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REVIEW
Characteristics of Ion Beams as Mutagens for Mutation Breeding in Rice and Chrysanthemums

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Abstract

Mutation breeding is a useful method to improve crops. More than 20 years have passed since mutation breeding with ion beams started in Japan, since which time many mutant varieties have been produced with ion beams. However, ion beams have not been sufficiently characterized in terms of mutagens for plant mutation breeding. This review introduces the characteristics of ion beams as mutagens for mutation breeding; investigated with three objectives: to obtain useful mutants with limited plant damage by irradiation treatment, the width of the mutated sector produced by irradiation, and the mutation spectrum, compared to gamma rays using rice and chrysanthemums. In addition, the optimum dose of ion beams and the criteria for an optimum irradiation dose are shown, based on which the irradiation treatment for the success of mutation breeding with ion beams is also described.

Discipline: Plant breeding
Additional key words: mutated sector, mutation spectrum, optimum dose

Introduction

Mutation breeding is an important method of crop improvement. Over the past 75 years, more than 3,200 mutant varieties have been produced worldwide. About 480 of these were produced in Japan, of which 46% are in rice (*Oryza sativa*), followed by chrysanthemum (*Chrysanthemum morifolium*) at 12%[7].

The type of mutagenic treatment and the methods used are important factors to obtain successful mutation breeding results. Physical mutagens such as gamma rays, X-rays and chemical mutagens such as ethyl methanesulfonate (EMS) have been widely used to induce mutations in various crops since the 1940s and 1960s, respectively[7,28].

Ion beams consist of ion particles accelerated by a cyclotron, and have been used for mutation breeding of various crops in Japan. It was shown that ion beams with high linear energy transfer (LET) have higher biological effects and mutagenesis on plants compared to low LET radiations, such as gamma rays, X-rays and electrons in the 1960s-70s[8,10]. Subsequently, plant mutation breeding with ion beams was started in the 1990s in Japan via cyclotron by the Japan Atomic Energy Agency (JAEA) and RIKEN. Over the past 20 years, mutation breeding by ion beam irradiation has been performed in various plants. Along with the increased use for mutation breeding, the molecular nature of mutation induced with ion beams has been gradually clarified. In addition to JAEA and RIKEN, at present, irradiation facilities at the Wakasa Wan Energy Research Center and National Institute of Radiological Sciences are also available for ion beam irradiation of plant materials.

Consequently of mutation breeding research with ion beams, many mutants were obtained, e.g. low cadmium rice[12], salt-resistant rice[1], self-compatible common buckwheat (*Fagopyrum esculentum*)[9], cherry blossom tree (*Cerasus* ‘Keio-zakura’) that blooms in all four seasons[2] and verbena (*Verbena* × *hybrida*) with improved flowering[13]. Moreover, mutant flower colors and shapes were obtained in various ornamental plants.

A successful mutant variety with ion beams is the ‘Aladdin’ of the standard-type chrysanthemum[27]. This variety was produced to save farmers’ labor involved in removing the axillary flower buds, and has few axillary flower buds compared to the original ‘Jinba’ variety. From approximately 10,000 regenerated plants, a plant with few axillary

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flower buds, good quality cut flowers and vigorous growth, was selected.

Ion beams have thus evolved as a new mutation method, but have not been sufficiently characterized in terms of mutagens for plant mutation breeding.

In this review, the mutagenic ‘efficiency’, mutation spectra and mutated sector size by ion beams were shown in comparison with gamma rays. In addition, the optimum irradiation dose and the criteria for the optimum dose were indicated. Based on these results, irradiation treatment for the success of mutation breeding was described.

A characteristic feature of ion beams is their ability to deposit high energy on a target, densely and locally, as opposed to low LET radiation, such as gamma- and X-rays[25,34]. In addition, relatively few ion particles penetrate through cells compared to gamma rays[25]. From those features, ion beams are expected to produce higher mutation frequencies. Moreover, it was thought that mutants involving a change in only the target trait, with the remaining genetic background unaffected, were obtained from the hypothesis that ion beams cause less damage to chromosomes compared to gamma- and X-rays.

