Rearrangements of the DNA in Carbon Ion-Induced Mutants of *Arabidopsis thaliana*

Naoya Shikazono, Atsushi Tanaka, Hiroshi Watanabe and Shigemitsu Tano

Plant Resources Laboratory, Department of Radiation Research for Environment and Resources, Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, Takasaki, Gunma, 370-1292, Japan

ABSTRACT

To elucidate the nature of structural alterations in plants, three carbon ion-induced mutations in *Arabidopsis thaliana*, gll-3, tt4(C1), and tgl1-21, were analyzed. The *gll*-3 mutation was found to be generated by an inversion of a fragment that contained GLI1 and Atpk1 loci on chromosome 3. The size of the inverted fragment was a few hundred kilobase pairs. The inversion was found to accompany an insertion of a 107-bp fragment derived from chromosome 2. The *tt4*(C1) mutation was also found to be due to an inversion. The size of the intervening region between the breakpoints was also estimated to be a few hundred kilobase pairs. In the case of *tgl*1-21, it was found that a break occurred at the *TTG1* locus on chromosome 5, and reciprocal translocation took place between it and chromosome 3. From the sequences flanking the breakpoints, the DNA strand breaks induced by carbon ions were found to be rejoined using, if present, only short homologous sequences. Small deletions were also observed around the breakpoints. These results suggest that the nonhomologous end-joining (NHEJ) pathway operates after plant cells are exposed to ion particles.

**STRUCTURAL** alterations of DNA, such as deletions, produce valuable null mutations for plant genetics. Structural alterations of the DNA encompassing a single gene are of considerable interest because one can directly clone a gene from a plant carrying the mutation using genomic subtraction or representative difference analysis (RDA; Sun et al. 1992; Lisitsyn et al. 1993). Thus, elucidation of the size and nature of the structural alterations induced is essential not only for the characterization of the mutation but for the application of such molecular techniques. Alternatively, analyses on the nature of structural alterations of the DNA in plants may provide important insights into DNA repair mechanisms.

Recent molecular analyses have revealed that X-ray and fast neutron irradiation and T-DNA integration could induce DNA rearrangements in the plant genome. Bruggemann et al. (1996) reported that 13 out of 18 *long hypocotyl 4* (*hy4*) homozygous mutants of *Arabidopsis* induced by fast neutrons carried deletions >5 kbp. It was also shown that complex rearrangements involving deletions, inversions, or insertions occurred in X-ray- and fast neutron-induced *tt3* and *tt5* mutants (Shirley et al. 1992). Laufs et al. (1999) found a 26-cM inversion bordered by two T-DNAs in a direct orientation. Nonhomologous end joining (NHEJ) of double-strand breaks was suggested to be involved in the formation of these rearrangements (Shirley et al. 1992; Laufs et al. 1999).

Characteristics of NHEJ include the use of short homologies for rejoicing, the occurrence of a short deletion at the broken site, and the presence of filler DNAs as well as direct or inverted repeats at the rejoined site. NHEJs were also observed at the rejoined sites of restriction enzyme-digested plasmids in tobacco protoplasts (Gorbunova and Levy 1997), at the rejoined sites of an integrated marker gene in tobacco cells that was digested with I-SceI (Salomon and Putcha 1998), at the integration of rearranged sites in transgenic rice and Arabidopsis after direct gene transfer (Takano et al. 1997; Sawasaki et al. 1998; Kohli et al. 1999), and at the border of T-DNA insertion sites (Gheysen et al. 1991; Mayerhofer et al. 1991; Laufs et al. 1999).

The killing and mutagenic effects of ion particles in higher plants have been studied extensively using various kinds of plants (see Smith 1972 for review). These studies demonstrated that plant cells were most efficiently inactivated or mutagenized per unit dose when the linear energy transfer (LET) of the ion particle was ~100–200 keV/μm. Similar results were obtained with other cell types such as bacteria and mammalian cells (Kraft et al. 1992; Goodhead 1995). LETs in this range are high and cannot be obtained with X rays or fast neutrons. A biologically important characteristic of ion particle irradiation is that it produces dense ionization within a localized area in the irradiated cell. This has been postulated to produce “clustered damage” on the DNA and to lead to an enhanced cell inactivation and mutation induction (Goodhead 1995). Therefore, re-
arrangements are expected to be more frequent after ion particle irradiation than after X-ray or fast neutron irradiation. After a rearrangement occurs, sequencing of the affected area can provide valuable information on the underlying DNA repair process that led to the rearrangement. Furthermore, as a result of such clustered damage, the sizes of deletions or inversions induced by ion particles may be smaller than those induced by low-LET radiation. If this is true, structural alterations induced by ion particles may provide new alleles that show unique phenotypes, and these alleles or phenotypes could be useful in plant genetics.

