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Effects of ion beam irradiation on size of mutant sector and genetic damage in *Arabidopsis*

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ABSTRACT

Size of mutant sector and genetic damage were evaluated in *Arabidopsis* to further our understanding of effective ion beam use in plant mutation breeding. *Arabidopsis* seeds, heterozygous for the *GLABRA1* (*GL1*) gene (*GL1*/*gl1-1*), were irradiated with 15.8 MeV/u neon ions (mean linear energy transfer (LET): 352 keV/μm), 17.3 MeV/u carbon ions (113 keV/μm), or ^{60}Co gamma rays. The frequency and size of glabrous sectors generated because of inactivation of the *GL1* allele were examined. The frequency and overall size of large deletions were evaluated based on the loss of heterozygosity of DNA markers using DNA isolated from glabrous tissue. Irrespective of the radiation properties, plants with mutant sectors were obtained at similar frequencies at the same effective dosage necessary for survival reduction. Ion beams tended to induce larger mutant sectors than gamma rays. The frequency of large deletions (>several kbp) increased as the LET value increased, with chromosome regions larger than 100 kbp lost in most large deletions. The distorted segregation ratio of glabrous plants in the progenies of irradiated *GL1*/*gl1-1* plants suggested frequent occurrence of chromosome rearrangement, especially those subjected to neon ions. Exposure to ion beams with moderate LET values (30–110 keV/μm) is thought effective for inducing mutant sectors without causing extensive genetic damage.

1. Introduction

Ion beams are a useful mutagen in plant mutation breeding processes, and have been used to develop various new plant varieties [1]. Ion beams have been extensively used for improvements of vegetatively propagating crops; this is because cross breeding is not applicable and high mutation frequency can be achieved, probably owing to high heterogeneity [2]. In most cases, cultured tissue, buds, or callus are irradiated and mutants screened from the regenerated plant population. Chimerism is unavoidable when multicellular tissue is treated with ionizing radiation. Therefore, cuttings are usually taken to extend the size of mutant sector and to obtain solid (non-chimeric) mutants [3–5]. Although sector size is an important factor in mutation breeding, few studies have investigated sector size generated by ion beam irradiation.

Enlargement of sector size by radiation treatment in tomato (*Solanum lycopersicum*) and *Antirrhinum majus* has been reported [6]. This is thought to be a result of the reduced cell numbers in shoot meristems contributing to shoot development; this phenomenon is termed 'internal disbudding'. Yamaguchi et al. [7] investigated segregation ratios of chlorophyll mutants in a M_2 generation of rice mutagenized using carbon ions and gamma rays, and demonstrated increased segregation ratios at higher dose ranges. This could be explained by a reduction of the number of initial cells contributing to seed development. Yamaguchi et al. [5] also investigated the chimeric structure of flower color mutants of chrysanthemum obtained following exposure to carbon ions and gamma rays. The flower color of mutants (reflecting the genotype of the L1 layer) and flower color of the plants regenerated from root tissue of mutants (reflecting the genotype of the L2 layer of mutants) were compared. All mutants obtained by gamma rays were periclinal chimera, while approximately half of the mutants obtained by carbon ions were solid mutants. This suggests the mutant sector expanded through the cell layer of the shoot meristem after carbon ion irradiation. These results suggest ion beams are more effective at enlarging sector size than gamma rays.

The greatest benefit of mutation breeding is the improvement of one or a few desirable traits without altering remaining characteristics [8]. Therefore, efficient mutagenesis with a reasonably low irradiation dose is preferable. In chrysanthemum, nuclear DNA content decreases as irradiation dose increases [9,10]. In addition,

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there is a correlative impairment of agronomic traits (leaf size and flower size) associated with reduction of nuclear DNA content, and irradiation with carbon ions with a linear energy transfer (LET) value of 107 keV/μm is more efficient than gamma irradiation in inducing flower mutations in relation to the reduction of nuclear DNA content [11]. However, the full effects of ion beams on sector size and genetic damage have not been fully evaluated.

In our previous study, we investigated glabrous mutant sectors generated in *Arabidopsis thaliana* plants heterozygous for the *GLABRA1* (*GL1*) gene (*GL1*/*gl1-1*), and demonstrated carbon ions near the range end (mean LET: 425 keV/μm) and neon ions (352 keV/μm) more frequently induced large deletions than carbon ions at 113 keV/μm, although the overall size of deletions remained to be elucidated [12]. Additionally, we found that ion beams with very high LET values more frequently induced large mutant sectors that extended across more than two rosette leaves [12]. This suggests ion beams with higher LET values induce larger mutant sectors. We feel this experimental system is useful for investigation of the size of mutant sector and assessment of genetic damage. In this study, we extended upon our previous work and further examined mutant sectors generated by ion beams and gamma rays. We evaluated the overall size of large deletions, which were not identified in the previous study. We also discuss the enlargement of mutant sector by ion beam irradiation in relation to genetic damage.

2. Materials and methods

2.1. Plant materials and growth conditions

F_1 hybrid *Arabidopsis* seeds heterozygous for *GL1* (At3g27920) were obtained by crossing a *gl1-1* mutant (Landsberg *erecta* (Ler) accession) with a wild type Columbia (Col-0) accession. Plants were grown in plug trays filled with MetroMix350 (Sun Gro Horticulture, Bellevue, WA), in a growth room (Koito Industries, Yokohama, Japan) at 23 °C under 16-h light/8-h dark photoperiod conditions.