In our study, three types of ion beams with different LETs: 220 MeV carbon ions (mean LET, 107 keV/μm), 320 MeV carbon ions (mean LET, 76 keV/μm), and 100 MeV helium ions (mean LET, 9 keV/μm) generated by an azimuthally varying field cyclotron at the ion-irradiation research facility of Takasaki Ion Accelerators for Advanced Radiation Application (Japan Atomic Energy Agency, Takasaki, Japan) were used. Gamma rays were irradiated with a dose of 10 Gy/h at the Institute of Radiation Breeding (National Institute of Agrobiological Sciences, Hitachiomiya, Japan).

The LET of 220 MeV carbon ions is 535 times higher than that of gamma rays (LET 0.2 keV/μm). When 220 MeV carbon ions are irradiated at a dose of 1 Gy, four tracks are produced in a cell[25]. In contrast, 2,000 spurs are produced in a cell at a dose of 1 Gy with gamma rays[25]. This means significant differences in the LET and the number of ion particles or gamma rays penetrating through cells between 220MeV carbon ions and gamma rays.

Rice (*O. sativa* cv. ‘Hitomebore’) and chrysanthemums (*C. morifolium* cv. ‘Taihei’) were used as the plant materials. Chlorophyll- and flower-color mutations were investigated in rice and chrysanthemums, respectively. Chlorophyll mutations were used in previous research about gamma rays[18], neutrons[18] and chemical mutagens[10,18,21] performed using rice and barley because of useful markers in mutation study[28]. There has also been considerable mutation research on flower color in chrysanthemums[5,11,19] because mutation is an important method of improving them. The spontaneous mutation rate with our method was 0.02% in chlorophyll mutations of rice (data not shown) and 0.6% in flower color mutation of chrysanthemums as shown in the non-irradiation of Table 1.

Table 1. Type and number of flower color mutants induced by ion-beam and gamma-ray irradiation

Radiation	Dose (Gy)	No. of plants investigated	No. of mutants	Mutation frequency (%)	No. of flower color mutants											
					Pale pink	Relatively pale pink	Deep pink	White	Pinkish white	Yellow	Pale yellow	Orange	Deep orange	Pale pink/ yellow[Z]	Pale yellow/ yellow[Z]	Other
220 MeV carbon-ion beam	5	159	23	14.5	5	0	0	5	2	0	2	6	0	0	3	0
	3	337	32	9.5	9	5	0	4	3	0	1	6	2	0	2	0
	2	354	29	8.2	11	1	0	5	4	0	1	4	1	0	2	0
	1	330	16	4.8	1	2	0	5	2	0	1	4	1	0	0	0
320 MeV carbon-ion beam	5	239	39	16.3	10	1	2	2	6	0	7	10	0	1	0	0
	3	320	23	7.2	7	0	1	3	2	0	3	1	6	0	0	0
	2	287	20	7.0	7	1	1	1	0	0	4	5	1	0	0	0
	1	268	6	2.2	1	0	0	0	0	0	2	0	3	0	0	0
100 MeV helium-ion beam	15	138	17	12.3	6	3	0	1	2	0	1	2	1	0	0	1
	10	225	14	6.2	2	1	0	6	1	1	0	2	1	0	0	0
	5	291	5	1.7	2	0	0	0	0	0	0	3	0	0	0	0
	2	275	3	1.1	2	0	0	0	0	0	0	1	0	0	0	0
Gamma rays	40	128	12	9.4	3	1	1	1	0	0	0	1	4	0	1	0
	30	184	13	7.1	3	0	1	1	2	0	1	5	0	0	0	0
	20	244	13	5.3	4	1	0	2	0	0	0	3	0	0	3	0
	10	399	15	3.8	6	0	0	2	1	0	2	2	0	0	2	0
Non-irradiated		937	6	0.6	2	0	0	0	0	0	0	0	0	1	2	1

Z: color of outer ray floret/color of inner ray floret.

Data shown in “Non irradiation” were taken from the regenerated plants from non-irradiated leaf explants.

Chrysanthemums (*C. morifolium* cv. ‘Taihei’), with pink flowers were used as the plant materials.

Data were cited from Yamaguchi et al.[33].

