Mutation Rate and Novel tt Mutants of Arabidopsis thaliana Induced by Carbon Ions

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ABSTRACT

Irradiation of Arabidopsis thaliana by carbon ions was carried out to investigate the mutational effect of ion particles in higher plants. Frequencies of embryonic lethals and chlorophyll-deficient mutants were found to be significantly higher after carbon-ion irradiation than after electron irradiation (11-fold and 7.8-fold per unit dose, respectively). To estimate the mutation rate of carbon ions, mutants with no pigments on leaves and stems (tt) and no trichomes on leaves (gl) were isolated at the M2 generation and subjected to analysis. Averaged segregation rate of the backcrossed mutants was 0.25, which suggested that large deletions reducing the viability of the gametophytes were not transmitted, if generated, in most cases. During the isolation of mutants, two new classes of flavonoid mutants (tt18, tt19) were isolated from carbon-ion-mutagenized M2 plants. From PCR and sequence analysis, two of the three tt18 mutant alleles were found to have a small deletion within the LDOX gene and the other was revealed to contain a rearrangement. Using the segregation rates, the mutation rate of carbon ions was estimated to be 17-fold higher than that of electrons. The isolation of novel mutants and the high mutation rate suggest that ion particles can be used as a valuable mutagen for plant genetics.

The mutagenic effect of ion particle irradiation, mainly on somatic mutations, has been investigated using various plant species (Smith 1972). The highest relative biological effectiveness (RBE) was found with LETs ~100–200 keV/μm. In contrast, few studies have been done on germline mutations. Mei et al. (1994) investigated germline mutations in rice and observed a high incidence of semidwarf mutants induced by argon and iron ions. In addition, they determined the mutation spectrum of argon ions by scoring the change of characters of panicles, spikelets, and grains, as well as changes in sheath color, plant size, and the timing of maturity. However, these previously identified germline mutations were mainly studied on mutated phenotypes for which the numbers of the corresponding genes were unknown.

For genetics and breeding, it is fundamentally important to know the germline mutation rate induced by a mutagen. Koornneef et al. (1982) studied the mutation rates of ionizing radiation in Arabidopsis and found that mutation rates per Gray for X rays and fast neutrons were within the range of 10−6–10−7 and 10−5, respectively. However, there are no reports, to our knowledge, of the germline mutation rate of plants after ion particle irradiation. As a first step to evaluate the use of ion particle mutagenesis in higher plants, the frequency of germline mutations after carbon-ion irradiation (LET = 113 keV/μm) was measured by Müller’s embryo test (Müller 1963), and further, the segregation and mutation rates for carbon-ion-induced Arabidopsis mutants
were estimated by isolating transparent testa (tt) and glabrous (gl) mutants.

MATERIALS AND METHODS

Plant material: Plants of A. thaliana ecotype Columbia were grown on hydroponics (HYDONEX, Osaka, Japan) or on rockwool (Nichiasu, Tokyo) at a temperature of 25°C ± 3°C in an air-conditioned greenhouse and were subirradiated at 3- or 4-day intervals with 0.03% HYPOX (HYDONEX). Seeds of the mutants used for the complementation test were provided from the Nottingham Arabidopsis Stock Center (stock nos. N64, N65, N66, N84, N85, N86, N87, N88, N89).

Irradiation: Irradiation of dry seeds was carried out as previously described (Tanaka et al. 1997a). The energy of carbon ions was 220 MeV and the mean LET within the seeds was calculated to be 113 keV/µm. The energy of electrons was 2 MeV and the mean LET was calculated to be 0.2 keV/µm. The dry seeds were irradiated with a dose of 150 Gy for carbon ions and with a dose of 750 Gy for electrons.

Embryo test: A total of 600 M1 plants were subjected to analysis for each mutagen treatment. Frequencies of embryonic lethals and of chlorophyll-deficient mutants were scored as previously described (Müller 1963; Mesken and Van der Veen 1968; Dellaert 1980a). In brief, the fifth siliques on the main stem of M1 plants were opened up just before ripening and their seeds were scored under a stereoscopic microscope. We classified the seeds of green color as normal, seeds with seed coat turned prematurely brown as embryonic lethal, and seeds of white/pale green color as chlorophyll-deficient mutants. Only the seeds on one side of the septum were counted for ease of handling. Embryonic lethality was calculated as a percentage of embryonic lethals among fertilized ovules, and chlorophyll deficiency as a percentage of chlorophyll-deficient mutants among nonlethal embryos.