Only a few studies have examined the structural alterations of DNA induced by ion particles using plants as materials. Meister et al. (1994) found that a rearrangement had occurred in the genome of an argon ion-induced dwarf mutant of rice by Southern blot analysis using random genomic clones as probes. Previously, we analyzed the characteristics of the carbon ion-induced mutations by PCR and Southern blot analyses and found that, out of four mutants, one had a total gene deletion, two contained inversions and/or translocations, and one had a point-like mutation (Shikazono et al. 1998b). The results of these studies suggested that ion particles frequently induce DNA rearrangements in plants. However, to our knowledge, there have been no reports on the analysis of the molecular nature of rearrangements at the sequence level after ion particle irradiation. In our study, DNA fragments from carbon ion-induced Arabidopsis mutants, which were found to contain rearrangements (Shikazono et al. 1998b), were cloned and subjected to sequence analysis.

**MATERIALS AND METHODS**

**Plant material:** Dry seeds of Arabidopsis thaliana ecotype Columbia (Col) were exposed to carbon ions (LET = 113 keV/μm) as described by Tanaka et al. (1997). The dry seeds were irradiated with a dose of 150 Gy, which was at the shoulder of the survival curve. Irradiated M1 seeds (26,200 lines) were sown and selfed to obtain 104,088 M2 seeds. From the screening of mutants and from the complementation test, six lines of glt1 mutant, six lines of ttg1 mutant, and four lines of ttg1-21 mutant, were designated glt1-3, ttg1(C1), and ttg1-21, respectively, and subjected to analysis (Shikazono et al. 1998b). The primers for mapping the DNA fragments were screened from this library by colony hybridization. Colony blots were prepared on positively charged nylon membranes (Hybond-N2; Amersham, Arlington Heights, IL). Digoxigenin-labeled probe I and probe II (Figure 1A), which were used for cloning fragments from glt1-3, were synthesized by PCR using primer 1, 5’-GAAATGGAATAAGGGAGAGATGAA-3’, and primer 2, 5’-GCCAGTCGTAAACATGACGGTG-3’; and primer 3, 5’-ATGTTGACTACTGCCATTTAG-3’, and primer 4, 5’-CCATGATCCGAAGAGACTAT-3’, respectively. In the present study, amplification was carried out at 94° for 3 min, followed by 50 cycles of 94° for 1 min, 50° for 1 min, and 72° for 2 min. At the end of the 50 cycles, the samples were incubated at 72° for another 10 min for complete extension. The probe for cloning the fragment containing the 107 bp from Col was synthesized by PCR using primer 5, 5’-AACTAGGACAGCGGTATTAG-3’, and primer 6, 5’-TTAGGGTACTAGTATTTGTATG-3’. Hybridization was conducted according to the protocol of the manufacturer (Boehringer Mannheim). After hybridization, filters were washed once with 2× SSC, 0.1% SDS and then once with 0.1× SSC, 0.1% SDS at 68°. Hybridized probes were detected by a color reaction using nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indoly-phosphate (Boehringer Mannheim).

For the analysis of the rejoined sites of ttg1(C1) and ttg1-21, thermal asymmetric interlaced (TAIL) PCR was carried out as described by Liu et al. (1995). The specific primers DSP1, 5’-CTGCAGGGTAGCTTCTTGGG-3’, DSP2, 5’-TGCCGACTGTAACCCTCTGAGAA-3’, and DSP3, 5’-CTCAGCTCCTGGCATCACACAG-3’, and the arbitrary degenerate primer AD1, 5’-(A/T)GTGNAG(A/T)ANCANAGA-3’, were used to amplify the downstream sequence of the break in ttg1(C1); and the specific primers USP1, 5’-ACGAAAGGACGACACCAGACGACCAG-3’, USP2, 5’-CTTCATCTGCGCTCTTCTTTGCC-3’, and USP3, 5’-CGATGTCGCTGTGCTGTTGGTCC-3’, and the arbitrary degenerate primer AD2, 5’-NGTCA(G/C)(A/T)GAA(A/T)GAA-3’, were used to amplify the upstream sequence of the break in ttg1(C1). The specific primers DSP1, 5’-AGCCTCCTCCCGAATCTCTCCTT-3’, DSP2, 5’-CTCTGCTCCGAGGATCTGCTCTGTTCTT-3’, and DSP3, 5’-GAGATCTCTTAATCCTCC-3’, and DSP4, 5’-GAGATCTCTTAATCCTCC-3’, were used to amplify the upstream sequence of the break in ttg1-21; and the specific primers USP1, 5’-CGAATATCGAGAATCACAAC-3’, USP2, 5’-TTAGAATTCCTCATCAAATCGTAGCC-3’, and USP3, 5’-TTCCGGCTTCATACCATCGTTCAATCG-3’, and the arbitrary degenerate primer AD2, 5’-NGTCA(G/C)(A/T)GAA-3’, were used to amplify the upstream sequence of the break in ttg1-21.

