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Induction of Mutants Related to Agronomic Characters 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 which shows a remarkable morphological diversity[6, 10, 16]. Recent advances in plant genetics show that most cereals including foxtail millet have a similar genome in terms of gene content and gene order, irrespective of chromosome number and genome size[3, 4]. This means that genetic information of one cereal can be applied to another with ease and that genes conferring advantages on millets may be exploited in major crops. We proposed that foxtail millet could be a model C_4 grass plant for genetic and molecular studies[5]. Mutants are good tools for genetics and developmental study. They are also genetic resources and have been used in plant breeding. However, there have been very few reports on foxtail millet mutants[1, 2]. In our previous paper[5], the effect of irradiation conditions on mutation rate induced by gamma-ray irradiation was examined. Mutation rate was estimated using chlorophyll deficiency mutants. In the following experiments, we obtained several mutants related to agronomic characters. In the present paper, we describe the induction and genetics of these mutants.

Materials and Methods

Selection of mutants related to agronomic characters in the M_2 generation

Out of our collection, Nagasaki 253 (N253) was selected for use in the present study. Gamma-ray irradiation and growth of the M_1 generation have already been described[5]. The M_2 seeds of the panicle on the M_1 generation, from which seed fertility was examined in the previous study, were sown in field soil in pots 15cm in radius and 18cm in depth. Then, seedlings were grown in a greenhouse on the Experimental Farm of the Faculty of Agriculture, Kagoshima University, Kagoshima, Japan (31° 30' N). The soil was 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. In the present study, progenies that were derived from the same panicle are called line. Sowing dates were July 3, 4 and 11, 2000. Two weeks after sowing, seedlings were transplanted to the Experimental Farm of the Faculty of

Agriculture, Kagoshima University. The number of plants in a line was 15, and planting density was 4cm × 40cm. Heading date (days to heading: DH) was recorded for each plant. Presence or absence of abnormality in plant height, panicle length, panicle shape, panicle number, seed set and anthocyanin pigmentation was checked for each plant. When possible mutants were discovered in a line, panicles of all plants in the line were harvested two months after heading date.

Progeny test using the M_3 generation

The M_3 seeds on the harvested panicles of the M_2 generation were sown in the soil sterilized as described above. Eight M_3 lines were examined for each M_2 possible mutant line. Sowing date was July 5, 2001. Two weeks after sowing, seedlings were transplanted to the farm as described above. The number of plants in a line was 15 and plant density was 5cm × 60 cm. The traits shown by possible mutants in the M_2 generation were observed in each plant in a line.

Two early heading plants appeared in one M_2 line. They were earlier than other M_2 plants by two weeks. To evaluate the earliness of the respective M_3 lines (hereafter called EH1-1 and EH1-2), cultivars showing different DH were grown under the same conditions as reference cultivars: Gai 29 (G29), Gai 53 (G53), Iwate 264 (I264), Genpeiawa (GPA) and Kuromochi (KM) (Table 1).

Table 1. Origin and earliness of reference cultivars

Cultivar	Abbreviation	Origin	Earliness
Gai 29	G29	Poland	Extremely early
Gai 53	G53	Russia	Extremely early
Iwate 264	I264	Iwate, Japan	Early
Genpeiawa	GPA	Nagano, Japan	Medium
Kuromochi	KM	Kagoshima, Japan	Late

EH1-1, EH1-2, N253 and reference cultivars were also grown with different sowing dates in the greenhouse as described above. Twenty seeds of these cultivars and lines were sown on the 20th of every month from June to September in 2001. They were sown in field soil sterilized by microwave in pots 15cm in radius and 18cm in depth. A week after sowing, every cultivar and line was thinned to five seedlings. Two replications were made. DH and leaf number on the main culm (LN) were recorded for each plant.

One low seed set mutant was obtained in the M_2 generation. This mutant was easily distinguished from normal plants because the mutant did not hang down its panicle. Seed set was calculated in the M_3 line derived from the low seed set plant (LSS1) and a sibline in which all plants hung down their panicles. Seed set was calculated as the frequency of fully ripened grains in about one hundred spikelets.

Results and discussion

Mutation rates of agronomic characters are shown in Table 2. Increase in panicle number, early heading, low seed set and aberrant panicle shape were observed in the M_2 generation. Except for early heading, the number of possible mutants in a line was smaller than that of normal plants, suggesting that recessive genes confer possible mutation traits. The segregating ratios of mutants observed in the M_2 generation are shown in Table 3.