Isolation of tt and gl mutants: Irradiated M1 seeds were sown and selfed to obtain M2 seeds. M2 seeds from 100 M1 lines were pooled, and 400–500 seeds from each pool were sown and screened for mutants. A total of 292 M1 pools for carbon ions and 96 M1 pools for electrons were screened in the present study. tt and gl mutants were identified at the frequency of embryonic lethals and of chlorophyll-deficient mutants in the 50 cycles, the samples were incubated at 72°C for another 7 min to complete extension. The amplified DNA fragments were analyzed by 1.5% agarose gel electrophoresis in TAE buffer and were visualized by ethidium bromide staining. Fragments that showed no apparent size alterations were further analyzed by direct sequencing. Sequences of both DNA strands of PCR fragments were determined by the dideoxynucleotide chain termination method (Sanger et al. 1977) with an automated sequencer (ABI Prism 310 genetic analyzer, Perkin-Elmer, Norwalk, CT). Analyses of the sequences were performed with the GENETYX MAC program (version 10.0, Software Development, Tokyo).

RESULTS

Electrons used in the present study have a LET of 0.2 keV/µm. Since this value is the same as the LET of γ-rays, biological effects of electrons could be regarded as equivalent to those of γ-rays. The RBE of carbon-ion exposures against electron exposures on the survival of Arabidopsis is approximately five (Tanaka et al. 1997a). Little effect on survival was observed up to 200 Gy for carbon ions and 1000 Gy for electrons, but survival rates were significantly reduced beyond those doses for each radiation. The doses for mutagenesis were determined to be 150 and 750 Gy for carbon ions and electrons, respectively. These doses were irradiated to give little effect on survival and an identical amount of lethal damage, thus minimizing the effects of lethal damage to the plant. Mutation frequencies per Gray or mutation rates per Gray were compared on the basis of the finding that radiation-induced mutation rates increase linearly with increasing dose (Koornneef et al. 1982).

Embryonic lethals and chlorophyll mutants: To study the mutational effect of carbon ions, we first looked at the frequency of embryonic lethals and of chlorophyll-deficient mutants in M1 siliques (Müller’s embryo test; Table 1). Embryonic lethals and chlorophyll mutants were found even without irradiation, although the frequencies were low (both <1%). The induced frequencies of embryonic lethals and chlorophyll-deficient mutants after irradiation were estimated by subtracting the percentage of unirradiated samples and were found to be 6.0 and 8.4% for carbon ions of 150 Gy and 2.6 and 5.4% for electrons of 750 Gy, respectively. That is, per unit dose, the frequencies of embryonic lethals and chlorophyll-deficient mutants induced by carbon ions were 11-fold and 7.8-fold higher, respectively, than those induced by electrons.

tt18 and tt19 mutants: A total of 62 tt mutants and 26 gl mutants were isolated after carbon-ion irradiation (see Table 4). From the complementation test, one line of tt3, seven lines of tt4, two lines of tt3, three lines of tt6, five lines of previously unidentified tt mutants, six lines of gl1, one line of gl2, and four lines of ttg1 were identified. On the other hand, 4 tt mutants and 7 gl mutants were isolated after electron irradiation. One line each of tt3 and tt4 and three lines of gl2 were
identified. However, five carbon-ion-induced tt mutant lines in the present study were found to complement all of the tt3, tt4, tt5, tt6, and tt7 mutants, which indicated that mutations had occurred in other loci. These five mutant lines were crossed to each other and sorted into two complementation groups. Each of the two loci was named tt18 and tt19.