Characteristics of ion beams

1. Mutation efficiency

When some chemical mutagens, such as methyl methanesulfonate (MMS) and EMS[15], were first used for mutation induction in the 1960s, mutation ‘efficiency’ and mutation spectra were compared to those of gamma- or X-rays using barley or rice. The term ‘efficiency’ is defined as the proportion of specific desirable mutagenic changes to plant damage due to mutagen treatment, such as chromosomal aberrations in mutation breeding[15,18,21]. Konzak *et al.* (1965)[15] suggested that the usefulness of any mutagen in plant breeding depends not only on its mutagenic effectiveness but also its mutagenic efficiency. Ion beams have been shown to exhibit higher mutation induction effects compared to gamma rays[8,17], X-rays[10,35], and electrons[24]. However, the efficiency of ion beams has not been compared to that of gamma- or X-rays.

To evaluate efficiency, the relationship between mutation frequency and chromosome aberration was examined. As the index of chromosome aberration in rice, sterility was used because the sterility in plants originating from irradiated rice seeds was considered due to chromosomal aberrations, similar to that for barley irradiated with neutrons[6] and X-rays[6]. In chrysanthemums, a reduction in nuclear DNA content, which is a serious chromosomal aberration and easily detectable by flow cytometry, was used as the index[33].

The relationship between the frequency of the chlorophyll mutation in the M_2 generation and fertility in rice are shown in Fig. 1. There were significant negative linear relationships between the fertility and mutation frequency per M_2 plant in the three types of ion beams. A similar relationship was also found in gamma rays, although it was not significant. 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 the 320 MeV carbon-ion beam and gamma rays were lower, i.e., 1.1%. Thus, the 220 MeV carbon-ion beam and the 100 MeV helium-ion beam induced higher frequencies of mutation based on fertility than gamma rays, suggesting that their ‘efficiencies’ exceeded that of gamma rays in rice.

The relationship between the frequency of flower color mutations and the nuclear DNA contents in plants regenerated from a callus induced on irradiated leaf segments in chrysanthemums is shown in Fig. 2. The mutation frequencies due to the 220 MeV carbon-ion beam, 320 MeV carbon-ion beam, and gamma rays were similar when based on a reduction in nuclear DNA content, whereas those of the 100 MeV helium-ion beam were lower than those of the other radiation types. It thus appears that the efficiencies of 220 and 320 MeV carbon-ion beam equaled or exceeded that of gamma rays, although the 100 MeV helium-ion beam had lower efficiencies than gamma rays in chrysanthemums.

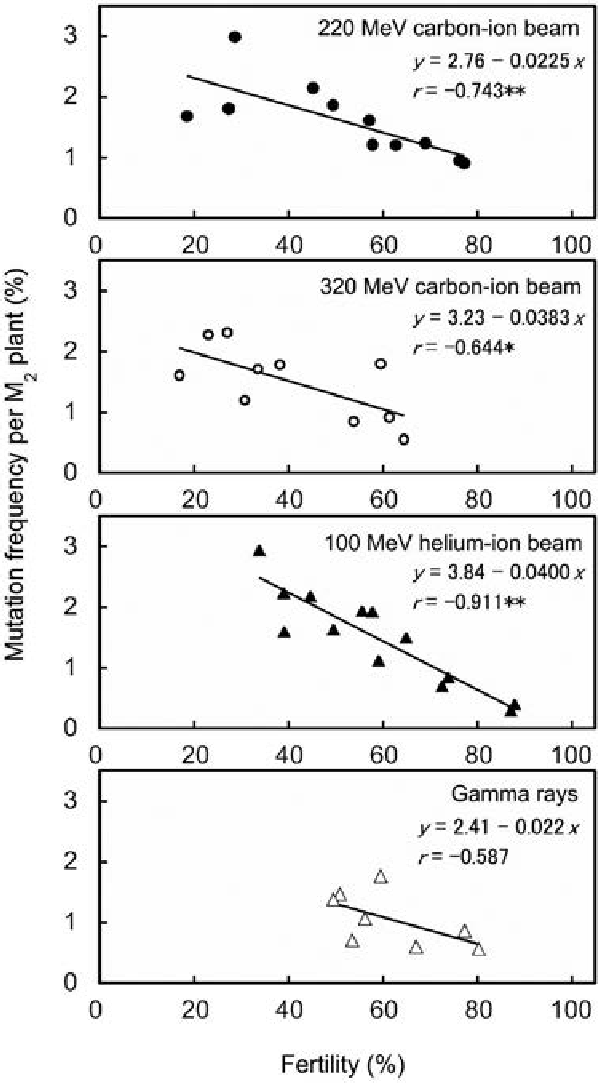

Fig. 1. Relationship between fertility and mutation frequency per M_2 plant in rice

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 randomly selected for each treatment.

