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Mutagenic effects of ion beam irradiation on rice

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We investigated the usefulness of ion beams for mutation breeding in rice (*Oryza sativa* L.) by comparing the efficiency (i.e., the ratio of desirable mutations to plant damage such as lethality and sterility), mutation rate, spectrum, and optimum dose to that of gamma rays. Rice seeds were irradiated with carbon ions (mean linear energy transfer = 76 and 107 keV/μm), helium ions (9 keV/μm), and gamma rays, and their survival and fertility were examined in the M_1 generation. The frequency of chlorophyll mutations and their types (albina, xantha, and viridis) were examined in the M_2 generation, using the M_1-plant progeny method. The efficiency of ion beams either equaled or exceeded that of gamma rays. In addition, the mutation rate of ion beams was higher than that of gamma rays. Thus, ion beams appeared to efficiently induce mutants with little radiation damage. No remarkable difference was observed in the relative frequencies of each type of mutation among 3 types of ion beams and gamma rays, thus suggesting that there was no difference in the spectrum. A shoulder dose, which hardly affected survival, was sufficient to efficiently obtain mutants for both types of radiation.

Key Words: gamma rays, ion beams, mutation frequency, optimum dose, rice, spectrum.

Introduction

Mutation breeding is a useful method for crop improvement. The type of mutagenic treatment is an important factor to obtain successful results in mutation breeding. Physical mutagens, such as gamma rays and X-rays, have mainly been used to induce mutations, and many mutant varieties have been released.

Ion beams have recently attracted attention as mutagens. A characteristic feature of ion beams is their ability to deposit high energy on a target, densely and locally, as opposed to low linear energy transfer (LET) radiation such as gamma rays and X-rays (Yang and Tobias 1979, Tanaka 1999). Due to this difference, ion beams are expected to result in different mutation induction effects and induction of novel mutants that have not been previously obtained.

Mutation induction with ion beams, using various plants, has been attempted since the 1990s in Japan. Until now, it has been demonstrated in Arabidopsis that ion beams induce mutations with high frequency and show a broad mutation spectrum, and novel mutants have been obtained (Hase *et al.* 2000, Shikazono *et al.* 2003, Tanaka *et al.* 1997, Tanaka *et al.* 2002). Furthermore, various mutants have been obtained in many crops, mainly ornamental plants (Hamatani *et al.* 2001, Hara *et al.* 2003, Kanaya *et al.* 2008, Kazama *et al.* 2008, Miyazaki *et al.* 2002, Miyazaki *et al.* 2006, Nagatomi *et al.* 1996, Okamura *et al.* 2003, Yamaguchi *et al.* 2003). Specific flower color mutants that could not be obtained by gamma rays, could be induced using ion beams, particularly in chrysanthemum (Nagatomi *et al.* 1996) and carnation (Okamura *et al.* 2003).

Thus, the characteristics of ion beams have been gradually clarified, and ion beam irradiation has evolved as a new mutation method. However, in terms of the mutagen for mutation breeding, the characteristics of ion beams, especially in comparison to gamma rays and X-rays, and the criterion of optimum irradiation dose for practical use have not been sufficiently clarified.

To compare the mutagenic effects of different mutagens, 2 terms, *effectiveness* and *efficiency*, have been used (Konzak *et al.* 1965, Mikaelsen *et al.* 1971, Nilan *et al.* 1965). *Effectiveness* is defined as the number of mutations

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produced per unit dose, whereas *efficiency* is defined as the ratio of specific desirable mutagenic changes to plant damage in the M_1 generation, such as lethality and sterility. Konzak *et al.* (1965) suggested that the usefulness of any mutagen in plant breeding depends not only on its mutagenic effectiveness, but also on its mutagenic efficiency. In this sense, ion beams have been shown to exhibit higher effectiveness compared to gamma rays (Fujii *et al.* 1966, Mei *et al.* 1994), X-rays (Hirono *et al.* 1970, Yang and Tobias 1979), and electrons (Shikazono *et al.* 2003). However, the efficiency of ion beams has not been compared to that of gamma rays or X-rays.

In the present study, using rice (*Oryza sativa* L.), we investigated the efficiency and effectiveness of ion beams and the spectrum of induced mutants in comparison to gamma rays. Furthermore, we attempted to identify the optimum dose of ion beams for use in irradiation treatment.

Materials and Methods

Oryza sativa L. cv. Hitomebore was used as our experimental material.

Hulled dry seeds of rice were placed on 6-cm-diameter petri dishes, with the embryos facing the irradiation source. The samples were irradiated with 220 MeV carbon ions (LET 107 keV/μm) at doses of 10, 20, 30, 40, 50, and 60 Gy; 320 MeV carbon ions (LET 76 keV/μm) at doses of 20, 40, 60, 80, 100, and 150 Gy; and 100 MeV helium ions (LET 9 keV/μm) at doses of 50, 100, 150, 200, 250, and 300 Gy, generated by an AVF-cyclotron (Japan Atomic Energy Agency, Takasaki, Japan). Gamma rays were applied to unhulled dry seeds of rice at doses of 100, 150, 200, 250, 300, 350, 400, and 450 Gy, with a dose rate of 10 Gy per h, in the gamma room at the Institute of Radiation Breeding (National Institute of Agrobiological Sciences, Hitachi-omiya, Japan). Irradiation treatments were repeated 4 times for 220 and 320 MeV carbon-ion beams, 3 times for 100 MeV helium-ion beam and gamma rays, using 300–1000 seeds per irradiation treatment.

