EFFECTS OF ION BEAM IRRADIATION ON MUTATION INDUCTION IN RICE.

Yoriko Hayashi*, Hinako Takehisa, Yusuke Kazama, Hiroyuki Ichida, Hiromichi Ryuto, Nobuhisa Fukunishi, Tomoko Abe, RIKEN Nishina Center, Saitama, Japan

Chiaki Kamba, Tadashi Sato, Tohoku University, Miyagi, Japan.

Abstract

Heavy- ion beam irradiation is an effective technique for mutation breeding to produce new cultivars. We evaluated the conditions of heavy-ion beam irradiation on mutation induction in rice (*Oryza sativa* L.). Rice seeds were irradiated by C or Ne ions accelerated to 135MeV/u within a dose range of 10 to 400 Gy. The LET (linear energy transfer) range of the C-ion beam was 23 to 100keV/µm and that of the Ne-ion beam was 60 to 100keV/µm. To evaluate the effect of irradiation, survival rate and the seed fertility were examined in M_1 plants and the frequency of chlorophyll deficient mutants (CDM) was examined in M_2 plants. The conditions of C-ion and Ne-ion irradiation to maintain high survival rate were up to 20Gy with 23-40keV/µm, and up to 10Gy with 60-80 keV/µm, respectively. Seed fertility tended to be unaffected by LET values of 23-70 keV/µm with the same dose of irradiation. The highest frequency of CDM was observed in 10Gy at C or Ne ion irradiation with 60-70 keV/µm. In this study, we isolated several mutant lines such as salt-tolerant, bronzing, crinkled-dwarf, necrosis and chlorosis. These mutants could be important as genetic resources for research in plant functional genomics.

INTRODUCTION

Radiation treatment of plants is one of the most common techniques for induction of plant mutantions. These mutants are useful for developing new plant varieties as well as for functional studies of genes. Recently, heavy-ion beam irradiation has been established as an effective method of inducing mutations in many plant species because of its high frequency and broad spectrum. At RIKEN RI Beam Factory (RIBF), heavy ion-beams such as carbon, nitrogen and neon (maximum energy 135 MeV/nucleon) have been used to irradiate plants. Heavy-ion beams for the irradiation to biological samples are accelerated using the RIKEN ring cyclotron (RRC), together with an AVF cyclotron. The range in water of these heavy-ion beams is sufficient to penetrate various plants organs. In 2005, an automatic irradiation system for biological samples was constructed. This system can select LET (linear energy transfer) using a range shifter that consists of twelve energy absorbers[1]. We have applied ion-beam bombardment to Rice seeds since 2000. Rice is an important plant not only as a staple farm crop but also as a model plant for genomic research.

In this report, we evaluate the effect on the mutation induction of rice and introduce some characteristic mutations.

* yorihayashi@riken.jp

MUTATIONAL EFFECT

Rice seeds (*Oryza sativa* L. cv. Nipponbare) were exposed to Ne-ion or C-ion accelerated to 135MeV/u by RRC within a dose range of 5 to 400Gy (Fig. 1). The LET values of these ions were calculated at the surface of the seeds. After irradiation, seedlings were transplanted into soil in pots, and grown in a greenhouse at 25°C in the daytime (12 hrs) and 20°C at night (12 hrs). One month in 2002, we reported that after irradiation, plants were transplanted to a paddy field. M_1 plants were harvested approximately 5 months after irradiation. M_2 seeds were harvested separately from each M_1 plants. These seeds were sown on seedbeds and grown in a greenhouse for one month to observe mutants.

Figure 1 Imbibition seeds of rice prepared for irradiation

Effect of pretreatment of seeds

In our previous investigation the most effective soaking period to induce CDM was 3 days[2]. The phenotypes of CDM were albina (albino), xanta (yellow leaves), chlorina (pale green leaves) and striata (striped leaves). The survival rate was not affected by a soaking period of 1 to 3 days (Table 1). In wheat and barley, the optimal dose of N-ion irradiation to dry seeds was about ten-fold that to imbibition seeds[3]. In this study, we investigated the difference in sensitivity between dry seeds and imbibed seeds of rice (Nipponbare). These seeds were exposed to C ions (135Mev/u, 22.6KeV/µm) within a dose range of 10 to 400Gy. Survival rates of the M_1 progeny were observed one month after irradiation. As shown in Fig. 2, imbibition seeds are more sensitive to ion-beam irradiation than dry seeds. Ion beam irradiation induced a high mutation rate at a relatively low dose without severe damage to plants. At the dose indicated by arrows, survival rate did not decrease but growth disturbance of the plants were observed. Based this findings, it was suggested that the optimal irradiation dose using C ions was around 150Gy for dry seeds, and around 20Gy for imbibition seeds.