The seeds of the three tt18 mutants were yellowish-brown and the tt18 locus was mapped at 1.9 cm (±1.0, standard error) from the AG marker (63.2 cm) on chromosome 4. It is known that LDOX is required for converting flavan-3,4-diols to 3-OH-anthocyanidins, which is one of the enzymatic steps involved in the anthocyanin biosynthetic pathway (Pelletier et al. 1997, 1999). Since tt18 had reduced pigmentation on leaves and stems and the LDOX gene was on the bacterial artificial chromosome (BAC; ATCHRIV58, accession no. AL16-1558) that was located near the map position mentioned above, it was thought that the mutation may have been in this gene. The LDOX gene was amplified by PCR in these mutants to clarify whether the mutation of the LDOX gene is responsible for this mutated phenotype. It was found that the entire locus could not be amplified in one of the mutants (tt18-3), indicating that a rearrangement or a deletion took place at the LDOX gene in this mutant line (Figure 1). We further analyzed the tt18-3 mutation in more detail by PCR and sequence analysis and found that (1) the downstream region from the middle of exon 2 of the LDOX gene was rejoined to the region on BAC T3F17, which locates at the bottom of chromosome 2, and (2) the upstream region >3.9 kb from the translation initiation site of the LDOX gene was present in the mutant (data not shown). We have quite extensively performed thermal asymmetric interlaced-PCR (Liu et al. 1995) and suppression PCR (Steber et al. 1995; Miyao et al. 1998) to identify the rejoined region to this upstream fragment of the LDOX gene, but have been unable to obtain an amplified fragment. We speculate that the failure of cloning the rejoined fragment was due to the sequence context of the rejoined fragment. For instance, if a fragment, which has extremely high/low GC content or short tandem repeats resulting in low affinity to the primer and is resistant to restriction enzyme cleavage, were rejoined to the upstream region of the LDOX gene, this fragment would be refractory to amplification by these two PCR methods. Although we are still far from full characterization of the mutation of tt18-3, we can at least conclude from these results that the tt18-3 mutation contains a rearrangement. A study to reveal the overall structural alteration in this mutant is now in progress. The other two lines (tt18-1, tt18-2) showed no apparent alterations of the amplified fragments with those from the wild type. We further carried out sequence analysis and demonstrated that there was a small deletion in each of these two mutant lines (Table 2; tt18-1, accession no. AB084467; tt18-2, accession no. AB084468). Both of these deletions seem to generate premature stop codons in the coding sequence, which would result in truncated proteins. From this PCR and sequence analysis, it was concluded that the mutation of the LDOX gene was responsible for the tt phenotype in these tt18 mutants.

Two tt19 mutants were also isolated. These showed a phenotype of no or very reduced level of pigmentation on leaves and stems. The seeds of tt19 have a brown-yellowish color that is different from brown-seeded wild type. The TT19 locus was mapped at 1.6 cm (±1.0, standard error) from TT4 (29.5 cm) on chromosome 5. Although TT4 and also TT7 are located close to the map position of TT19, both tt4 and tt7 complemented tt19, indicating that neither was allelic to TT19. The flavonol synthase 1 (FLSI) gene, which encodes an enzyme known to catalyze the conversion of dihydroflavonols to flavonols, also maps close to the TT19 locus (Pelletier et al. 1997; Wisman et al. 1998). To determine whether a mutation in the FLSI gene is responsible for the tt19 phenotype, we sequenced the genomic DNA encompassing the entire FLSI gene. (The gene was sequenced from 375 bp upstream of the translation initiation site to 577 bp downstream of the translation termination site.) However, no mutation was found in FLSI in either of the two tt19 alleles. Furthermore, Wisman et al. (1998) reported that the seed color of a fls1 null mutant is brown and is indistinguishable from that of the wild type. On the basis of these facts and the lack of other mutants defective in flavonoid biosynthesis

### Table 1

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>No. of M1 plants</th>
<th>No. of normal seeds</th>
<th>Embryonic lethals</th>
<th>Chlorophyll mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Frequency (×10⁻³)¹</td>
<td>Frequency (×10⁻³)²</td>
</tr>
<tr>
<td>Unirradiated</td>
<td>600</td>
<td>11,927</td>
<td>119</td>
<td>0.98</td>
</tr>
<tr>
<td>Electrons (750 Gy)</td>
<td>600</td>
<td>6,397</td>
<td>256</td>
<td>3.6</td>
</tr>
<tr>
<td>Carbon ions (150 Gy)</td>
<td>600</td>
<td>2,986</td>
<td>247</td>
<td>7.0</td>
</tr>
</tbody>
</table>

¹ Calculated as a percentage of embryonic lethals among fertilized ovules.
² Frequency/Gy = (Frequency after irradiation − Frequency without irradiation)/Irradiated dose (Gy).
³ Calculated as a percentage of chlorophyll-deficient mutants among nonlethal embryos.
mapped close to tt19, it is likely that tt19, generated by carbon-ion irradiation, is a novel mutant.