One hundred seeds from each irradiation treatment were sown on moistened rock wool to investigate the effects of ion beams on survival. After a 4- or 5-day incubation at approximately 30°C, they were moved into a greenhouse. The number of seedlings that survived 3 weeks after sowing was counted, and the survival rate was expressed as the number of seedlings from irradiated seeds divided by the number of seedlings from non-irradiated seeds. The germination rate of the non-irradiated controls was greater than 93% in both hulled and unhulled seeds.

In the irradiation treatment with 220 MeV carbon ions at doses of 10, 20, 30, and 40; 320 MeV carbon ions at doses of 20, 40, 60, 80, and 100; 100 MeV helium ions at doses of 50, 100, 150, 200, and 250; and gamma rays at doses of 100, 150, 200, 250, and 300, surviving seedlings (M_1 plant) were cultured in a paddy field, and the panicles (M_2 seeds) of the longest culm in each M_1 plant were harvested. Fertility and mutation were investigated using lots, which consisted of more than 150 M_1 plants derived from M_1 seeds irradiated with each dose on the same day, and the fertility and mutation frequency were calculated for each lot.

Fertility was determined based on seed set in the panicles of 50 M_1 plants selected at random from each lot.

Chlorophyll mutation was investigated in the M_2 generation, using the M_1-plant progeny method; twenty-five M_2 seeds from each M_1 plant were sown individually. After germination, the number of chlorophyll mutants and germinated seedlings, and their type [albina (white), xantha (yellow), viridis (light green or yellow-green), and others such as striata (longitudinal white or yellow stripes) and maculata (green or yellow spots distributed over the leaf)] was investigated.

The mutation frequency per M_2 plant shown in Fig. 3, Fig. 4 and Fig. 5 was determined as the number of chlorophyll mutants divided by the number of investigated M_2 plants. The mutation frequency per M_1 plant shown as supplemental data in ESMs 1–2 was determined as the number of M_1 plants that produced chlorophyll mutants in their progeny (M_2 plant) divided by the number of investigated M_1 plants. The number of mutated M_1 plants per sown M_1 seed shown in Fig. 6 was determined as the number of M_1 plants that produced chlorophyll mutants in their progeny (M_2 plant) divided by the number of sown M_1 seeds. The frequency of spontaneous mutation was estimated using approximately 49000 non-irradiated plants.

The segregation frequency was investigated using strains of which 16–25 M_2 seedlings germinated, and they were calculated as the number of chlorophyll mutants divided by the number of germinated M_2 plants in each strain.

Results

Survival

In both ion beams and gamma rays, the dose-response curves had a shoulder at a certain dose, and survival rates rapidly decreased with doses higher than that corresponding to the shoulder (Fig. 1). The dose that corresponds to the shoulder was 20 Gy with the 220 MeV carbon-ion beam, 60–80 Gy with the 320 MeV carbon-ion beam, 150 Gy with the 100 MeV helium-ion beam, and 200 Gy with gamma rays. The 50% lethal dose was 35 Gy with the 220 MeV carbon-ion beam, 100 Gy with the 320 MeV carbon-ion beam, 240 Gy with the 100 MeV helium-ion beam, and 300 Gy with gamma rays. Thus, the effect on lethality increased with increasing LET.

Fertility

The fertility decreased linearly with increasing irradiation dose (Fig. 2). The effect on fertility also increased with increasing LET in the following order: 220 MeV carbon-ion beam < 320 MeV carbon-ion beam < 100 MeV helium-ion beam < gamma rays.

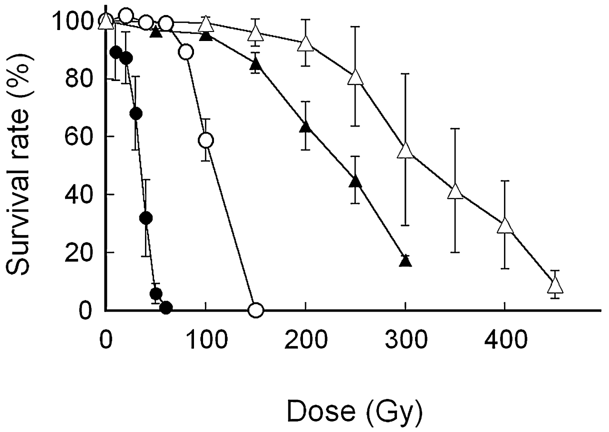

Fig. 1. Effect of ion beam and gamma ray irradiation on the survival of rice seeds. Survival rate is expressed as the number of seedlings from the irradiated seeds divided by the number of seedlings from the non-irradiated seeds. ●: 220 MeV carbon-ion beam; ○: 320 MeV carbon-ion beam; ▲: 100 MeV helium-ion beam; △: gamma rays. Vertical bars indicate SE ($n=4$ for the 220 and 320 MeV carbon-ion beams; $n=3$ for the 100 MeV helium-ion beam and gamma rays).