Table 2. Agronomic character mutants obtained in M_2 and M_3 generations

Irradiation condition	Dose (Gy)	No. of lines	No. of lines in which possible mutants were obtained	Mutation rate (%)	Mutation traits
Dry	25	115	0	0.00	
	50	103	1	0.97	Multiple panicles
	100	131	2	1.53	Aberrant panicle, multiple panicles
	Total	349	3	0.86	
Wet	25	99	0	0.00	
	50	120	1	0.83	Multiple panicles
	100	82	2	2.44	Earliness, semi-sterility
	Total	301	3	1.00	

Early heading mutants

In the early heading mutant lines, EH1-1 and EH1-2, the M_3 lines derived from the two early M_2 plants were composed of only early heading plants. On the other hand, another line from a normal heading M_2 plant was composed of both early and normal heading progeny (Table 3). This result indicated that the early heading is controlled by recessive gene(s).

EH1 headed earlier than G29 or G53, both of which are among the earliest strains in our collection, both under field conditions and under different sowing date conditions in the greenhouse (Table 4). Natural daylength decreases after the summer solstice on June 23. Since foxtail millet is a short-day plant, DH is shortened according to the decrease in natural daylength. However, the DHs of N253 and EH1 are stable irrespective of daylength. DH is determined by two factors: basic vegetative growth period, which is not influenced by photoperiod, and photoperiod sensitivity, which is largely influenced by it. Our results suggest that the earliness of EH1 is caused by reduction in basic vegetative growth period. LN of the EH1 line was also the least among the lines examined.

EH1 has another morphological trait. It keeps elongating internodes just after germination (Figs. 1 and 2). No recombinant between early heading and elongating internodes has appeared in more than one hundred plants of M_3 lines segregating in these traits. This fact suggests that the early heading time gene(s) has a pleiotropic effect on internode elongation. To our knowledge, no such mutant genes conferring early heading and internode elongation have been reported in foxtail millet or other cereals. We harvested the panicle of the M_3 line segregating in heading date (and internode elongation). Using M_4 and M_5 generations, the genetic mechanism controlling these traits will be clarified.

Low seed set mutant

The M_3 line derived from the one low seed set M_2 plant (LSS1) was composed of only low seed set progeny (Table 3). Four M_3 lines derived from normal seed set plants were composed of both normal and low seed set plants while the other three M_3 lines from normal seed set plants were composed of only normal seed set plants. This result indicated that the low seed set was controlled by recessive gene(s). The average seed sets of the M_3 line fixed in low seed set and an M_3 line fixed in normal seed set were 0.461 and 0.708, respectively. Since the number of M_3 lines and plants in a line was small in this experiment, we could not decide how many mutant genes conferred these traits. A number of male-sterile mutants have been obtained in rice. Most of them were controlled by a recessive gene. In contrast, genetic information on female sterility mutants is meager[13]. It should be

Table 3. Inheritance of agronomic character mutants obtained in the present study

Traits of mutants	Irradiation condition	Dose (Gy)	Ratio in M_2 generation		Ratio in M_3 generation		
			No. of mutants	No. of normal plants	Traits of the parental plant[1)]	No. of mutants	No. of normal plants
Multiple panicles	Dry	50	1	14	M	2	12
					N	0	15
					N	0	14
					N	0	14
					N	0	13
					N	0	15
					N	0	14
Multiple panicles	Dry	100	1	10	M	1	14
					N	0	15
					N	0	12
					N	0	14
					N	0	14
					N	0	13
					N	0	15
					N	0	14
Aberrant panicle	Dry	100	2	13	M	3	11
					M	2	13
					N	1	12
					N	1	9
					N	0	13
					N	0	9
					N	0	11
					N	0	15
Multiple panicles	Wet	50	2	11	M	2	11
					N	0	14
					N	0	15
					N	0	15
					N	0	14
					N	0	14
					N	0	14
					N	0	15
Earliness	Wet	100	2	2	M	14	0
					M	15	0
					N	0	12
					N	5	7
Low seed set	Wet	100	1	14	M	14	0
					N	0	11
					N	0	14
					N	0	12
					N	5	8
					N	1	9
					N	3	12
					N	5	8

1) M and N stand for mutant and normal, respectively.

Table. 4. Mean days to heading (DH) and leaf number on the main culm (LN) of EH1 and reference cultivars

Cultivar and line	DH					LN			
	Farm	Greenhouse				Greenhouse			
		Sowing date				Sowing date			
	Jul. 5	Jun. 20	Jul. 20	Aug. 20	Sep. 20	Jun. 20	Jul. 20	Aug. 20	Sep. 20
EH1-1	29.1	30.3	33.6	34.3	34.7	8.8	9.0	8.0	7.8
EH1-2	29.5	30.8	34.4	33.8	34.6	9.3	9.8	8.3	8.1
N253	52.4	54.9	50.2	46.9	49.7	17.7	17.4	14.4	11.1
G29	31.5	37.3	36.1	36.2	35.8	14.0	13.3	10.7	9.0
G53	33.5	38.0	37.4	35.9	35.3	14.0	13.4	11.2	8.1
I264	46.2	53.8	51.6	44.0	39.6	17.6	17.2	11.9	7.9
GPA	47.0	62.4	50.3	47.2	43.9	17.4	15.3	12.0	8.5
KM	75.5	97.8	79.6	53.8	47.0	21.2	17.6	13.8	9.8

Fig. 1. Seedlings of N253 (*left*) and EH1-1 (*right*).