**Segregation rates:** To convert the observed mutant frequencies into mutation rates, the segregation rate had to be known. Therefore, we surveyed the segregation rate of the mutants in the F2 population, which derived from the cross between parental Columbia plants and the radiation-induced M3 plants. The average segregation rates were found to be 0.250 (± 0.008, standard error) and 0.256 (± 0.012, standard error) for carbon-ion-induced and electron-induced mutants, respectively (Table 3).

**Mutation rates:** Due to the finding of tt18 and tt19 mutants, the number of loci responsible for the tt phenotype was considered to be 7. Recently, Johnson et al. (2002) reported on a novel mutant, ttg2, which would have been found as a gl mutant in the present screen. The total number of loci responsible for tt and gl phenotypes in this study, therefore, was regarded as 12. Using the average value obtained for segregation rate, average mutation rates per dose for carbon- and electron-induced mutants were estimated to be $1.9 \times 10^{-6}$ and $0.11 \times 10^{-6}$, respectively (Table 4). The present result demonstrates that the mutation rate of carbon ions was 17-fold higher than that of electrons. It should be noted that all mutants isolated in this study had no additional visible phenotypes.

**DISCUSSION**

**Mutagenic effect of carbon ions:** It was demonstrated from the present study that the frequencies of embryonic lethals and chlorophyll-deficient mutants induced by carbon ions were 11-fold and 7.8-fold higher, respectively, than those induced by electrons (Table 1) and that the mutation rate per Gray of carbon ions $(1.9 \times 10^{-6}/Gy)$ was 17-fold higher than that of electrons (Table 4). These values are comparable with the RBEs (12–35) estimated on somatic mutation in Arabidopsis after ion particle irradiation with similar LETs (74–230 keV/μm; Fujii et al. 1966, 1967; Hirono et al. 1970). It is known that carbon ions have a LET 500-fold higher than that of electrons. The high frequency of embryonic lethals and chlorophyll mutants and the high mutation rate after carbon-ion irradiation indicate that damage produced by a single carbon ion is more mutagenic than that produced by 500 tracks of electrons. Since all loci, except tt7 and gl3, were found to be mutated by carbon-ion irradiation, it is likely that carbon ions could randomly mutate the genome (Table 4).

The mutation rate of electrons appeared to be approximately threefold lower than that of X rays (Koornneef et al. 1982). It should be noted that, to obtain an averaged value, the mutation rate of X rays was recalculated under an assumption that a total of 80 loci correspond to the phenotypes of the screened mutants and that the seeds were irradiated at a single dose of 273.13 Gy. We speculate this discrepancy is due to the irradiation conditions: they irradiated imbibed seeds, while we used dry seeds. In the case of carbon ions, the mutation rate per Gray also seemed to be three- to fourfold less than that of fast neutrons (Koornneef et al. 1982). (The mutation rate of fast neutrons was also recalculated with a dose of 45.39 Gy, as mentioned above.) Considering the difference of irradiation conditions mentioned above, we estimate that the potential of carbon ions to induce germline mutations is similar to that of fast neutrons. The frequencies of embryonic lethals and chlorophyll-deficient mutants were not discussed in detail in this context, because the values differed signifi-

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**Figure 1.**—PCR analysis of the tt18 mutants. (A) Schematic representation of the LDOX locus. Putative exons are shown in solid boxes and the numbers below show the positions of the exons, where the translation initiation site is set as 1. The primers are shown as solid arrowheads. The size of the PCR products from the wild type is also shown. (B) Gel electrophoresis pattern of PCR-amplified fragments from the three tt18 mutant lines. The primer sets used for amplification are shown above each lane.
Mutagenesis of Arabidopsis by Carbon Ions

### TABLE 2

<table>
<thead>
<tr>
<th>Allele</th>
<th>Position of the mutation</th>
<th>Altered nucleotide sequence</th>
<th>Predicted changes of amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>tt18-1c</td>
<td>1083–1084</td>
<td>TT → deletion</td>
<td>Phe334Ala335Gln336His337 → Cys334Ser335Thr336Val337Stop</td>
</tr>
<tr>
<td>tt18-2d</td>
<td>1052–1056</td>
<td>TGGAG → deletion</td>
<td>Glu324Ser325 → Ser324Gly325Stop</td>
</tr>
</tbody>
</table>

* The first nucleotide of the translation initiation codon was set as 1.