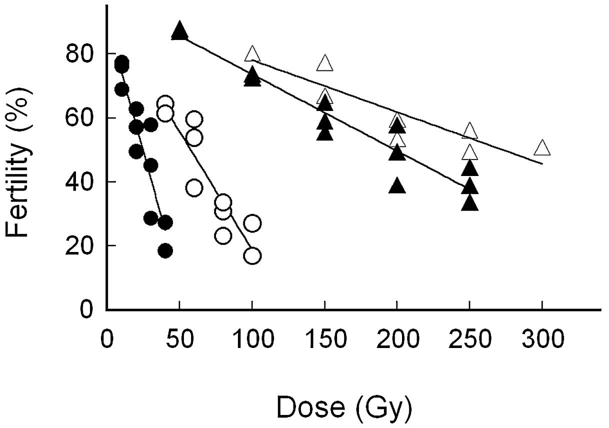

Fig. 2. Effect of ion beam and gamma ray irradiation on fertility. Fertility is determined based on seed set in the panicles of 50 M_1 plants selected at random from each lot, which consisted of more than 150 M_1 plants derived from M_1 seeds irradiated with each dose on the same day. ●: 220 MeV carbon-ion beam, $y=90.6-1.64x$, $r=-0.918$***; ○: 320 MeV carbon-ion beam, $y=92.6-0.738x$, $r=-0.913$***; ▲: 100 MeV helium-ion beam, $y=97.6-0.240x$, $r=-0.959$***; △: gamma rays, $y=94.2-0.162x$, $r=-0.896$**. ** and *** Significant at 1% and 0.1% levels, respectively.

Effectiveness

To evaluate the "effectiveness", the relationship between the irradiation dose and the mutation frequency per M_2 plant is shown in Fig. 3. The frequency of chlorophyll mutation increased linearly with increasing irradiation dose. The dose required to obtain the same mutation frequency increased in the following order: 220 MeV carbon-ion beam < 320 MeV carbon-ion beam < 100 MeV helium-ion beam < gamma rays. Thus, the effectiveness also increased with LET, indicating that the "effectiveness" of ion beams was higher than that of gamma rays. The frequency of spontaneous mutation was approximately 0.02%.

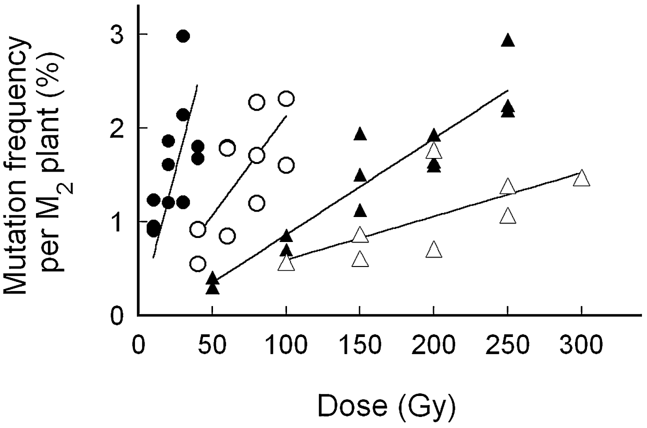

Fig. 3. Effect of ion beam and gamma ray irradiation on mutation induction. The mutation frequency is determined as the number of chlorophyll mutants divided by the number of M_2 plants investigated, using the M_1-plant progeny method. ●: 220 MeV carbon-ion beam, $y=0.876+0.0305x$, $r=0.563$; ○: 320 MeV carbon-ion beam, $y=0.145+0.0193x$, $r=0.696$; ▲: 100 MeV helium-ion beam, $y=-0.171+0.0103x$, $r=0.934$**; △: gamma rays, $y=0.124+0.00464x$, $r=0.685$. ** Significant at 1% level.

Eefficiency

To evaluate the "efficiency" on the basis of lethality, the relationship between the mutation frequency and the survival rate is shown in Fig. 4 and ESM 1. Even if the same radiation type was applied at the same dose, the survival rate and the mutation frequency differed among irradiation treatments, as shown in Fig. 1 and Fig. 3, respectively. Therefore, the mutation frequency and the survival rate of each irradiation treatment were plotted, and the relation between them is shown.

The mutation frequency per M_2 plant increased inversely with the survival rate following the irradiation treatment (Fig. 4). The relationship between the mutation frequency per M_2 plant and survival rate was not linear; in the range where the survival rates were 90–100%, the mutation frequencies increased markedly. In contrast, in the range where the survival rates were 90% or less, the mutation frequency increased gradually. Therefore, the mutation frequencies were compared at 70% and 90% survival rates. At a 90% survival rate, the mutation frequency per M_2 plant with the 3 types of ion beams was approximately 1.7%, whereas that of gamma rays was lower than those of the ion beams, approximately 1.0%. At 70% survival rate, the 320 MeV carbon-ion beam showed the highest mutation frequency (2.0%), followed by the 100 MeV helium-ion beam (1.9%), 220 MeV carbon-ion beam (1.8%), and gamma rays (1.3%).