2.2. Irradiation

GL1/*gl1-1* heterozygous seeds were irradiated with 15.8 MeV/u neon ions or 17.3 MeV/u carbon ions using an azimuthally varying field cyclotron, or by ^{60}Co gamma rays at the Takasaki Advanced Radiation Research Institute, National Institutes for Quantum and Radiological Science and Technology, Japan. The range and mean LET of ion beams were calculated using ELOSS M code [13]. The mean LET was 352 keV/μm (range in seeds: 315–399 keV/μm) for 15.8 MeV/u neon ions, and 113 keV/μm (107–121 keV/μm) for 17.3 MeV carbon ions. To determine the shoulder dose (*Dq*) of survival curve, irradiated seeds were sown and the survival rate determined three weeks after sowing. Three replications of 25 or 50 seeds were used for each dose. Survival curves were drawn and *Dq* determined as previously described [12].

2.3. Detection of glabrous sectors and estimation of deleted regions

GL1/*gl1-1* heterozygous seeds were irradiated with a half dose of *Dq*. Irradiated seeds were grown as described above, and glabrous sectors on rosette leaves were continuously observed until the bolting stage. Leaf tissues of glabrous sectors were excised using a scalpel under a stereomicroscope (MZ6, Leica Microsystems, Tokyo, Japan). Excised tissue was macerated in solution (0.5% SDS, 250 mM NaCl, 25 mM EDTA, 200 mM Tris-HCl, pH 7.5) using a micro pestle, and the supernatant subjected to isopropanol precipitation. The pellet was rinsed with 70% ethanol, and genomic DNA dissolved in 100 μl distilled water. Polymorphic DNA markers that could distinguish Col-0 and Ler chromosome sequences were used. The *GL1* and *gl1-1* allele are on Col-0 and Ler chromosomes, respectively, in *GL1*/*gl1-1* heterozygous plants. PCRs were performed in a GeneAmp PCR System 9700 (Applied Biosystems, Tokyo, Japan) using 1 μl of genomic DNA solution, 150 μM each dNTP, 0.1 μM each of the relevant primer pairs listed in Table S1, 0.5 unit of Ex Taq polymerase (Takara Bio, Shiga, Japan), and 1 × Ex Taq buffer in a 40 μl total volume. PCR conditions consisted an initial denaturation at 94 °C for 5 min, followed by 40 cycles of 94 °C for 20 s, 57 °C for 20 s, and 72 °C for 40 s, and a final extension at 72 °C for 2 min. The amplified fragment was purified using MinElute PCR Purification Kit (Qiagen, Tokyo, Japan), and sequences determined using a 3500 Genetic Analyzer (Applied Biosystems). Polymorphic sites on the Col-0 chromosome were considered deleted when the DNA markers showed a loss of heterozygosity (LOH), i.e., when no or very little peak for the Col-0 sequence was detected against the peak for the Ler sequence.

2.4. Segregation ratio for glabrous plants

The *GL1*/*gl1-1* heterozygous plants used for the observation of glabrous sectors were self-pollinated. More than 100 seeds were collected from each plant and sown, the segregation ratio for glabrous plants was determined.

2.5. Statistics

Statistical comparisons of fraction of sectors were made using a chi-squared test. Distributions of segregation ratios were compared using an *F* test, and the mean of segregation ratios compared using a Mann-Whitney *U* test.

3. Results

3.1. Frequency of plants with glabrous sectors

GL1/*gl1-1* heterozygous seeds were irradiated with 15.8 MeV/u neon ions, 17.3 MeV/u carbon ions, or gamma rays and the frequency of emergence of glabrous mutant sectors compared. Irradiation doses at each radiation were determined based on the survival curve of irradiated seeds. The *Dq* of the survival curve for gamma rays was determined to be 2292 Gy (Supplementary Fig. 1). The *Dq* values for neon and carbon ions were determined in our previous study to be 89 and 227 Gy, respectively [12]. *GL1*/*gl1-1* heterozygous seeds were irradiated with a half dose of *Dq* for each radiation, this was 45, 113, and 1146 Gy for neon ions, carbon ions, and gamma rays, respectively. The irradiated seeds were grown and observed for glabrous mutant sectors (Fig. 1). Emergence of glabrous sectors is likely due to the inactivation of the *GL1* gene, this is because the *GL1* gene is in the heterozygous state; no mutant sectors were observed in the non-irradiated population [12]. The frequencies of plants with glabrous sectors were 2.8%, 2.7%, and 3.0% for neon ions, carbon ions, and gamma rays, respectively (Table 1). Frequencies for neon and carbon ions were similar to those observed in our previous study at 3.1% and 2.7% for neon and carbon ions, respectively [12]. These results suggest doses with the same effect on survival reduction are almost equivalent for inducing plants with glabrous sectors, regardless of the properties of ionizing radiations, including gamma rays.

3.2. Size and distribution of glabrous sectors

Plants with glabrous sectors were further grown and their chimeric structure examined. For neon ions, 32.3% of glabrous sectors extended across more than two leaves, this fraction was signifi-