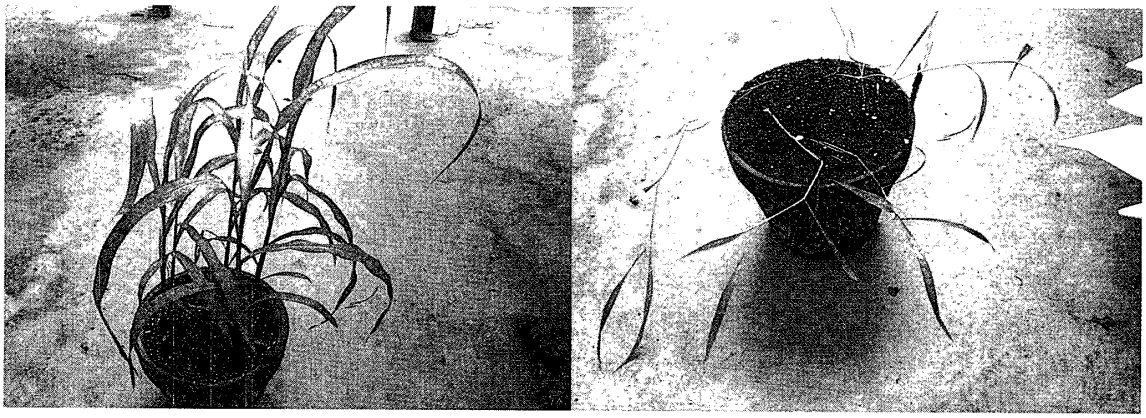

Fig. 2. Stands of N253 (left) and EH1-1 (right) three weeks after sowing.

determined whether pollen or eggs are sterile in our mutant.

Aberrant panicle mutant

Two aberrant panicle plants were obtained in one M_2 line. One or more splits were observed on the top of the aberrant panicles. Such panicle shape has been observed in some of our collection[14, 15], but the original cultivar N253 has no splits on the panicles. The ratio of possible mutants in the respective M_2 lines was small, suggesting that these traits were controlled by recessive gene(s). If this assumption was correct, the M_3 lines from the respective mutants should produce

only mutants. However, they produced both mutants and normal plants. These results indicated that the expression of gene(s) conferring aberrant panicles is unstable, strongly influenced by environmental factors. Aberrant panicles seen in some of our collection strains are stably inherited. It remains unsettled as to whether or not the gene(s) conferring aberrant panicles of our new mutant is different from that of our collection strains.

Multiple panicle mutants

Multiple panicle mutants were obtained in three M_2 lines (Table 1). The ratio of possible mutants in the respective M_2 lines was small, suggesting that these traits were controlled by recessive gene(s) (Table 3). If this assumption was correct, the M_3 lines from the respective mutants should produce only mutants. However, they produced both mutants and normal plants. The other M_3 plants produced only normal plants. These results indicated that the expression of gene(s) conferring multiple panicles is unstable, strongly influenced by environmental factors.

Future prospects

We obtained two stably inherited mutations related to agronomic characters. An early-heading mutant EH1 will contribute to the study of heading response. This mutant will also shed light on the interaction between flower formation and internode elongation. No early heading cultivars have the internode elongation trait[7, 8, 9, 14, 15], indicating that the early heading mutant gene(s) was not distributed in our collection. Gibberellin application is known to cause internode elongation in rice and other grasses. The internode elongation of EH1 may be caused by dysfunction of gibberellin-mediated signal transduction or gibberellin biosynthesis. A mutant showing aberrant internode elongation was discovered in rice, and it was named Awa-Odori[11]. The internode elongation of Awa-Odori was inhibited by application of uniconazole, a chemical inhibiting gibberellin biosynthesis[12]. Similar physiological approaches should be applied to EH1 in order to understand the cause of aberrant internode elongation. A low seed set mutant will contribute to the study of the development of reproductive organs. Whether pollen or eggs are sterile should be determined in the near future.

Summary

Several mutants related to agronomic characters were induced by gamma-ray irradiation in foxtail millet. In the present study, inheritance and features of these mutants were analyzed. The mutants obtained were: one early heading mutant, one low seed set mutant, one abnormal panicle mutant and three multiple panicle mutants. The early heading mutant headed earlier than cultivars planted in Europe, which are considered to be among the earliest cultivars. The earliness of the mutant was caused by recessive gene(s). The early heading mutant keeps elongating internodes just after germination. No recombinant between early heading and elongating internodes has appeared, suggesting that the early heading time gene(s) has a pleiotropic effect on internode elongation. The other mutant traits were also caused by recessive genes. The multiple panicle mutants and the aberrant panicle mutant showed complicated inheritance, suggesting the expression of genes conferring these traits are unstable, strongly influenced by environmental factors.

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