The relationship between the mutation frequency per M_1 plant and the survival rate was similar to the case of the mutation frequency per M_2 plant with each radiation (ESM 1). At a 90% survival rate, the 320 MeV carbon-ion beam showed the highest mutation frequency (9.5%), followed by the 220 MeV carbon-ion beam (9.3%), gamma rays (8.4%), and 100 MeV helium-ion beam (8.1%). At a 70% survival

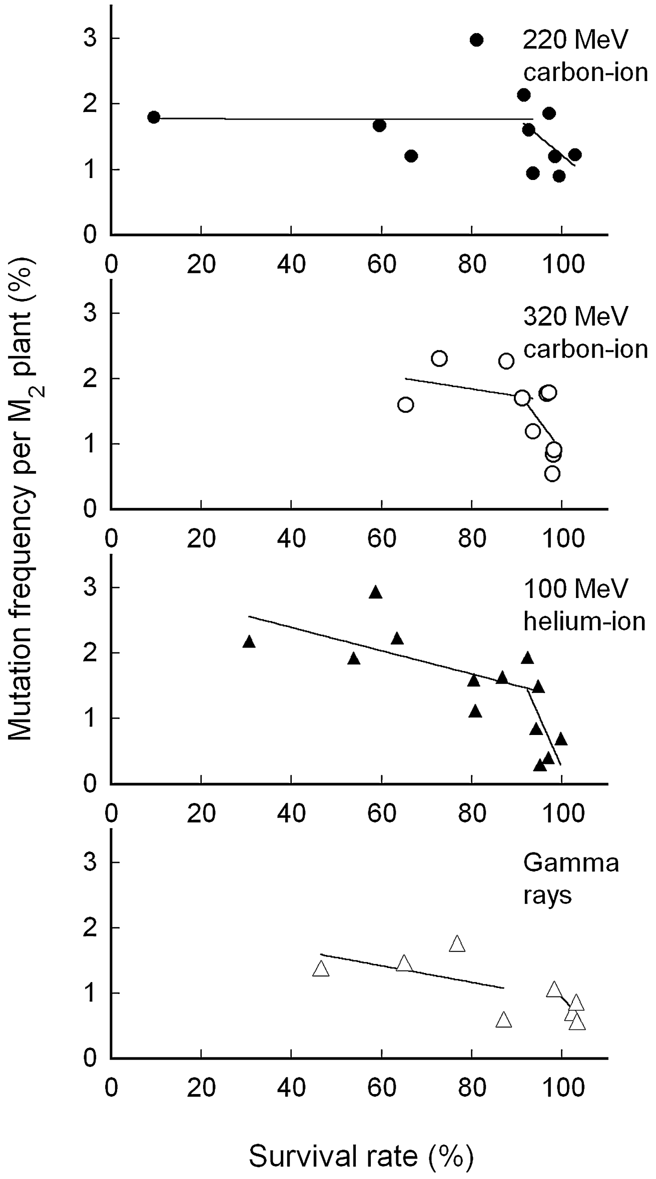

Fig. 4. Relationship between survival rate and mutation frequency. The mutation frequency is determined as the number of chlorophyll mutants divided by the number of M_2 plants investigated, using the M_1-plant progeny method. Survival rate is expressed as the number of seedlings from irradiated seeds divided by the number of seedlings from the non-irradiated seeds. The regression lines are calculated in the range of 60%–95% survival and 90%–105% survival individually. 220 MeV carbon-ion beam, $y=1.77-0.0001x$, $r=-0.005$ (for 60%–95% survival) and $y=6.91-0.057x$, $r=-0.496$ (for 90%–105% survival); 320 MeV carbon-ion beam, $y=2.70-0.011x$, $r=-0.280$ (for 60%–95% survival) and $y=10.2-0.093x$, $r=-0.492$ (for 90%–105% survival); 100 MeV helium-ion beam, $y=3.1-0.018x$, $r=-0.635$* (for 60%–95% survival) and $y=15.8-0.156x$, $r=-0.614$ (for 90%–105% survival); gamma rays, $y=2.18-0.013x$, $r=-0.445$ (for 60%–95% survival) and $y=8.36-0.074x$, $r=-0.825$ (for 90%–105% survival). * Significant at 5% level.

rate, the 100 MeV helium-ion beam showed the highest mutation frequency (10.2%), followed by the 220 MeV carbon-ion beam (9.3%), 320 MeV carbon-ion beam (8.1%), and gamma rays (6.6%).

Thus, it was suggested that the "efficiency" on the basis of lethality of ion beams was higher than that of gamma rays. The difference of "efficiency" on the basis of lethality between ion beams and gamma rays was clear in the mutation frequency per M_2 plant in comparison with the mutation frequency per M_1 plant.

To evaluate the "efficiency" on the basis of fertility, the relationship between the mutation frequency and the fertility is shown in Fig. 5 and ESM 2. The fertility was also differed among irradiation treatments even if the same radiation type was applied at the same dose, as shown in Fig. 2. Therefore, the mutation frequency and the fertility of each irradiation treatment were plotted, and their relationship is shown. The fertility and the mutation frequency per M_2 plant showed a negative linear relationship for each type of radiation. The mutation frequency of ion beams increased significantly with decreasing fertility, and the same tendency was observed for gamma rays. The mutation frequencies at 60% fertility of the 220 MeV carbon-ion beam and the 100 MeV helium-ion beam were 1.4%, whereas those of 320 MeV carbon-ion beam and gamma rays were lower, i.e., 1.1%.

The fertility and the mutation frequency per M_1 plant also showed a negative linear relationship (ESM 2). The mutation frequency increased significantly and inversely with fertility in 220 MeV carbon-ion beam, the 100 MeV helium-ion beam and gamma rays. At 60% fertility, the 100 MeV helium-ion beam showed the highest mutation frequency (8.0%), followed by the 220 MeV carbon-ion beam (7.5%), 320 MeV carbon-ion beam (7.2%), and gamma rays (6.7%).