* and ** Significant at 5 and 1% levels, respectively. Rice (*O. sativa* cv. ‘Hitomebore’) was used as the plant materials. Data were cited from Yamaguchi et al.[31].

The most ‘efficient’ ion species differed between rice and chrysanthemums. The 100 MeV helium-ion beam with LET 9 keV/μm was most effective for inducing chlorophyll mutants in rice[27], while the 320 MeV carbon-ion beam with LET 76 keV/μm was the most effective in inducing flower color mutants in chrysanthemums[29]. It was thus thought that there was a difference in the most ‘effective’ ion species,

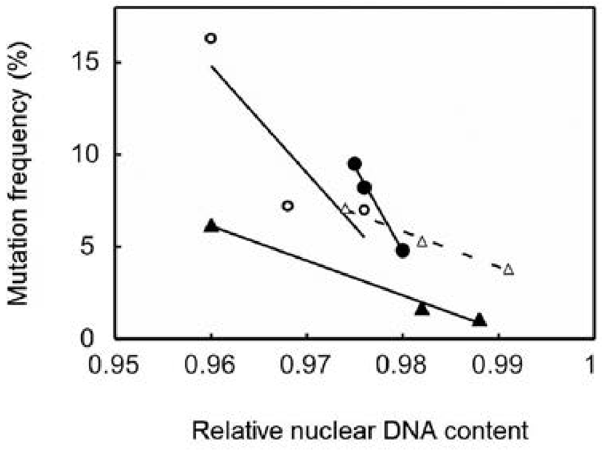

Fig. 2. Relationship between the nuclear DNA content and frequency of flower color mutations in chrysanthemums

●: 220 MeV carbon-ion beam, $y = 900 - 914x$, $r = -0.997^*$; ○: 320 MeV carbon-ion beam, $y = 572 - 581x$, $r = -0.875$; ▲: 100 MeV helium-ion beam, $y = 187 - 188x$, $r = -0.995$; △: gamma rays, $y = 196 - 194x$, $r = -0.996$. * Significant at 5% level. Chrysanthemum (*C. morifolium* cv. 'Taihei') was used as the plant materials. Data were cited from Yamaguchi et al.[33].

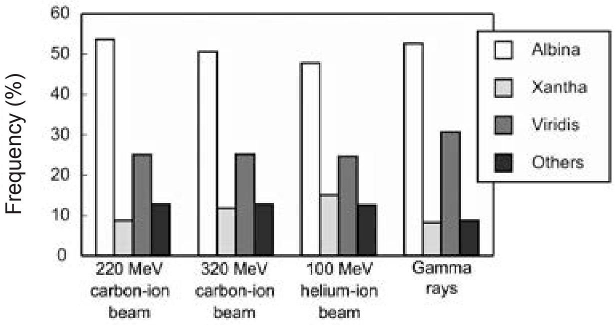

Fig. 3. Frequency of different types of chlorophyll mutants induced by ion-beam and gamma-ray irradiation in rice

The frequency is determined as the number of each type of chlorophyll mutants divided by the total number of chlorophyll mutants induced by each radiation type. Chlorophyll mutation was investigated in irradiation treatment with 220 MeV carbon ions at doses of 10, 20, 30, and 40 Gy; 320 MeV carbon ions at doses of 40, 60, 80, and 100 Gy; 100 MeV helium ions at doses of 50, 100, 150, 200, and 250 Gy; and gamma rays at doses of 100, 150, 200, 250, and 300 Gy. Rice (*O. sativa* cv. 'Hitomebore') was used as the plant material. Data were cited from Yamaguchi et al.[34].

