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Effect of Irradiation Conditions on Mutation Rate Induced by Gamma-Ray Irradiation in Foxtail Millet (*Setaria italica* (L.) P. Beauv.)

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Introduction

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is a highly self-pollinated diploid grass of Eurasia that shows a remarkable morphological diversity[5, 10, 18]. It is an old staple cereal in Eurasia, and is considered to have been the main cereal in the early period of agriculture. It is now cultivated sporadically on a small scale in scattered local areas throughout Eurasia as a relict crop[6]. The situation is similar in Japan. Foxtail millet was once grown throughout Japan, especially in the southern Kyushu region, however, the area of land devoted to its cultivation decreased rapidly in 1950's. No official nationwide survey on this matter has been performed since 1970 due to the decline in the growing area and economic value of foxtail millet.

Millets, including foxtail millet, have some advantages over major crops such as rice, wheat and maize: (1) Millets can be grown well under poor conditions such as sterile land and dry weather. (2) They produce a relatively stable yield irrespective of growth conditions. (3) Grains can be stored for a longer time for future food supply[13]. Recently, most cereals, including foxtail millet, have been shown to have similar genomes in terms of gene content and gene order, irrespective of chromosome number and genome size[1, 3, 4]. This means that genetic information on one cereal can be applied to another with ease and that genes conferring such advantages of millets may be exploited in major crops. Artificial hybridization of foxtail millet was difficult because the floret was too small to make emasculation and pollination. An efficient artificial hybridization technique, however, has been developed recently[17].

These facts combined make foxtail millet a suitable cereal for genetic and molecular studies. The knowledge obtained in the study of foxtail millet can be easily transferred to major crops such as rice (*Oryza sativa* L.) and maize (*Zea mays* L.) and *vice versa*, which will eventually lead to stable food supply in the world. The majority of terrestrial plants, including many important grasses such as rice and wheat, are classified as C_3 plants that assimilate atmospheric CO_2 directly through the C_3 photosynthetic pathway while C_4 plants such as maize and sugar cane (*Saccharum officinarum* L.) evolved from C_3 plants, acquiring the C_4 photosynthetic pathway to achieve high photosynthetic performance and high water- and nitrogen-use efficiencies[11]. Foxtail millet is classified as a C_4 plant. Many C_4 type grasses such as maize, sugar cane and sorghum (*Sorghum bicolor* Moench) have a tall stature and long growth period, inadequate for laboratory studies, while some foxtail millet lines have a very small stature with very short growth period. These traits may boost this millet to the status of 'model C_4 grass plant' as rice is the model C_3 grass plant.

Kagoshima University has been collecting foxtail millet from around the world since 1951. To date, over 600 strains have been collected. We have evaluated many agronomic characters of the strains, and found that they are diversified according to the seed collecting areas[7, 8, 9, 15, 16]. However, the genetic bases of these characters have been little known. Recently, we have started genetic studies of agronomic characters and artificial mutants, both of which have been studied little in foxtail millet. Many mutants have been induced by gamma-ray irradiation in major crops. In the present paper, effects of irradiation conditions on mutation rate induced by gamma-ray irradiation in foxtail millet are reported.

Materials and Methods

Plant materials

Out of our collection of strains, Nagasaki 253 (N253) was selected for use in this study. N253 was collected in Nagasaki Prefecture, Japan, in 1965. Some of its agronomic traits are as follows: vigorous early growth, short stature with one long panicle and anthocyanin pigmentation at the base.

Gamma-ray irradiation

The seeds just harvested in the fall of 1998 were poured into small glass bottles. Each bottle contained 100 seeds. Then, they were sent to the Tokai Research Establishment of the Japan Atomic Energy Research Institute (JAERI), located in Ibaraki Prefecture, Japan. One of two treatments was applied to the seeds two days before gamma-ray irradiation. One involved soaking the seeds in their bottles in tap water at room temperature (*wet condition*). The other involved keeping the seeds dry inside of their bottles until sowing date (*dry condition*). Then, the seeds were irradiated with gamma-rays. The gamma-ray irradiation was carried out using the JAERI (Tokai) CO-60 facility supported by the Inter-University Program for the Joint Use of JAERI Facilities. Doses of gamma-ray irradiation were 0Gy (Control), 25Gy, 50Gy and 100Gy. In total, eight kinds of irradiation conditions were tested. Two thousand seeds were examined under each condition.