Thus, on the basis of fertility, ion beams induced higher frequencies of mutation than gamma rays, suggesting that the "efficiency" on the basis of fertility of ion beams was higher than that of gamma rays. Also in the efficiency on the basis of fertility, the difference between ion beams and gamma rays was clear in the mutation frequency per M_2 plant in comparison with the mutation frequency per M_1 plant.

Optimum irradiation dose

The optimum irradiation dose for obtaining the highest number of mutants from the irradiated seeds was clarified from the relationship between the irradiation dose and the number of mutated M_1 plants per sown M_1 seed (Fig. 6). In both ion beams and gamma rays, the number of mutated M_1 plants per sown M_1 seed reached a maximum at a certain dose; at higher doses, the number of mutated M_1 plants per sown M_1 seed decreased with increasing dose. When quadratic curves were fitted to the plots for each radiation, the maximum numbers of mutated M_1 plants per sown M_1 seed was 0.085 at 22 Gy with the 220 MeV carbon-ion beam, 0.094 at 73 Gy with the 320 MeV carbon-ion beam, 0.076 at 187 Gy with the 100 MeV helium-ion beam, and 0.058 at 209 Gy with gamma rays. Thus, the maximum numbers of mutated M_1 plants per sown M_1 seed of the 3 types of ion beams were higher than that of gamma rays. The dose at which the number of mutated M_1 plants per sown M_1 seed

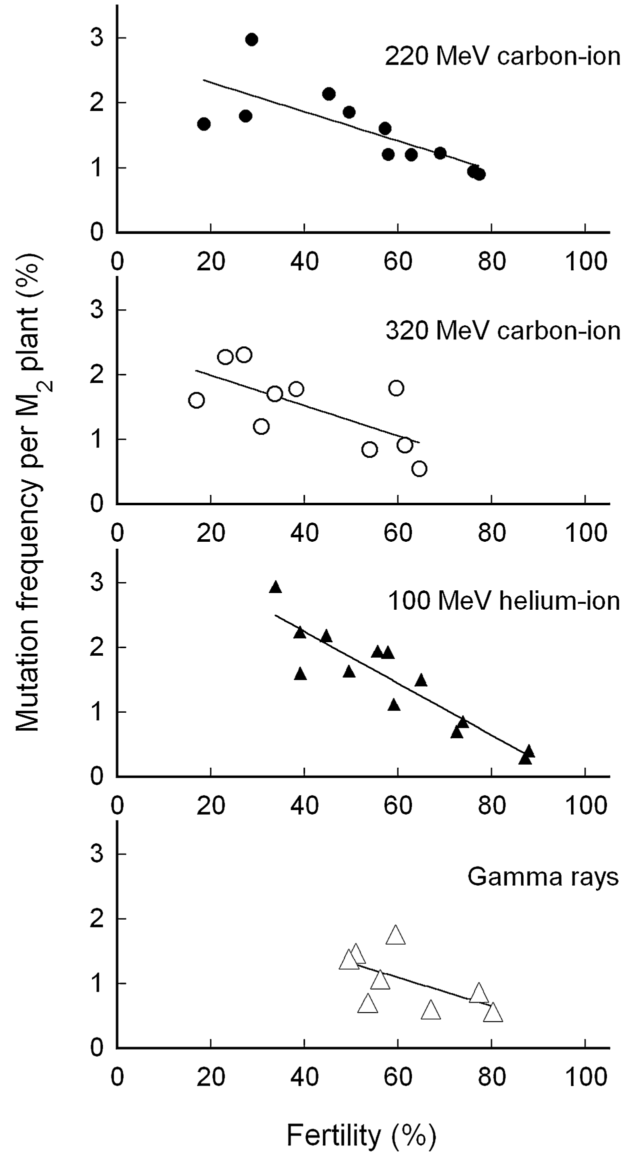

Fig. 5. Relationship between fertility and mutation frequency. The mutation frequency is determined as the number of chlorophyll mutants divided by the number of M_2 plants investigated, using the M_1-plant progeny method. Fertility is based on seed set in panicles of the longest culm in 50 M_1 plants selected at random in each treatment. 220 MeV carbon-ion beam, $y=2.76-0.0225x$, $r=-0.743$**; 320 MeV carbon-ion beam, $y=3.23-0.0383x$, $r=-0.644$*; 100 MeV helium-ion beam, $y=3.84-0.0400x$, $r=-0.911$**; gamma rays, $y=2.41-0.022x$, $r=-0.587$. * and ** Significant at 5 and 1% levels, respectively.

was highest almost corresponded to the shoulder appearing in the survival curves for both ion beams and gamma rays (Fig. 1).

Spectrum of chlorophyll mutation

Both ion beams and gamma rays induced albina, xantha, viridis, and other mutants such as striata (longitudinal white or yellow stripes) and maculata (green or yellow spots distributed over the leaf). Regardless of radiation type and irradiation dose, the frequency of albina was highest, followed by viridis (Fig. 7). When totaled at each radiation type, the frequency of albina was about 50%, whereas that of xantha ranged from 8%–15%, that of viridis ranged from 25%–30%, and that of other mutations ranged from 8%–13% in each radiation type. Thus, no remarkable difference in the relative frequencies of each type of mutation was observed among the 3 types of ion beams and gamma rays.

