 R/day were applied. Since gamma-rays induced marked decrease in the seed set, the seeds which survived were grown to maturity without irradiation in the next year to obtain enough seeds to continue the repeated irradiation experiments. Then the autotetraploid plants were divided into two groups, and gamma-rays were applied each year to either of the two groups.

The recurrent acute irradiation of tetraploid plants (Nipponbare) in their successive generations was started in 1982. Forty-day-old seedlings were exposed to 0.25, 0.5, 1.0 and 2.0 kR of ^{137}Cs gamma-rays (Cesium 137 Gamma-ray Irradiation Facilities, Research Center for Nuclear Science and Technology, The University of Tokyo). All the doses were applied within two minutes. The seedlings were transplanted in a paddy field and grew to maturity.

About 300 plants of tetraploid Fukunishiki and about 800 plants of tetraploid Nipponbare were exposed to gamma-rays every year. Each panicle which bore fertile seeds was harvested in the autumn and about 5 seeds per panicle were sown in the next spring.

Results and Discussion

The tetraploid plants showed deep green and stiff leaves. They were shorter than the diploid plants. They had large and awned spikelets and the number of spikelets per panicle was lower than that of the diploids. The mean panicle fertility was about 30 percent, ranging from 0 to about 70 percent. Attempts at amphidiploidizing the autotetraploid rice plants by gamma-irradiation were not successful.

Fully fertile individuals occurred occasionally in the irradiated tetraploid populations. They appeared like the original diploid plants without exception and two diploid-like plants and six diploid-like plants were obtained in Fukunishiki and Nipponbare, respectively. Their generations were advanced without gamma-irradiation and the segregation of visible characters in the progeny was examined. The mutant characters observed in the segregants of the diploid-like plants are listed in Tables 1 and 2. The results indicated that the diploid-like plants with multiple mutant characters could be obtained and that dominant characters, *i.e.* awned spikelet and colored coleoptile, apiculus, stigma and so on, were included in the mutant characters. As mentioned above, tetraploids have awned spikelets unlike the diploids. This phenomenon is considered to be a manifestation of gene dosage effect. The diploid-like plants which have awned spikelets seem to inherit the character, namely the partially duplicated chromosome carrying the genes of the character, from gamma-irradiated tetraploids. Since another dominant mutation (coloration) was induced, the possibility that the gene expression mechanisms had changed could not be excluded.

It is considered that the decrease of nuclear or chromosomal DNA amounts which is brought about in an evolutionary process as well as the increment of DNA amounts (REES and JONES 1967, JONES and BROWN 1976), must play an important role in the domestication of the genus *Oryza* and *Glycine* (NAGATO and YAMAMOTO 1981, YAMAMOTO and NAGATO 1984). KING and WILSON (1975) and WILSON *et al.* (1975) suggested that the evolution of organisms largely depends on the alteration of control mechanisms of gene expression induced through the rearrangement of the chromosome organization. JONES and BROWN (1976) reported that both chromosome rearrangement and DNA deletion had occurred in *Crepis* evolution.

Table 1. Mutant characters of diploid-like plants derived from gamma-irradiated tetraploids of Fukunishiki¹⁾.

Plant	Year ²⁾	Exposure dose ³⁾	Mutant characters	Segregants in progeny
1	1984	10 kR (ca 80 R/day)	Plant height Fertility	shorter than the diploids range from zero to normal
2	1985	25 kR (ca 120 R/day)	Plant height Panicle length Spikelet Grain Fertility Heading date	tall, medium (slender type) to short (dwarf) long to short panicles (slender and dwarf type) colored apiculus and sterile glume awned spikelet glutinous endosperm, narrow grain (slender type), small grain (dwarf type) normal to sterile plants (slender type) normal to about 30 days late heading plants

¹⁾ The irradiation was started in 1979.²⁾ Year when the diploid-like plants were observed.³⁾ Accumulated doses to which the diploid-like plants had been exposed before they were obtained.Table 2. Mutant characters of diploid-like plants derived from gamma-irradiated tetraploids of Nipponbare¹⁾.

Plant	Exposure dose (kR)	Year ²⁾	Mutant characters	Segregants in progeny
1	0.25	1985	none	
2	0.25	1985	Coloration Spikelet Grain Leaf Fertility Heading date	purple apiculus sterile glume stigma internode leaf apex leaf sheath coleoptile awned spikelet stigma exsertion glabrous hull (leaf) narrow grain glabrous leaf (hull) ranges from normal to about 10 percent normal to late-heading plants
3	0.5	1986	Plant height Leaf Chlorophyll	taller than the diploids long flag leaf albino
4	1.0	1985	none	
5	2.0	1987	no test	(The progeny shall be tested in 1988)
6	2.0	1987	no test	(The progeny shall be tested in 1988)

¹⁾ The irradiation was started in 1982.²⁾ Year when the diploid-like plants were observed.

Based on these considerations, it can be anticipated that when artificially reduplicated chromosomes of the tetraploids were irradiated, they were restructured through the induction of chromosomal aberrations, such as translocation, inversion and deletion and they also decreased in number due to the disappearance of the damaged chromosomes. A considerable rearrangement of the chromosome organization can be undertaken without loss of viability, because the artificial tetraploids exhibit genetic redundancy. Consequently, the segregants of the diploid-like plants obtained may have a chromosome constitution which differs from that of the original diploid plants and display gross phenotypic changes caused by the alteration of gene expression mechanisms and/or the formation of new genes. If this assumption is valid, the tetraploid-irradiation technique may become a useful means for inducing gross changes (macromutations). The chromosome constitution of all the diploid-like plants should be analysed before conclusions can be reached.

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イネの人為同質四倍体にガンマ線照射して得られた二倍体様植物体の形質の変異

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コルヒチン処理によって得られたイネ(フクニシキ, 日本晴)の同質四倍体にガンマ線を照射したところ, 外見的に二倍体と考えられる植物体が出現した. この植物体を二倍体様植物体と呼ぶことにした. その後代を無照射で栽培し, 形質の変異を調査した. これまでに得られた8個体の二倍体様植物体には, 二倍体のフクニシキおよび日本晴と形態的に見分けのつかぬものから, 多くの変異形質を持つものまで種々の変異型のあることが分かった. また変異の中には優性方向のもの(有芒, 着色)もあり, これらは遺伝子の発現制御機構の変化や部分的に重複した遺伝子の遺伝子量効果によって生じたのではないかと考えた.

種の形成が遺伝子突然変異の集積ではなく, 染色体構造の変化に伴う遺伝子発現機構の再組織化を通して行われたのではないかという意見が進化学の分野において提出されている. この考えに基づけば, 人為的に染色体を倍加した植物体に放射線を照射することにより, 転移, 欠失, 逆位などの染色体異常を誘発し, 遺伝的に冗長性を持つ染色体を再構成することを通して多くの変異を持ちながらなおかつ正常の稔性を持つ植物体を得ることが期待される. もし, このことが現実となれば, 変異の幅をひろげる良い手段となるだろう. 本実験によって得られた二倍体様イネがこのような植物体に相当するかどうか, 染色体の観察によって確認しなければならない.