Growth of M_1 generation and estimation of sensitivity to gamma-ray irradiation

The day after the irradiation, 300 randomly selected germinated seeds (M_1 generation) of the *wet condition* group for each gamma-ray dose were sown in field soil in pots 15cm in radius and 18cm in depth. Fifteen seeds were sown in each pot. The soil had been sterilized by microwave treatment (high output for 30 minutes) using a microwave oven (NE-1021 type: National, Osaka, Japan) to avoid contamination of the soil with seeds of other foxtail millet cultivars or weeds. About one month after irradiation, the seeds of the *dry condition* group were soaked in tap-water for three days. Then, 300 randomly selected germinated seeds for each gamma-ray dose were sown in the same manner as those of the *wet condition* group. Seedlings were grown in a greenhouse on the Experimental Farm of the Faculty of Agriculture, Kagoshima University, Kagoshima, Japan (31° 30' N). The air temperature in the greenhouse was kept between 20 and 25 ℃. Thirty days after sowing, HYPONex (mixed fertilizer; HYPONex, Osaka, Japan) solution (0.05 %) was supplied at a rate of 50 ml per pot every five days as an additional fertilizer. Heading date (days to heading), panicle length and culm length were examined for each plant. Culm length was expressed as the length from the base to the highest node of the main culm. Panicle length was expressed as the length from the top of the panicle to the highest node of the main culm. Each panicle was harvested two months after heading date. One side of each panicle was threshed and seed set was examined. The other side was used for the estimation of mutation rate in the M_2 generation. Sensitivity to gamma-ray irradiation was estimated by the difference in the values of the examined characters between control and treatment plots.

Estimation of mutation rate in the M_2 generation

The seeds on the panicles of the M_1 generation were sown in the soil sterilized as described above. Two weeks after sowing, leaf color was examined to check for the occurrence of chloroplast mutants. In the present study, chloroplast mutants were classified into albino (white leaves), xantha (yellow leaves) and viridis (yellow green to pale green leaves) (Fig. 1), according to the classification by Walles[19].