**Sequencing:** Nucleotide sequences of both DNA strands of the cloned fragments were determined by the dideoxyribonucleotide chain termination method (Sanger et al. 1977) with an automated sequencer (ABI Prism 310 and 310 Genetic analyzer; Perkin-Elmer, Norwalk, CT). The assembly and editing of the DNA sequences were performed with the Sequencher program (Takara, Japan) and Autoassembler program (Perkin-Elmer).

Homologous sequences were obtained from the database using the BLAST program at the Arabidopsis Information Resource (http://www.arabidopsis.org/blast/). Analyses of the sequences of the cloned fragments were performed with the GENETYX MAC program (version 10.0; Software Development Co., Japan).

**Mapping:** DNA fragments were mapped by the cleaved amplified polymorphic sequences (CAPS) method developed by Konieczny and Ausubel (1993). In brief, DNA from F2 plants derived from a cross between Landsberg erecta (Ler) and Col were amplified by PCR. PCR was carried out at 94° for 3 min, followed by 50 cycles of 94° for 1 min, 50° for 1 min, and 72° for 2 min. At the end of the 50 cycles, the samples were incubated at 72° for another 10 min for complete extension. The primers for mapping the glt1 locus were primers 3 and 4. The primers for mapping the 107 bp fragment were primer 7, 5’-TTCAAGATATTCAATACCAAAC-3’, and primer 8, 5’-ATATTTAAAAAGGACACCAAGGTT-3’. The primers for mapping Atphk7 were primer 9, 5’-TTATGTTCGTGTTTTGAGGT TTT-3’, and primer 10, 5’-CCGGTGACAGCGGTTACTT-3’.
Rearrangements in Carbon-Induced Mutants

TAG-3’. The amplified fragment at the GL1 locus and the fragment at the Atpk7 locus were digested with TaqI for 1 hr at 65°C, and the amplified fragment containing the 107 bp was digested with BfI for 1 hr at 37°C. The reaction mixtures were subjected to agarose gel electrophoresis to observe the polymorphisms. The linkage analysis was done using the MAPL mapping program, which was downloaded from http://peach.ab.a.u-tokyo.ac.jp/~ukai/mapl1.htm.

RESULTS

We have isolated a gl1-3 mutant from carbon ion-mutagenized M2 plants and further found, by Southern blot and PCR analyses, that a break occurred within exon 3 of the GL1 gene and then a rearrangement took place in this mutant (SHIKAZONO et al. 1998a, accession no. AB006078; SHIKAZONO et al. 1998b). As a first step to analyze the structural alteration of gl1-3 in more detail, the HindIII fragment containing the GL1 gene was cloned from the wild type (Col; Figure 1A). This fragment was sequenced and was found to be 12,367 bp in length. PCR-amplified probes that hybridize to the upstream and downstream regions of the break were designated probe I and probe II, respectively (Figure 1A). The results of cloning and sequencing of the hybridizing fragments for each probe in the mutant are schematically shown in Figure 1, B and C. The EcoRI fragment cloned by probe I was 10,209 bp and the HindIII fragment cloned by probe II was 7230 bp in length. From the homology search, it was found that the sequence upstream of the break at the third exon of GL1 was rejoined to the region containing the Atpk7 gene (Hayashida et al. 1992, accession no. D109910). The sequence downstream of the break at the third exon of GL1 was found to be rejoined to the region downstream of the Atpk7 gene, with an unknown 107-bp sequence inserted in between these two regions. No point mutations were found in these fragments cloned from gl1-3.