namely the most 'effective' LET, to mutation induction between rice and chrysanthemums. It was reported that the mutation induction effect was LET-dependent, regardless of ion species in *Arabidopsis*[14], meaning the difference in the most 'effective' ion species between rice and chrysanthemums was not due to ion species but due to the LET of each ion beam. Kazama *et al*. (2008)[14] reported that a LET of 30 KeV/µm was most effective for inducing albino mutants in the M_2 generation of *Arabidopsis*. Consequently, it is possible that the optimum LET for mutation induction differs among plant species, whereas the differences in DNA damage by ion species or plant species remain unclear.

2. Mutation spectra

Different mutation spectra from currently used mutagens, namely, the induction of novel mutants, are expected via the new mutagen. Therefore, the mutation spectrum for ion beams is compared to gamma rays in the chlorophyll mutation of rice and the flower color mutation of chrysanthemums.

According to previous reports[28], the relative frequencies of each type of chlorophyll mutation, 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 in rice among the three types of ion beams and gamma rays. Regardless of radiation type and irradiation dose, the frequency of albina was highest, followed by viridis. When totaled in terms of each radiation type, the frequency of albina was about 50%, whereas that of xantha ranged from 8-15%, viridis from 25-30%, and other mutations from 8-13% per radiation type (Fig. 3). Thus, no remarkable difference in the relative frequencies of each type of mutation were observed among the three types of ion-beam and gamma-ray treatments.

It was reported that ion-beam-specific flower color mutants, which are not obtained using gamma rays, were obtained in carnations (*Dianthus caryophyllus*)[22] and chrysanthemums[19]. Nagatomi et al. (1998)[19] reported that ion-beam-specific flower color mutants such as complex and stripe types were obtained from an original 'Taihei' variety of chrysanthemum, with a pink flower color, using 220 MeV carbon-ion beams. We also used the same variety 'Taihei'. Consequently, twelve types of flower color mutants were obtained (Table 1). However, ion-beam-specific mutants reported in Nagatomi et al. (1998)[19] were not observed, and no differences were also found in the relative frequencies of each flower color mutation between three types of ion beams and gamma rays.

Our results therefore suggest that there was no difference in the mutation spectrum between ion-beam and gamma-ray irradiation treatments.

It was reported that the frequency of flower color mutations differed according to the irradiated plant materials in the acute irradiation of gamma rays to 'Taihei'; flower color mutants were obtained at a higher frequency when cultured petals were irradiated rather than cultured leaves[20]. This result suggests that mutagenesis in genes related to flower color differs between petal and leaf[20]. Nagatomi et al. (1998)[19] used petals, while we used leaves in the present study. This difference might be the reason for not obtaining ion-beam-specific flower color mutants in our study.

3. Mutated sector size

To obtain mutants, seeds (M_2) or buds (vM_2) must originate from mutated cells of irradiated seeds (M_1) or buds (vM_1). If a mutation occurs in a cell of the apical meristem, a lineage of mutated cells develops as a sector, embranching any leaves and buds. If the mutated sector is narrow, there is little possibility of any flowers or buds forming within the mutated sector, which hinders efforts to isolate a mutant. Consequently, a wide mutated sector is desirable to efficiently establish and obtain mutants.

In rice, the panicle of the main culm from an irradiated seed is chimeric, because the generative tissues of the panicles in main culm were derived from the initial cells of the embryo; consisting of a maximum of about 5 or 6 cells[23]. Accordingly, the ‘segregation frequency’ of chlorophyll mutants in M_2 seeds from the main culm, which were calculated as the number of chlorophyll mutants divided by the number of germinated M_2 plants in each strain, is expected to become less than 0.25 from the ‘segregation ratio’ of 3:1[23]. The increase in segregation frequency in rice was due to the decline in initial cells by irradiation as discussed previously[23,29]. The mutated sector size can be estimated from the segregation frequency of chlorophyll mutants in M_2 seedlings having originated from seeds of the main culm.