*Wild-type LDOX protein consists of 356 amino acids.

Accession no. AB084467.

Accession no. AB084468.

Significantly between experiments (Mesken and Van Der Veen 1968; Dellaert 1980a). However, the RBES of fast neutrons estimated with these endpoints (embryonic lethality and chlorophyll deficiency) were found to be fairly constant, ~6–7 (Timofeev-Resovskii et al. 1971; Dellaert 1980a). These results led us to suppose that the criteria of scoring mutants vary among different scorers but the ratio of the frequency itself in each experiment is accurate and meaningful. On the basis of this interpretation and provided that the frequency of mutation increases linearly with dose, similar values between fast neutrons (6–7) and carbon ions (7.8–11) support the conclusion that both types of radiation have a similar mutagenic effect on Arabidopsis seeds.

Koornneef et al. (1982) also looked at the mutagenic effect of EMS and isolated mutants with a frequency ~0.20 × 10⁻³ mutants/locus under an experimental condition of 10 mM for 24 hr. Our study showed that carbon ions had a frequency of 0.08 × 10⁻³ mutants/locus. Thus, in Arabidopsis, carbon ions at a dose of 150 Gy seem to be severalfold less mutagenic than treatment with 10 mM EMS for 24 hr. Similarly, regarding the frequency of embryonic lethals, a 150 Gy of carbon-ion irradiation seems to be three times less mutagenic than a treatment of 8.3 mM EMS for 24 hr (Mesken and Van Der Veen 1968).

It is noteworthy that the high mutation rate by carbon-ion irradiation was observed at a relatively low dose (150 Gy) at which virtually all plants survive. This characteristic of ion particle mutagenesis is quite useful from the viewpoint of plant genetics and breeding.

**Segregation rate of carbon-ion-induced mutants:** Dellaert (1980b) concluded that any deviation from 25% in the segregation frequency of radiation-induced mutants could be ascribed to the reduced frequency of fertile gametes and not to chimerism or to the reduced viability of the M1 plants. On the basis of this evidence, the segregation rates in this study were estimated in the F2 generation of a cross with Columbia (Table 3). The average segregation frequency calculated from eight

### TABLE 3

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>F2 phenotype</th>
<th>Mutant</th>
<th>Wild type</th>
<th>Total</th>
<th>Segregation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carbon ions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tt4(C1)</td>
<td></td>
<td>54</td>
<td>170</td>
<td>224</td>
<td>0.241</td>
</tr>
<tr>
<td>tt4(C2)</td>
<td></td>
<td>55</td>
<td>165</td>
<td>220</td>
<td>0.250</td>
</tr>
<tr>
<td>tt6-2</td>
<td></td>
<td>44</td>
<td>176</td>
<td>220</td>
<td>0.200</td>
</tr>
<tr>
<td>tt18-1</td>
<td></td>
<td>61</td>
<td>165</td>
<td>226</td>
<td>0.270</td>
</tr>
<tr>
<td>tt18-2</td>
<td></td>
<td>95</td>
<td>277</td>
<td>372</td>
<td>0.255</td>
</tr>
<tr>
<td>tt18-3</td>
<td></td>
<td>57</td>
<td>168</td>
<td>225</td>
<td>0.253</td>
</tr>
<tr>
<td>gl1-3</td>
<td></td>
<td>60</td>
<td>165</td>
<td>225</td>
<td>0.267</td>
</tr>
<tr>
<td>gl1-4</td>
<td></td>
<td>110</td>
<td>302</td>
<td>412</td>
<td>0.267</td>
</tr>
<tr>
<td><strong>Average segregation frequency = 0.250 ± 0.008</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Electrons** |             |        |           |       |                       |
| tt3-2  |             | 57     | 170       | 227   | 0.251                 |
| gl2-4  |             | 65     | 160       | 225   | 0.289                 |
| gl2-5  |             | 51     | 170       | 221   | 0.231                 |
| gl2-6  |             | 56     | 166       | 222   | 0.252                 |
| **Average segregation frequency = 0.256 ± 0.012** |

* Mean ± SE.
lines of carbon-ion-induced mutants was found to be quite close to the theoretical Mendelian value for a single recessive allele.