Southern blot analysis using the 107-bp fragment as a probe detected a single HindIII fragment (data not shown), indicating that this fragment is not a repetitive sequence. The hybridizing HindIII fragment was cloned and then sequenced (Figure 2A). The clone was 3047 bp in length and contained the complete 107-bp sequence. To locate this 3047-bp HindIII fragment, a marker for the CAPS method was generated within this region. A BfI fragment of 561 bp was specific to Col, and BfI fragments of ~400 and 160 bp in length were specific to Ler. Using this polymorphism, the 107-bp fragment was found to lie on chromosome 2 between markers PHYB and COP1 (Figure 2B). PHYB and COP1 map at 34.4 cM and at 63.3 cM (http://www.arabidopsis.org/maps.html), respectively. The location of the 107-bp fragment was further confirmed from the physical map position of the sequenced BAC F8N16 (accession no. AC005727), in which a sequence identical to the 3047-bp HindIII fragment was found.

To determine whether this 107-bp fragment was cut out from the original site and then inserted between the downstream region of GL1 and the downstream region of the Atpk7 gene in the gl1-3 mutant, PCR was

![Figure 1](image1.png)

**Figure 1.**—Cloning and sequencing of fragments containing the GL1 gene sequence from wild type (Col) and gl1-3. (A) A schematic representation of the HindIII fragment containing the GL1 gene from wild type (Col). Exons are shown as solid boxes. Probes used to clone fragments from gl1-3 are shown below as thick solid lines at the corresponding location. The break that occurred in gl1-3 locates between probes I and II. Bg, BglII; E, EcoRI; H, HindIII; Sc, ScaI; Xb, XbaI. (B) A fragment from gl1-3 cloned by probe I. (C) A fragment from gl1-3 cloned by probe II.

![Figure 2](image2.png)

**Figure 2.**—Characterization of the inserted 107-bp fragment. (A) Cloning and sequencing of the HindIII fragment containing the 107-bp sequence from the wild type (Col). The 107-bp region is shown as a solid box. Primers of the CAPS marker for this region are shown as solid arrowheads, E, EcoRI; H, HindIII; Xb, XbaI. (B) Linkage of the 107-bp region to other CAPS markers. Forty-six F2 plants were subjected to the mapping experiment.
Figure 3.—Nucleotide sequence of the rearranged sites in carbon ion-induced mutants. Short homologous sequences involved in rejoining are shown as gray letters. Boxed sequences were deleted during the rejoining process. Potential topoisomerase I recognition sequences (thick lines) and cleavage sites (arrowheads) are shown above the sequence. Direct repeats are shown as black horizontal arrows, whereas imperfect direct and/or inverted repeats are shown as gray horizontal arrows below the sequence. (A) Nucleotide sequence of the rearranged site in gl1-3. (B) Nucleotide sequence of the rearranged site in tt4(C1). (C) Nucleotide sequence of the rearranged site in ttg1-21.

The Atpk7 gene was recently placed on the BAC MMJ24 (accession no. AB-025626). The position of the Atpk7 gene is indicated to be a few hundred kilobase pairs north of GL1 (http://www.arabidopsis.org/maps.html).

Nucleotide sequences of the rejoined sites in gl1-3 are shown in Figure 3A. Comparing the sequence of the rejoined site of the upstream sequence of the GL1 and the Atpk7 gene with the sequences of these genes in the wild type demonstrated that 4 bp of homology (TGAT) was involved when DNA strand breaks were rejoined. In the case of the rejoined site of the downstream sequence of the GL1 gene, the 107-bp fragment, and the downstream region of the Atpk7 gene, it was found at the rejoined site. Since the location of the Atpk7 gene was unknown, a novel CAPS marker for the Atpk7 locus was generated. After PCR and digestion with TaqI, two fragments, ~430 and 290 bp in length, were observed in Col, while a single undigested fragment was found in Ler. Out of 46 F2 plants, no recombination was observed between the Atpk7 and the GL1 marker. It is shown that GL1 locates at 48.4 cM on chromosome 3 (http://www.arabidopsis.org/maps.html). The result of mapping suggests that Atpk7 lies near GL1 on chromosome 3. Consistent with this conclusion, the Atpk7 gene was recently placed on the BAC MMJ24 (accession no. AB-025626). The position of the Atpk7 gene is indicated to be a few hundred kilobase pairs north of GL1 (http://www.arabidopsis.org/maps.html).
have a break within the first and the second exon of their genes, respectively, suggesting that a translocation or an inversion took place in each mutant (Shikazono et al. 1998b). To analyze the structural alteration of these mutations in more detail, the rejoined sites were cloned by TAIL PCR and were sequenced.