Table 1 Frequency of CDM in M_2 plants induced by C-ion irradiation

Phenotype	Treatment					
Soaking period	1		2		3	
Dose (Gy)	20	40	20	40	20	40
Fertile M_1 line	128	128	129	102	129	128
Seed fertility (%)	85.3	85.3	86.0	68.0	86.0	85.3
Albina/ Xanta	3	4	3	2	2	5
Chlorina	1	1	2	2	1	3
Striata	0	0	0	1	1	3
Total CDM	4	5	5	5	4	11
CDM (%)	3.1	3.9	3.9	4.9	3.1	8.6

Harvest of 2002

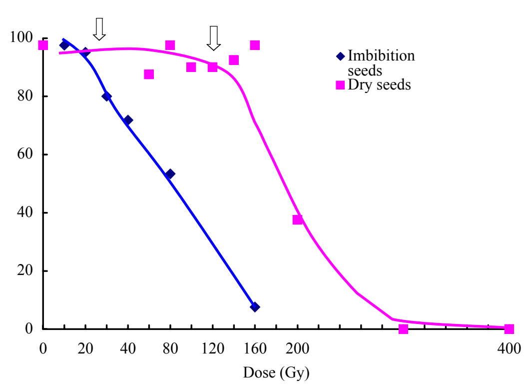

Figure 2 Effect of C-ion beam irradiation on dry seeds and imbibition seeds

Cultivar difference

It is reported that there is a cultivar difference in resistance to ultraviolet-B (UVB: 280-320 nm) in rice (*Oryza sativa* L.). Norin 1 is more sensitive than Sasanishiki, and this difference is derived from DNA repair ability by photolyase[4]. Sensitivity against heavy-ion beam irradiation differs with the status of plants such as species, target organ, developmental stage[5][6][7]. In this study, we examined the difference in sensitivity against C-ion beam irradiation of three cultivars of rice (Nipponbare, Norin 1, Sasanishiki). The imbibition seeds were irradiated within a dose range of 5 to 40Gy (23.6keV/μm) and observation of M_1 and M_2 progenies were described above. The survival rate of Sasanishiki was lowest at any dose of irradiation (Fig. 3). The frequency of CDM was higher than that of other cultivars (Fig. 4). These results indicate that Sasanishiki was most sensitive to C-ion beam irradiation.

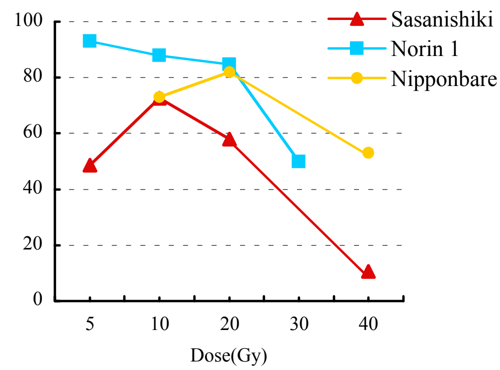

Figure 3 Survival rate of Sasanishiki, Norin 1, and Nipponbare irradiated with C-ion beams