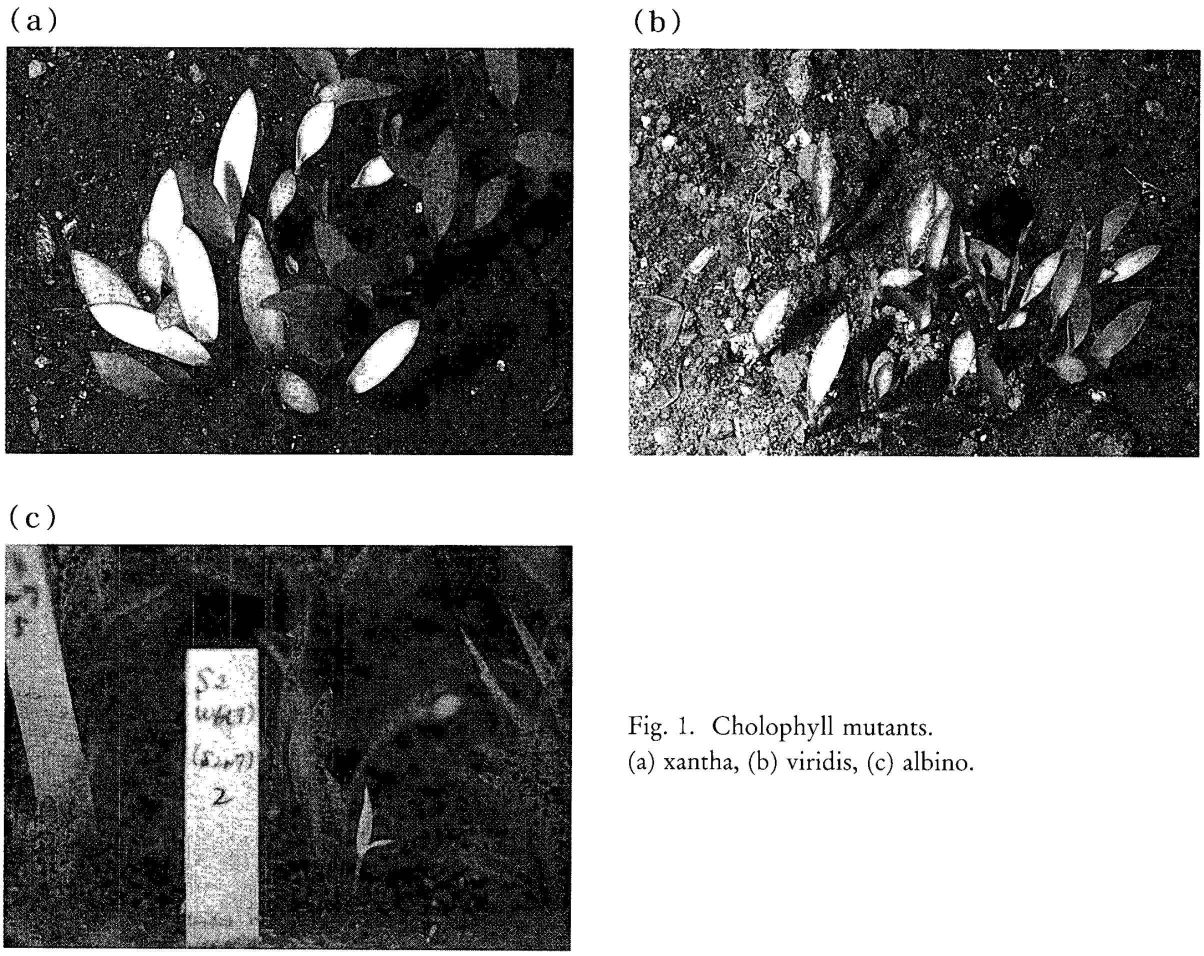

Fig. 1. Cholophyll mutants.
(a) xantha, (b) viridis, (c) albino.

Results and Discussion

Sensitivity to gamma-ray irradiation

The number of plants examined, mean days to heading, panicle length and culm length for the M_1 generation by group (*wet or dry condition*) and by irradiation dose are shown in Table 1. About one third of plants of each plot died before heading. Most of them died just after sowing, probably because coleoptiles and roots were elongated by pregermination treatment and seedlings could not take root. There seems no definite relationship between the number of plants examined and irradiation treatments. There seemed to be a tendency for the number of days to heading to increase, and for values related to other characters to decrease for both the *wet* and *dry condition* groups according to increases in the gamma-ray dosage. However, no definite effects related to gamma-ray irradiation dosage or irradiation conditions on plant growth were observed. According to the review by Fujii[2], wet condition plant seeds in general show higher radiosensitivity than dry condition seeds. Our result suggests that in the case of foxtail millet, tolerance to gamma-ray irradiation under the wet condition is comparable to that under the dry condition.

Effect of irradiation conditions on mutation rate

Mutation rates estimated by the frequency of chloroplast mutants are shown in Table 2. There seems to be a slight tendency for the frequency of mutants to increase according to the elevation of the gamma-ray dose. Mutants occurred twice as frequently in the *wet condition* group as in the *dry condition* group. Yamashita[21] compared the mutagenic effects between irradiation on dry seeds and

Table 1. Effects of gamma-ray irradiation on the growth of the M_1 generation

Irradiation condition	Dose (Gy)	No. of M_1 plants that survived	Panicle length (cm)	Culm length (cm)	Days to heading (day)	Seed set (%)
Wet						
	0	222	3.11±0.86[1]	21.40±7.28	57.8±6.8	57.8±17.5
	25	196	3.00±1.03	19.70±8.26	59.8±9.2	52.7±18.2
	50	230	2.51±0.97	17.54±8.22	60.7±7.7	56.7±17.3
	100	186	2.60±0.96	17.56±8.49	61.3±7.8	47.1±19.8
Dry						
	0	199	2.93±0.92	19.23±6.54	57.7±7.4	48.8±21.3
	25	217	2.99±1.04	16.51±6.21	55.7±8.5	43.8±19.2
	50	200	2.89±1.03	18.45±6.67	56.4±7.8	43.8±17.9
	100	251	2.84±0.76	15.24±5.08	63.5±7.6	42.9±19.6

1) Mean±standard deviation

Table 2. Chlorophyll mutation induced by gamma-ray irradiation in the M_2 generation

Irradiation condition	Dose (Gy)	No. of M_1 plants	No. of M_2 lines in which chlorophyll mutation occurred	Mutation rate (%)	Chlorophyll mutation		
					xantha	viridis	albino
Wet							
	25	239	8	3.35	5	3	
	50	260	8	3.08	2	4	2
	100	222	12	5.41	7	5	
	Total	721	28	3.88	14	12	2
	0 (Control)	245	1	0.41	1		
Dry							
	25	160	2	1.25	2		
	50	161	3	1.86	3		
	100	206	5	2.43	3		2
	Total	527	10	1.90	8		2
	0 (Control)	155	0	0.00			

that on growing plants, using many crops such as foxtail millet, rice and tabacco (*Nicotiana tabaccum*), though he made no detailed description of irradiation methods. According to Yamashita[21], the gamma-ray irradiation on growing plants induced about three times as many chloroplast mutations as that on dry seeds did. Our result was consistent with Yamashita's[21], which indicated that mutants were obtained effectively by irradiation on germinated seeds. According to the review by Sato[14], among the chloroplast mutations in rice, the albino trait is induced five to ten times as frequently as the xantha trait by a chemical mutagen, N-methyl-nitorosourea (MNU). By contrast, our experimental results with foxtail millet showed that the xantha trait was induced four to seven times as frequently as the albino trait. The difference in frequency is thought to be due to the difference in the relevant gene loci rather than in the mutation frequency per gene locus[14]. Such logic can be applied to mutation induced by gamma-ray irradiation. If so, our results suggest that there may be a large difference between foxtail millet and rice in the number and type of genes involved in chloroplast formation. Considering the genome conservation in the grass family, our result has many implications.