In the case of tt4(C1), 1.3-kbp and 1-kbp fragments containing the upstream and downstream regions of the TT4 gene (accession no. M20308), respectively, were cloned by TAIL PCR (Figure 4B). Sequencing of these fragments revealed that the broken ends of the TT4 gene were rejoined to broken ends generated at the fourth exon of a putative gene on BAC F18O22 (accession no. AL163817; Figures 3B and 4B). The position of the breakpoint was estimated to be a few hundred kilobase pairs south of the TT4 gene (http://www.arabidopsis.org/maps.html). Comparison of the nucleotide sequences flanking the rejoined sites with those of the wild type revealed that 1 bp was deleted in both regions during the rejoining process. Further, the broken site at the TT4 gene contained a potential topoisomerase I cleavage site. There was an imperfect direct repeat at the rejoined site containing the downstream region of the TT4 gene. The rejoined sites revealed that no homologous end was used and that no point mutations occurred during the rejoining process.

In the case of ttg1-21, 600-bp and 700-bp fragments containing the upstream and downstream regions of the TTG1 gene (accession no. AJ133743), respectively, were cloned by TAIL PCR (Figure 4C). Sequencing of these fragments revealed that the broken ends of the TTG1 gene were rejoined to broken ends generated at the first intron of a putative gene on BAC F1C9 (accession no. AC011664; Figures 3C and 4C). The TTG1 gene and this putative gene lie at 50 cM on chromosome 5 and 6 cM on chromosome 3, respectively (http://

---

**Figure 3.—Continued.**
A sequence analysis of the carbon ion-induced mutations was carried out in our study. The structural alteration of the mutations is summarized in Figure 4. In the case of gl1-3, from the fact that both the upstream and downstream regions of the GL1 gene were rejoined to fragments at the Atpk7 locus and the fact that the Atpk7 locus locates quite near to GL1 on chromosome 3, it was concluded that an inversion took place in this mutant (Figure 4A). The size of the inverted fragment was a few hundred kilobase pairs. Furthermore, it was demonstrated that a 107-bp fragment from chromosome 2 was inserted at the rejoined site of the downstream region of the GL1 gene and the 3' downstream region of the Atpk7 gene. In the case of tt4(C1), since the location of TT4 is 29.5 cM on chromosome 5, and the position of the rejoined site is a few hundred kilobase pairs south of the TT4 gene, it was concluded that a fragment of a few hundred kilobase pairs in size was inserted in this mutant (Figure 4B). Similarly, since the breakpoint within TTG1 and the other breakpoint locate around 50 cM on chromosome 5 and 6 cM on chromosome 3, respectively, this mutant seems to contain a reciprocal translocation between chromosomes 3 and 5 (Figure 4C). It has been reported that low-LET radiation, such as X-ray and fast neutron irradiation, could simultaneously cause deletion, inversion, and/or insertion in the plant genome (Shirley et al. 1992). Our study showed that similar large and complex structural alterations could be induced by ion particles. Since high-LET radiation is much more mutagenic than low-LET radiation, ion particle irradiation can be a powerful tool for inducing genetic rearrangements in plants.

Rejoining of double-strand breaks via NHEJ was assumed to operate at the broken ends after X-ray and fast neutron irradiation of Arabidopsis seeds (Shirley et al. 1992). Several characteristics of NHEJ were also found from the sequence of the rejoining site after carbon ion irradiation. Short homologies of 1–4 bp were involved in 5 of 8 rejoined sites and small deletions were present at 7 of 8 breakpoints (Figure 3). Involvement of short homologies and/or deletions has been reported at a similar frequency during the rejoining process in plant cells (Gheysen et al. 1991; Mayerhofer et al. 1991; Gorbunova and Levy 1997; Takano et al. 1997). Direct repeats and imperfect inverted repeats were observed at the rejoined site in our study (Figure 3), indicating
that these sequences may have played some role in rejoining the DNA ends. Insertions of 2 and 107 bp were also observed after carbon ion exposure (Figure 3). This is consistent with previous findings that ~30–40% of the sites rejoined via NHEJ in plant cells are accompanied by filler DNAs (Mayerhofer et al. 1991; Gheysen et al. 1991; Gorbunova and Levy 1997; Takano et al. 1997). Consequently, these results strongly suggest that the NHEJ pathway of double-strand breaks operates after plant cells are exposed to both low- and high-LET radiation.