The segregation frequency of chlorophyll mutants induced by gamma rays was 0.17 by the irradiation dose resulting in a survival rate of 55-90% (Fig. 4). In contrast, segregation frequencies of mutants at the irradiation dose, which also resulted in a survival rate of 55-90%, were 0.19–0.20 by the 220 MeV carbon-ion beam and 0.18–0.22 by the 320 MeV carbon-ion beam respectively. The segregation frequencies produced with the 220 and 320 MeV carbon-ion beams thus exceeded those produced with gamma rays. Likewise, in the 100 MeV helium-ion beam, the segregation frequency seemed to exceed that of gamma rays, although the segregation frequency at a survival rate of 60% was lower than that by gamma rays. These results suggest that the mutated sector size produced by ion beams was wider than that by gamma rays.

In chrysanthemums, the mutated sector size can be estimated from the segregation ratio of flower color mutants in progenies produced by twice cutting back of shoots from irradiated lateral buds. In the sector size by this method, there were no differences among the 220 MeV carbon ion, 100 MeV helium ion and gamma rays[32]. However, analysis of the chimeric structure of flower color mutants showed a difference in the expansion of mutated sectors through different layers between ion beams and gamma rays[32].

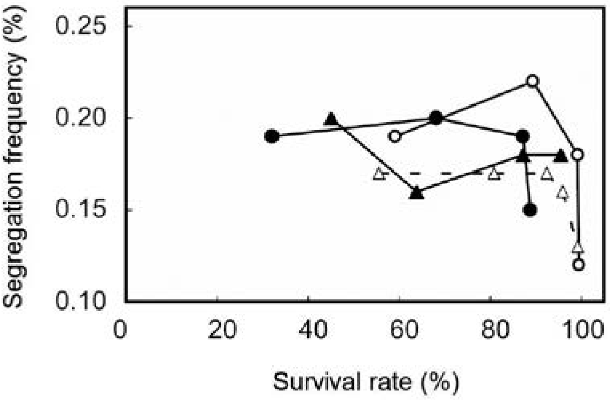

Fig. 4. Comparison of chlorophyll mutant segregation frequencies between ion beams and gamma rays in rice

●: 220 MeV carbon-ion beam; ○: 320 MeV carbon-ion beam; ▲: 100 MeV helium-ion beam; △: gamma rays. Rice (*O. sativa* cv. ‘Hitomebore’) was used as the plant materials.

A plant consisting of two or more genetically different somatic tissues is called a chimera[4]. Angiosperm plants consist of three layers LI, LII, and LIII, outer to inner[4]. A periclinal chimera plant is one in which only one of the three layers differs from others, or in which all three layers differ from each other. Periclinal chimeras are useful for producing various phenotypes, such as those based on various arrangements of genotypically different groups of cell layers, which would be expected in ornamental plants such as chrysanthemums[4]. Conversely, a plant in which mutated sectors have expanded through all layers is called a sectorial chimera. A solid mutant was considered to be produced by forming sectorial chimera from one mutated cell by expanding the mutated sector through layers, and by the subsequent release of chimera. In solid mutants, the risk of ‘back-sporting’ which is undesirable in commercial cut flower production, is far lower.

Since roots develop from the LIII tissue[4], the tissue genotype of the LIII layer can be determined from the flower color of plants derived from root culture. Therefore, whether a plant is a periclinal chimera or a solid mutant can be determined by comparing the flower color of donor mutant plants to that of plants derived from their roots.

On irradiating the apical meristem with gamma rays, periclinal chimera mutants are generally obtained[3,16]. In our study, all flower color mutants obtained with gamma rays were periclinal chimeras (Table 2). In contrast, some mutants obtained by ion beam irradiation seemed solid mutants.