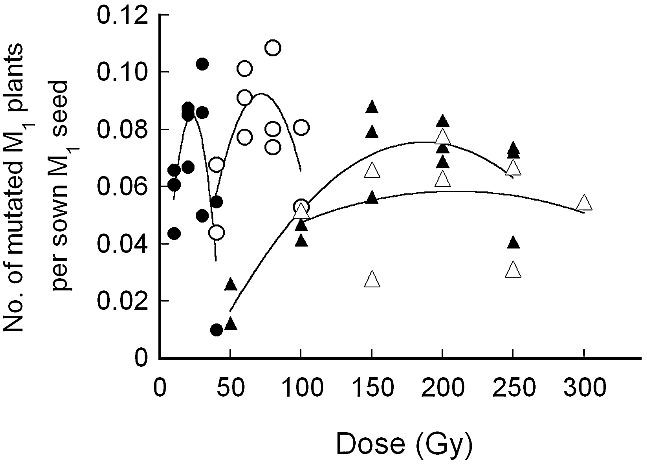

Fig. 6. Relationship between irradiation dose and the number of mutated M_1 plants per sown M_1 seed. The number of mutated M_1 plants per sown M_1 seed is determined as the number of M_1 plants that produced chlorophyll mutants in their progeny (M_2 plant) divided by the number of sown M_1 seeds sown after irradiation. ●: 220 MeV carbon-ion beam, $y=-0.696+0.780x-0.0174x^2$, $r=0.732$*; ○: 320 MeV carbon-ion beam, $y=-8.52+0.493x-0.0034x^2$, $r=0.726$*; ▲: 100 MeV helium-ion beam, $y=-3.462+0.118x-0.000315x^2$, $r=0.869$***; △: gamma rays, $y=1.83+0.0383x-0.0000916x^2$, $r=0.218$. * and *** Significant at 5% and 0.1% levels, respectively.

Segregation frequency

In each radiation type, the strain that showed high segregation frequency increased with irradiation of a higher dose. As a result, the average of the segregation frequency increased. The segregation frequencies produced by ion beams seemed to be higher than those produced by gamma rays; the segregation frequencies for chlorophyll mutants induced by gamma rays did not increase above 0.17 when the dose increased from 200 Gy to 300 Gy (Fig. 8). In contrast, segregation frequencies for mutants induced by 220 MeV carbon-ion beam were 0.19–0.20 in the range of 20–40 Gy, those by 320 MeV carbon-ion beam were 0.18–0.22 in the range of 60–100 Gy, and those by 100 MeV helium-ion beam were 0.16–0.20 in the range of 100–250 Gy.

Discussion

On the basis of fertility, the mutation frequency induced by ion beams equaled or exceeded that induced by gamma rays. On the basis of lethality, ion beams induced higher frequencies of mutation than gamma rays. Furthermore, the maximum number of mutated M_1 plants per M_1 seed in 3 types of

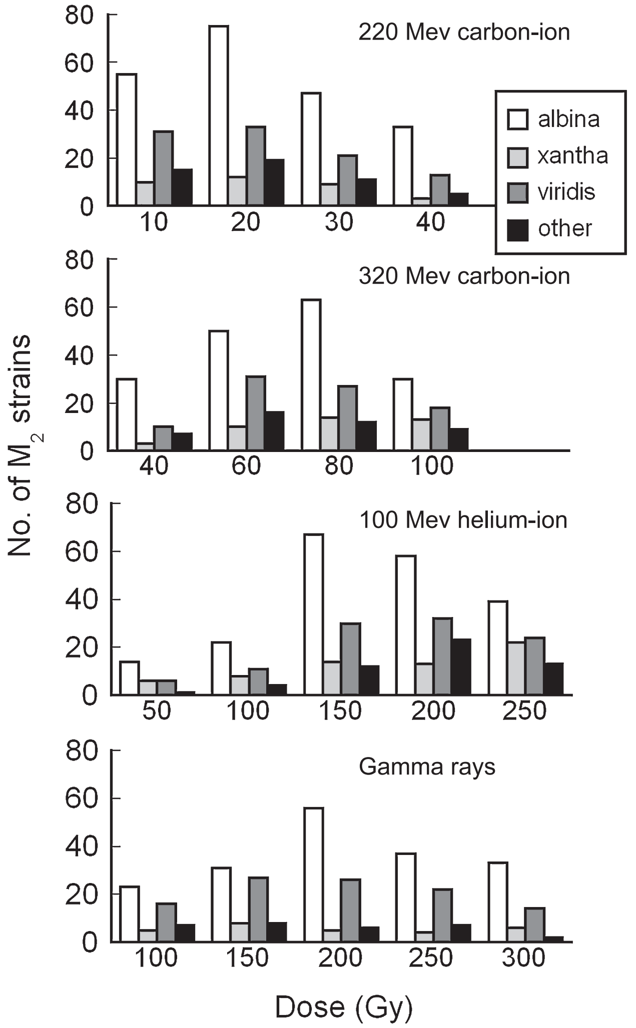

Fig. 7. Frequency distribution of the different types of chlorophyll mutants induced by ion beam and gamma ray irradiation.

ion beams were higher than that in gamma rays. Both in ion beams and gamma rays, number of mutated M_1 plants per sown M_1 seed reached a maximum at the shoulder dose, which did not affect survival markedly. Accordingly, this result also demonstrated that ion beams had high “efficiency” on the basis of lethality. Thus, it was suggested that the efficiency of ion beams was equal or superior to that of gamma rays.