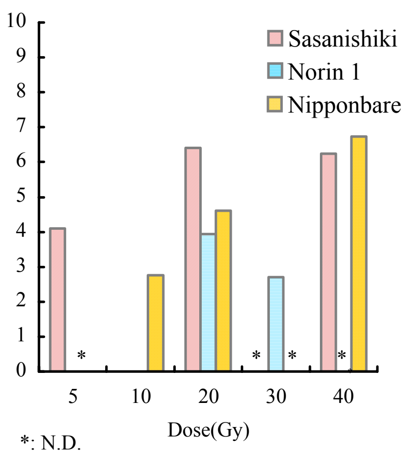

Figure 4 Frequency of CDM induced by C-ion irradiation

DOSE and LET response

In Arabidopsis, the lethality rate and flowering rate after ion-beam treatment are affected by LET[8]. We evaluated the dose and LET response to heavy-ion beam irradiation on mutation induction in rice (Nipponbare). Imbibition seeds were irradiated by C or Ne ions accelerated to 135MeV/u within a dose range of 10 to 40Gy. LET range of the C-ion beam was 23 to 100keV/μm, and Ne-ion beam was 60 to 100keV/μm. Survival rates and seed fertilities were observed in M_1 progenies. CDM rates were observed in M_2 progenies. High dose irradiation caused decreases in both survival and seed fertility rate (Table 2). It is important for mutation breeding to induce mutants that retain superior characteristics. And decrease of survival rates and seed fertility is undesirable for efficient induction of mutants. The highest frequencies of CDM with high survival rates were observed at 10-15Gy of C or Ne ion irradiation with 60-70 keV/μm (Table 2). These findings showed that it was possible to select more effective irradiation conditions for mutation induction by controlling the LET values.

Table 2 Survival rate and frequency of CDM induced by C & Ne-ion irradiation

Ion	LET* (keV/μm)	Dose (Gy)	Survival rate (%)	Seed fertility (%)	CDM (%)
C	22.59 – 22.86	10	73	88	2.8
		20	80	71	4.6
		40	40	66	9.0
	30.01 – 32.22	20	68	69	2.3
		30	71	46	4.7
	37.56 – 42.40	10	77	81	3.4
		20	53	67	3.4
	48.26 – 55.96	20	58	62	4.2
	60.73 – 86.71	10	41	74	9.7
		15	71	61	7.6
Ne	63.00 – 66.30	10	75	84	8.6
		15	86	73	6.2
	69.66 – 73.70	10	65	77	10.3
	77.87 – 85.40	10	68	71	2.0

*The range of LET when C & Ne ion-beam irradiated the sample with a thickness of 2.7mm.

MORPHOLOGICAL MUTANTS

In this study, we isolated several mutants of rice in M_2 and M_3 generations (Table 3 and Fig.5). Line 6-99L (a) was taller than wild type (WT) in normal paddy field (PF) and showed salt-tolerance in a saline paddy field (SPF). Line 40-S (b) was crinkled-dwarf mutant. Line 22-4 (c) was temperature dependent chlorosis mutant. This chlorosis was observed on only 1st to 4th leaves. Line 22-70(d) showed necrosis. Line 7-3b and line 11-29 (e) were lesion mimic mutant lines. Line 7-3b was resistant to rice blast disease[9]. These mutants were developed only two or three years. Such a short developing period is one of useful advantage of ion-beam treatment for breeding. And these mutants may also contribute to physiological and genetic studies of rice.

Table 3 Irradiation conditions to produce mutants

Line	Ion	Dose (Gy)	LET (keV/μm)
a) 6-99L	C	40	22.6
b) 40-S	C	40	22.6
c) 22-4	C	20	22.6
d) 22-70	C	20	29.9
e) 7-3b	Ne	10	63.8
11-29	C	20	22.6

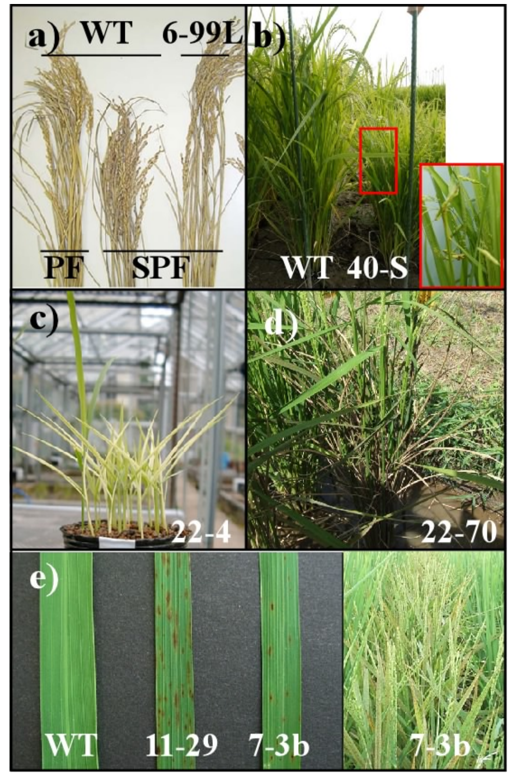

Figure 5 Morphological mutants produced by heavy-ion irradiation.
a) Tall; b) Crinkled-dwarf, c) Chlorosis; d) Necrosis e) Bronzing

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