In irradiation of 220 MeV carbon ions at 2 Gy, 100

Table 2. Comparison of the flower color of plants derived from roots of mutants with that of mutants induced with ion-beam and gamma-ray irradiation to lateral buds of chrysanthemum

Radiation	Dose (Gy)	Mutant no.	Flower color		Suggested chimera structure
			Mutants	Plants derived from roots	
220 MeV carbon-ion beam	2	#511	Pale pink	Pale pink	Solid mutant
		#513	Deep pink	Deep pink	Solid mutant
		#524	Pale yellow	Pale yellow	Solid mutant
		#531	Deep orange	Deep orange	Solid mutant
		#532	Deep orange	Deep orange	Solid mutant
		#515	Pale pink	Pink [Z]	Periclinal chimera
		#520	Wine red	Pink	Periclinal chimera
		#521	Pale pink	Pink	Periclinal chimera
		#601	Pale yellow	Pink	Periclinal chimera
100 MeV helium-ion beam	10	#546	Pale yellow	Pale yellow	Solid mutant
		#574	Deep orange	Orange	Periclinal chimera
		#545	Orange	Pink	Periclinal chimera
		#564	Deep orange	Pink	Periclinal chimera
		#565	Pinkish white	Pink	Periclinal chimera
		#566	Pinkish white	Pink	Periclinal chimera
		#568	Orange	Pink	Periclinal chimera
		#571	Deep orange	Pink	Periclinal chimera
		#572	Pinkish white	Pink	Periclinal chimera
100 MeV helium-ion beam	5	#503	White	White	Solid mutant
		#506	Orange	Orange	Solid mutant
		#537	Deep orange	Deep orange	Solid mutant
		#502	Rather pale pink	Pink	Periclinal chimera
		#507	Orange	Pink	Periclinal chimera
		#510	Orange	Pink	Periclinal chimera
		#519	Pale pink	Pink	Periclinal chimera
Gamma rays	80	#549	Orange	Deep pink	Periclinal chimera
		#550	Orange	Deep orange	Periclinal chimera
		#557	Rather pale pink	Pink	Periclinal chimera
		#558	Pale yellow	Pink	Periclinal chimera
		#581	Pinkish white	Pink	Periclinal chimera
		#584	Pale pink	Pink	Periclinal chimera
		#585	Orange	Pink	Periclinal chimera
		#587	Pale pink	Pink	Periclinal chimera
		#591	Pale yellow	Pink	Periclinal chimera
		#598	Yellow	Pink	Periclinal chimera

Z: The same flower color as the original cultivar 'Taihei.'
Chrysanthemums (*C. morifolium* cv. 'Taihei'), with pink flowers, were used as the plant materials. Data were cited from Yamaguchi et al.[34].

MeV helium ions at 10 Gy, and gamma rays at 80 Gy, there were no significant differences in the effects on survival and mutation induction (data not shown). However, solid mutants appeared only with ion beams (Table 2). Accordingly, the high biological effectiveness of ion beams could not account for solid mutant induction observed only in ion beams. Moreover, solid mutants were also obtained at 100 MeV helium ions of 5 Gy, at which point the mutation frequency was lower than that of 100 MeV helium ions at 10 Gy (Table 2). Therefore, the factor for obtaining solid mutants only with ion beam irradiation seemed to differ from that for inducing flower color mutation.

It was suggested that the mutated sector size produced by ion beams was wider based on the high segregation ratio in rice and expansion of the mutated sectors through different layers in chrysanthemums compared to gamma rays. It is unlikely that parts derived from the mutated cell would broaden relatively without reducing the number of initial cells when the irradiation dose increases. Consequently, it was thought that the number of initial cells in the apical meristems irradiated with ion beams was lesser than that with gamma rays.

Ion beams cause serious damage because of their high LET, and a relatively small number of ion beams penetrate through cells compared to the number of gamma rays[25]. It was thought that these characteristics of ion beams resulted in differences in mutated sector size to gamma rays. It was also assumed that some initial cells died, even with a low ion beam irradiation dose and that dead cells with serious damage would be mixed with live cells with little or no damage in apical meristems. Accordingly, the degree of radiation damage due to ion beams differs significantly between cells comprising the apical meristem. In contrast, it was assumed that chromosomes are uniformly irradiated at many points by gamma rays[35], and that gamma rays tend to produce lesions uniformly distributed throughout cells[35]. Consequently, the degree of damage would not differ significantly among initial cells; almost all initial cells were uniformly dead or alive depending on the irradiation dose, and the survival potential of only a few cells was low. Thus, this difference in damage to the initial cells by ion-beam and gamma-ray treatments resulted in a difference in the number of surviving initial cells; therefore, the mutated sectors produced with ion beams were wider than those produced with gamma rays.