Frequency of mutants in the M_2 generation

The frequency of chlorophyll mutants in the M_2 generation derived from M_1 panicles is shown separately in Table 3. No chlorophyll mutation appeared in the M_1 generation, indicating that the genes conferring chlorophyll mutation are all recessive. Most chlorophyll mutations in rice are due to monogenic recessive inheritance[14]. If this held true in foxtail millet, one fourth of plants derived from an M_1 panicle would be expected to show chlorophyll mutation. However, the frequency of chlorophyll mutants was less than 25 % in most cases. This result suggests that the panicle of foxtail millet is derived from plural initial cells, as indicated in rice by Osone[12]. In some cases, the frequency of chlorophyll mutants was close to 25% (W50X1, W50X2 etc.). This can be explained by the assumption that only a mutated initial cell survived during the early development while the other cells died. Yamamoto et al.[20] observed the size and pattern of the mutated sector of M_1 panicles after gamma-ray irradiation or ethylene imine (EI), a chemical mutagen, treatment. In most cases, chlorophyll mutants were scattered on the M_1 panicles, suggesting that the panicle is derived from one initial cell, though the details of mutation treatments and frequency of chlorophyll mutants were not mentioned. Our assumption made before the experiment was that the frequency of chlorophyll mutants would be less for the *wet condition* group than for the *dry condition* group; the *wet condition* seeds start cell division and germination before the irradiation so that the number of target cells of wet (germinated) seeds may be greater than that of dry seeds. However, our experimental results showed no clear difference in frequency of chlorophyll mutants between the two conditions.

Summary

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is a highly self -pollinated diploid grass of Eurasia, which shows a remarkable morphological diversity. Recent studies suggest that foxtail millet can be a suitable cereal for genetic and molecular studies. Mutants provide good information on the genetics of morphological diversity. In the present paper, effects of irradiation conditions on mutation rate induced by gamma-ray irradiation in foxtail millet are reported. Seeds were irradiated with gamma-rays under dry and wet conditions. Then the growth of M_1 plants, and the frequency and kind of chlorophyll mutants in the M_2 generation were examined. Difference in irradiation conditions influenced the growth of M_1 plants little, but it greatly influenced the rate of chlorophyll mutation.

Table 3. Ratio of chlorophyll mutants in M_2 lines induced by gamma-ray irradiation

Irradiation condition	Dose (Gy)	M_2 line	Kind of mutation	No . of mutants	No. of normal plants	Ratio of mutants (%)
Wet						
	0	W0X1	xantha	1	22	4.55
	25	W25X1	xantha	1	85	1.18
		W25X2	xantha	5	12	41.67
		W25X3	xantha	2	20	10.00
		W25X4	xantha	2	15	13.33
		W25X5	xantha	6	52	11.54
		W25V1	viridis	1	28	3.57
		W25V2	viridis	1	15	6.67
		W25V3	viridis	4	38	10.53
	50	W50X1	xantha	22	85	25.88
		W50X2	xantha	6	19	31.58
		W50A1	albino	5	11	45.45
		W50A2	albino	3	38	7.89
		W50V1	viridis	1	25	4.00
		W50V2	viridis	7	71	9.86
		W50V3	viridis	1	12	8.33
		W50V4	viridis	4	29	13.79
	100	W100X1	xantha	13	84	15.48
		W100X2	xantha	3	64	4.69
		W100X3	xantha	2	14	14.29
		W100X4	xantha	3	18	16.67
		W100X5	xantha	2	16	12.50
		W100X6	xantha	3	29	10.34
		W100X7	xantha	2	19	10.53
		W100V1	viridis	22	121	18.18
		W100V2	viridis	2	39	5.13
		W100V3	viridis	8	92	8.70
		W100V4	viridis	5	30	16.67
		W100V5	viridis	1	96	1.04
Dry						
	25	D25X1	xantha	3	29	10.34
		D25X2	xantha	1	11	9.09
	50	D50X1	xantha	2	41	4.88
		D50X2	xantha	3	31	9.68
		D50X3	xantha	1	27	3.70
	100	D100X1	xantha	2	33	6.06
		D100X2	xantha	3	20	15.00
		D100X3	xantha	2	12	16.67
		D100A1	albino	1	5	20.00
		D100A2	albino	1	12	8.33

The xantha trait was induced as four to seven times as frequently as the albino trait, which was opposite to the result obtained in rice. Considering the genome conservation in the grass family, this result has many implications. The frequency of chlorophyll mutants in M_1 panicles was less than 25

% in most cases, suggesting that the panicle of foxtail millet is derived from plural initial cells.

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