Vizir and Mulligan (1999) demonstrated, by analyzing diploid and triploid progeny obtained by pollinating γ-ray-irradiated haploid pollens to diploid or tetraploid multilocus lines of Arabidopsis, that 73% of the deletions were not transmitted to the diploid progeny. They concluded that large deletions could be rescued only in triploid progeny. Timpte et al. (1994) reported that a revertant of axr2-1 of Arabidopsis induced by 5 krad of γ-rays had a deletion that spanned at least 2 cM. This deletion was transmitted to the progeny at a reduced frequency through only the male and was not transferred through the female gametophytes. Bruggemann et al. (1996) analyzed null hy4 alleles that were lethal in homozygous Arabidopsis and found that they were rarely transmitted through male gametophytes. They showed that hy4 alleles did not affect the viability of female gametophytes. All of these hy4 alleles contained deletions >8 kb in size. These results imply that a large deletion in the gamete impairs the fertilization and thus reduces the segregation rate.

Considering the fact that the average segregation rate is around the expected Mendelian value (0.25), one may speculate that the size of deletions induced by carbon ions is relatively small under the present experimental conditions or that large deletions induced at M1 were infrequently transmitted through either or both gametophytes. In the latter case, those deletions could have rarely become homozygous in the progeny and thus evaded identification under our screening conditions. Consistent with this interpretation, we have not yet found large deletions but found two inversions, one reciprocal translocation (Shikazono et al. 2001), small (2- and 5-bp) deletions, and a rearrangement (present study) induced by carbon-ion irradiation. Further experiments identifying and analyzing heterozygotes of mutations, such as that of Bruggemann et al. (1996), would help to uncover whether large deletions are formed by carbon-ion irradiation in Arabidopsis.

Novel mutations induced by carbon ions: A collection of mutants of the flavonoid pathway have been identified in Arabidopsis and studies of these mutants have led to a detailed understanding of the enzymology, metabolic regulation, and physiological functions of the pathway (Shirley et al. 1995; Winkel-Shirley 2001).

In the present study, three tt18 mutants, which complemented all the previously identified tt mutants that lack or have little pigment on leaves and stems, were isolated from the carbon-ion-mutagenized M2. Each of these mutants was found to have a mutation at the LDOX gene (Figure 1 and Table 2). Therefore, we concluded that disruption of the functional LDOX protein is responsible for the observed phenotype in these tt18 mutants. LDOX converts flavan-3,4-diols to 3-OH-anthocyanidins and is one of the important enzymes required for constructing pigments (anthocyanin derivatives) from the precursor phenylalanine (Pelletier et al. 1997, 1999). The identification of mutations in the LDOX gene completes the series of mutations for the key enzymes involved in anthocyanin biosynthesis in Arabidopsis. A study to identify and characterize the TT19 gene is now being undertaken. Further investigations of these novel Arabidopsis tt18 and tt19 mutants would provide a more detailed description of flavonoid metabolism.

In addition to tt18 and tt19, the induction of additional novel mutations by ion particles has been reported in plants, such as ast mutation in Arabidopsis, which shows spotted anthocyanin pigmentation in testa (Tanaka et al. 1997b), UV-B-resistant mutants of Arabidopsis (Tanaka et al. 2002), frtl1 mutation in Arabidopsis resulting in altered shape of sepal and petal (Hase et
al. 2000), mutations showing a complex color in petals of Chrysanthemum (NAGATOMI et al. 1995), and PVR-resistant mutations in tobacco (HAMA et al. 1999). Although one cannot rule out the possibility that these novel mutations have arisen merely because the mutations had not been saturated, the identification of these ion-particle-induced novel mutations may suggest that ion particles and widely used low LET radiation induce different mutation spectra. As a first step to verify this hypothesis, a comparison of the frequencies of point mutations, rearrangements, and deletions induced by carbon ions with those of electrons is now in progress. We thank B. Winkel-Shirley for her valuable comments on the manuscript and also for providing the names of the authors. M. D. Marks for providing the names of the alleles. We also thank A. Sakamoto and T. Hirose and Y. Hase for helpful discussions.

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