Ekberg (1969) reported that 82% of sterility induced by ionizing radiations (neutrons and X-rays) was predominantly caused by chromosome aberrations (translocations and inversions) in barley. Furthermore, the sterility caused by recessive mutations does not appear until the M_2 generation. Therefore, it was believed that the sterility induced by ion beams and gamma rays also was mainly caused by chromosome aberrations, as with neutrons and X-rays, though these factors of sterility were not clarified from our study. Consequently, it was believed that high “efficiency” on the basis of fertility means that mutation frequency is high in the degree of chromosome aberrations. Yang and Tobias (1979) suggested that gamma rays cause more deleterious effects to cells than high-LET radiation when a certain density of genetic lesions is required to produce a given mutation, and as a result, heavy ions could potentially induce viable mutants at a higher efficiency than gamma rays. Thus, ion beams appear to efficiently induce mutants with little radiation damage.

Some of the translocations and inversions were transmittable to the next generation (Ekberg 1969), thus causing sterility in later generations. Consequently, lower sterility in the M_1 generation is desirable for seed propagated crops. Also, in vegetative propagated crops, lower chromosome aberrations are desirable because the M_1 generations are used directly as mutants. For these reasons, it was believed that a mutagen of “high efficiency” on the basis of fertility is better for obtaining a useful mutant.

On the other hand, the relationship between the mutation frequency and irradiation dose showed that irradiation with a low dose of ion beams can induce the same mutation frequency induced by gamma rays. Therefore, the mutation frequencies per unit dose were higher with ion beams than with gamma rays, thus indicating that ion beams had a higher mutation effectiveness than gamma rays, which confirms the results of previous studies (Fujii *et al.* 1966, Mei *et al.* 1994).

The difference of the mutation frequency between ion beams and gamma rays was slightly small in the mutation frequency per M_1 plant in comparison with the mutation frequency per M_2 plant. It was thought that it was due to the difference in the number of initial cells after irradiation between ion beams and gamma rays; the number of initial cells after ion beam irradiation was less than that after gamma ray irradiation, as discussed later. The expected mutation frequency per M_1 plant differs due to the number of initial cells in the seed; mutation frequency per M_1 plant rises with increasing cells. Therefore, the mutation frequency per M_1 plant of gamma rays was evaluated higher than that of ion beams even if the mutation frequencies per initial cells were the same between ion beams and gamma rays. For this reason, it was thought that the mutation induction effect should be compared using the mutation frequency per M_2 plant considering the number of initial cells after treatments of mutagens.

For rice, Kawai advised to harvest M_2 seeds from early developed panicles in order to obtain many mutants of independent genetic origin (qtd. in van Harten 1998). Osone (1963) reported that the generative tissues of the panicles in main culm were derived from corpus initial cells of the embryo, which consists of about 5 or 6 cells in maximum. Accordingly, the panicle of the main culm is chimeric, and therefore the segregation frequency of chlorophyll mutation in M_2 seeds from main culm becomes less than 0.25 (Osone 1963). In our study, we harvested the panicles (M_2 seeds) of the longest culms in each M_1 plant, probably which were main culms, and the averages of the segregation frequency with every irradiation treatment were less than 0.25.

The number of strains with high segregation frequency increased with the irradiation doses. The segregation frequency shows the ratio of parts derived from the mutated

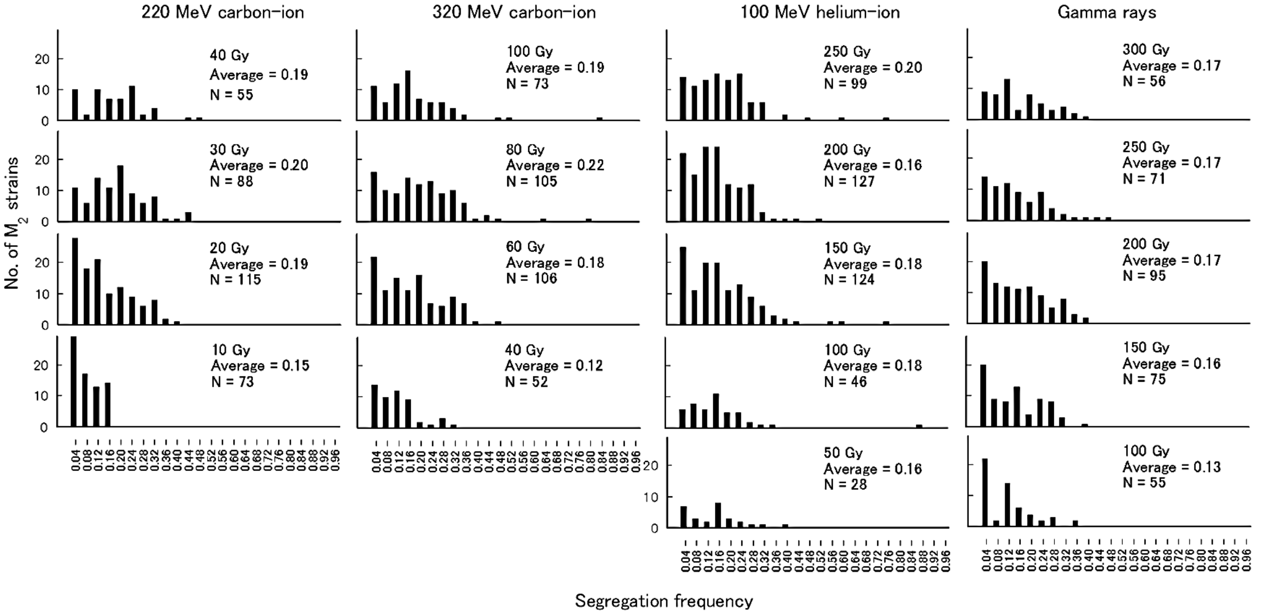

Fig. 8. Frequency distribution of segregation frequency of chlorophyll mutants induced by ion beam and gamma ray irradiation.

cell in the panicle, i.e., the width of the mutated sector. It is unlikely that the parts derived from the mutated cell broaden without a change in the number of initial cells when the irradiation dose increases. It was thought that the increase of the segregation frequency was due to the decreasing number of initial cells as previously discussed by Yamaguchi (1962) and Osone (1963).