The underlying mechanisms of the formation of DNA rearrangements are largely unknown in plants. Potential topoisomerase I cleavage sites (5’ G/C-A/T-T 3’; Been et al. 1984) were observed around the breakpoints in this study (Figure 3). This result suggests the involvement of topoisomerase I in generating DNA rearrangements. The involvement of topoisomerase I in DNA rearrangements was also implied by sequence analysis after γ-irradiation of human fibroblast cells (Forrester and Radford 1998) and after particle bombardment of Arabidopsis (Sawasaki et al. 1998). It has been reported that topoisomerase I cleavage was stimulated by the presence of a nick or a gap on the opposite strand several bases downstream of the cleavage site (Pourquier et al. 1997; Kingma and Osheroff 1998). Since ion particles would frequently produce nicks or gaps, cleavage of topoisomerase I would have often resulted in a double-strand break and this might have served as a template for NHEJ. Zhu and Schiestl (1996) reported that the overexpression of topoisomerase I in yeast led to a 6- to 12-fold increase of nonhomolo-
gous integration of transformed DNA compared with that in yeast with repression or deletion of topoisomerase I, suggesting that topoisomerase I plays some role in integration. Further study is needed to elucidate the initial DNA damage and the subsequent rejoicing process that leads to large structural changes such as deletion, inversion, and translocation in plants.

Since high-LET radiation would cause dense ionization within a localized area in the nucleus, the damage specifically induced by high-LET radiation, referred to as “locally multiply damaged sites” or “clustered damage,” is assumed to occur (Ward 1994; Goodhead 1995). It seems plausible to hypothesize that, due to this type of damage, some base changes occur near the broken end after the cells are exposed to ion particles. However, no such changes were observed in our study. This result could be explained by assuming that, even though there were some sites of base damage near the broken ends, these damaged sites were removed by exonuclease activity during the rejoicing process. Since deletions of several base pairs are observed at broken ends, this explanation seems plausible. Alternatively, it is possible that such mutations are rarely detected simply because the clustered damage is mainly nonreparable, resulting in cell death.

It was suggested from the efficient induction of large and complex structural alterations that ion particle irradiation is a powerful tool for inducing valuable null mutants for plant genetics. However, it remains unclear whether there is any difference between the nature of the mutations induced by high- and low-LET radiation in plants. It was reported that the distribution of double-strand breaks induced after high-LET radiation was nonrandom both theoretically and experimentally in mammalian cells on scales of kilobase to megabase pairs (Holly and Chatterjee 1996; Löbrich et al. 1996; Rydberg 1996; Sachs et al. 1998). It seems plausible to assume that the characteristics of the structural alterations of the genome induced by high-LET radiation would reflect these nonrandom clustered distributions of the damage. Although no clustered mutations on a scale of 10 bp were found in our study, it seems possible that clustered structural alterations on scales of kilo- or megabase pairs are frequently induced by ion particles. Consistent with this assumption, the intragenic deletions containing multiple exons at the HPRT locus in CHO-K1 cells were reported to be more frequent after α-particle exposure than after γ-irradiation (Schwartz et al. 1994). Further, Chaudhry et al. (1996) found that the proportion of radon-induced mutants with relatively small deletions involving the TK locus alone or the TK locus with one or both of the two closest markers was significantly higher than in the case of X-ray-induced mutants. From these results, one may speculate that ion particle irradiation efficiently induces moderate-sized deletions (of several kilobases) in plants, which could be directly applied to cloning a gene using molecular techniques such as genomic subtraction or representative difference analysis (Sun et al. 1992; Lisitsyn et al. 1993). Thus, it appears to be quite important to comparatively analyze the high- and low-LET radiation-induced mutations at the molecular level in plants and to clarify the character of high-LET radiation-induced mutations.

We thank Dr. A. Sakamoto, Dr. T. Hirose, and Mr. Y. Hase for helpful suggestions and discussions. We also thank Dr. A. R. Walker for providing the name of the tigf allele.

**LITERATURE CITED**


Communicating editor: C. S. Gasser