Optimum ion-beam radiation dose

An appropriate dose of irradiation is essential for successful mutation breeding, and the practical use of ion beams requires information about effective and efficient doses. In gamma rays meanwhile, an M_1 seedling growth reduction of 30-50% or a survival rate of 40-60% in control plants has often been considered the criterion for promising gamma-ray irradiation treatment. However, no experiment-based reports exist on the optimum irradiation dose of gamma rays, although gamma rays have been used for mutation breeding for a long time. Therefore, suitable irradiation doses were examined in rice, based on efforts to obtain the maximum number of mutant lines from seeds sown after irradiation with ion beams and gamma rays[31]. Consequently, the number of mutated M_1 plants per M_1 seed sown peaked at a dose which almost corresponded to the shoulder appearing in the survival curves in the three types of ion beams and gamma rays. The result of the 220 MeV carbon-ion beam is shown in Fig. 5.

The survival rate at the shoulder dose on the survival curves was approximately 90% and hence lower in comparison to the 50% lethal dose. Sterility in rice was induced by ion beams and gamma rays and was mainly due to chromosomal aberrations. Therefore, alterations in the genetic background by irradiation at the shoulder dose appeared lower than those irradiated at the 50% lethal dose. Consequently, the shoulder dose was a more suitable criterion to efficiently obtain useful mutants without considerably altering their genetic background.

Also in chrysanthemums[33] and roses (*Rosa* x *hybrida*)[30], mutants were obtained, even at low doses of ion beam irradiation that did not affect shoot regeneration or survival, respectively. Our results demonstrated that lower-dose irradiation enables the production of mutants with a relatively unmodified genetic background, making them useful. Tanaka et al. (2010)[26] described how the shoulder dose which hardly affected survival would suffice to obtain mutants efficiently. Furthermore, Ueno[27] pointed out that lower-dose irradiation was important to produce the chrysanthemum variety with few axillary flower buds, 'Aladdin'. We also believe lower-dose irradiation is vital to obtain single-point mutants, especially for radiation breeding of vegetatively propagated crops, because the mutants thus obtained would be directly used as new cultivars.

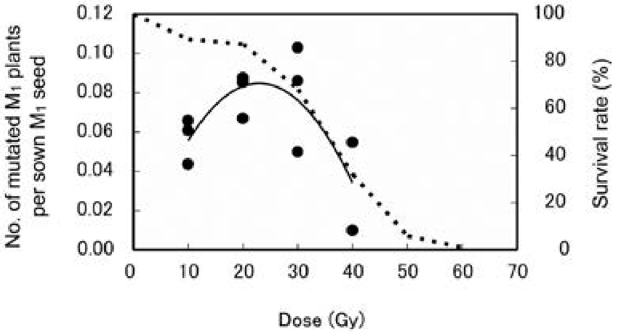

Fig. 5. Relationship of the number of mutated M_1 plants per M_1 seed sown and survival rate to an irradiation dose with a 220 MeV carbon-ion beam

The number of mutated M_1 plants per M_1 seed sown was determined as the number of M_1 plants that produced chlorophyll mutants in their progeny (M_2 plant) divided by the number of M_1 seeds sown after irradiation. ● Solid line: the number of mutated M_1 plants per M_1 seed sown: Dotted line: survival rate. Rice (*O. sativa* cv. 'Hitomebore') was used as the plant material.

Mutation breeding is mainly used to alter only a specific trait when improving crops. The high 'efficiency' based on chromosomal aberration indicates a high mutation frequency in the target trait to the change of genetic background; the low possibility of mutation induction in two different traits at the same time. In our study, the efficiencies of ion beams equaled or exceeded that of gamma rays. Consequently, ion beams are considered more suitable for the induction of point mutation compared to gamma rays.

The LET was indicated as an important factor for mutation induction. The LET of ion beams can be controlled, and we can select the type of ions and their LET, while mutation breeding with ion beams is used in various crops. There was the possibility of the optimum LET for mutation induction differing among plant species. Therefore, the possibilities that ion beams can be optimized to obtain useful mutants efficiently remains. Further investigation is expected to optimally utilize ion beams.

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