The segregation frequencies of ion beams were higher than those of gamma rays. This result indicates that the number of initial cells in seeds after irradiation with ion beams was smaller than that with gamma rays. This difference between ion beams and gamma rays may have been due to the following reason; ion beams cause serious damage because of their high LET, and compared to the number of gamma rays, a relatively small number of ion beams penetrate through cells (Tanaka 1999). It was assumed that some initial cells died even with a low irradiation dose of ion beams, and dead cells with serious damage would be mixed with live cells with little or no damage in apical meristems irradiated with ion beams. In contrast, it was assumed that chromosomes are uniformly irradiated at many points by gamma rays (Yang and Tobias 1979), and as a result, the degree of damage would not differ greatly among initial cells; almost all initial cells were uniformly dead or alive according to the irradiation dose. Thus, it was believed that this difference between ion beams and gamma rays causes the difference in the number of initial cells.

A wide sector is also useful because it facilitates the screening and establishment of mutants in mutation breeding, and it was shown that ion beams were superior with regard to this point.

New mutagens are expected to have a different spectrum from the mutagens presently used. In the comparison of the spectrum of chlorophyll mutations, significant differences between ion beams and gamma rays were not revealed, because the relative frequency of each type of chlorophyll mutation induced by ion beams was similar to that induced by gamma rays; the frequency of albina was highest, followed by viridis. This tendency was previously observed in rice treated with gamma rays (Ando 1968, Yamaguchi 1962), X-rays (Matsuo *et al.* 1958), and thermal neutrons (Matsuo *et al.* 1958, Yamaguchi 1962). The same results were obtained using gamma rays (Doll and Sandfaer 1969), X-rays (Gustafsson 1969), and neutrons (Gustafsson 1969) in similar studies performed with barley. However, the spectrum of mutations induced in barley by 2 chemical mutagens, ethylene oxide (Gustafsson 1969) and ethyl methanesulfonate (Doll and Sandfaer 1969), differed from that induced by radiation; the frequency of viridis was higher than that of albina. The results of our study suggest that the relative frequency of each chlorophyll mutation induced by radiation did not vary among types of radiation, and it is likely to differ from the frequencies produced by chemical mutagens.

We found no difference in the spectrum of chlorophyll mutations between ion beams and gamma rays. Naito *et al.* (2005) showed that both high-LET carbon ions and low-LET gamma rays generate large deletions, regardless of the irradiation dose, and hypothesized that most deletions are not transmitted to progeny. In our study, the comparison of the spectrum was performed in M_2 generation only on the mutations caused by deletions that were transmitted. This might be the reason why there were no differences in the spectrum between radiation type or irradiation dose. In chrysanthemum (Nagatomi *et al.* 1996) and carnation (Okamura *et al.* 2003), it has previously been reported that specific flower color mutants that could not be obtained by gamma rays, could be induced using ion beams. Naito *et al.* (2005) also described that non-transmissible mutations can survive over generations in vegetative propagated crops and have probably contributed to the genetic improvement of such crops. The differences of the spectrum between ion beams and gamma rays might have appeared because both transmissible and non-transmissible mutations can be detected in vegetative propagated crops such as chrysanthemum and carnation. However, further investigations are necessary to reveal differences in the spectrum of mutations, and it might be clarified during its use for mutation breeding aimed at various target traits in many crops.

For practical uses of ion beams, the criteria for the irradiation dose are necessary. With regard to the optimum irradiation dose, to our knowledge, there are no experiment-based reports on gamma rays. Therefore, in the present study, we examined suitable irradiation doses from the perspective of obtaining the maximum number of mutant lines from the seeds sown after irradiation in both ion beams and gamma rays. As a result, the number of mutated lines per irradiated seed was highest at a dose that corresponded to the shoulder appearing in the survival curves in both ion beams and gamma rays. Irradiation at the shoulder dose does not affect survival markedly, approximately 90% survival. Our result demonstrated that irradiation with such low dose was enough to efficiently produce mutants. For gamma ray treatment, a growth reduction for M_1 seedlings of 30%–50% or a survival rate of 40%–60% in control plants has often been considered the criterion for a promising treatment. In order to avoid considerable change in the genetic background (van Harten 1998), treatments are nowadays performed with lower doses than the doses used for those criteria. The shoulder dose is lower in comparison to the 50% lethal dose, which has been considered a criterion for a promising treatment in gamma rays. Therefore, the change of the genetic background appeared to be lower, but this is outside the scope of our study. Consequently, the shoulder dose is considered to be a more suitable criterion for the assessment of irradiation treatment for efficiently obtaining useful mutants without considerably changing their genetic background.

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