Constructing *S*-locus deletion mutant in common buckwheat by using heavy-ion-beam irradiation

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In *Fagopyrum esculentum* (common buckwheat), the plants exhibit short-styled or long-styled flowers, showing distyly. The floral morphology and intra-morph incompatibility are both determined by a single genetic complex named *S*-locus. Plants with short-styled flowers are heterozygous (*S/s*) and plants with long-styled flowers are homozygous recessive (*s/s*) at *S*-locus. Previously we discovered a new gene, *S-LOCUS EARLY FLOWERING 3* (*S-ELF3*), which is a candidate gene for short-styled phenotypes of distyly, and its flanking region of about 500 kbp has already been sequenced[1]. Recombination around the *S*-locus is supposed to be restricted, because no recombination between floral morphology and intra-morph incompatibility was observed. Thus, genetic mapping is not possible to determine the genomic region containing the *S*-locus. The purpose of this study is to construct mutants that lack the genomic region around the *S*-locus by heavy-ion-beam irradiation, in order to use the mutants for narrowing down the *S*-locus in the future.

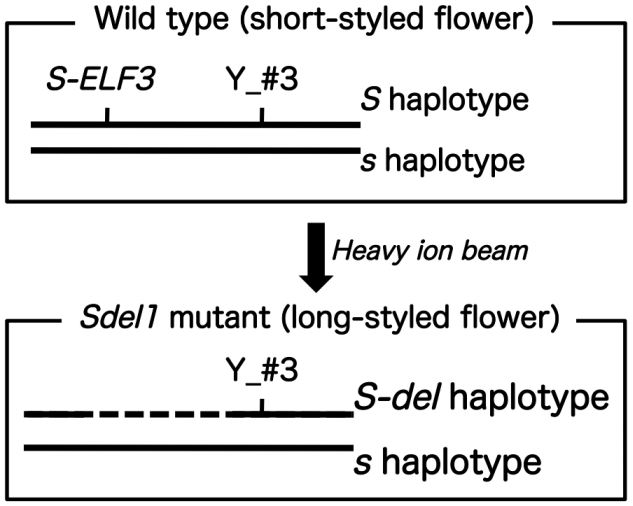

Fig. 1. Schematic diagram of the screening of *S-del* haplotypes. New mutant *S-del1* possessed only Y_#3 marker, which is tightly linked to *S*-locus, i.e., no *S-ELF3* was found. The flower phenotypes of *S-del1* were long styled. Dashed line indicates the genomic region deleted by heavy-ion-beam irradiation.

For obtaining an *S*-locus-deletion plant, buckwheat seeds were irradiated with accelerated $^{12}C^{6+}$ ions in doses ranging from 100 Gy to 125 Gy. The linear energy transfer (LET) range of $^{12}C^{6+}$ was from 22.5 keV/μm to 30 keV/μm. The total DNA was extracted from 1,152 plants of M_2 growing in the experimental room, and their flower types were investigated. For screening of the *S*-locus-deletion plant, PCR was performed using an *S*-haplotype specific primer set (Y_#3) obtained using cDNA-Amplified Fragment Length Polymorphism (AFLP) analysis (Yasui et al., in preparation). The Y_#3 PCR marker showed perfect linkage with the *S*-locus in 1,400 mapping population and was amplified only with short-styled buckwheat plants collected from all over the world. *S-ELF3* and Y_#3 marker were located physically distant to each other, because the DNA sequence of Y_#3 marker could not be found on the 500 kbp BAC contig flanking of *S-ELF3* (Yasui et al., in preparation). Further, if short-styled plants lack genomic region only around the *S*-locus, the flower type of the plant is expected to become long-styled, but must possess the Y_#3 marker (Fig. 1).

In 1,152 plants investigated, one showed both positive Y_#3 PCR amplification and long-styled flowers and was named *S-del1*. Furthermore, *S-ELF3* and six dominant PCR markers covering the 500 kbp sequence flanking *S-ELF3*[1] produced no PCR products with *S-del1* DNA. It is considered that the large genomic region (>500 kbp) harboring the *S*-locus was deleted in the *S-del1* mutant and that the flower type of *S-del1* changed from short-styled to long-styled (Fig. 1). These results imply that *S-ELF3* or its flanking gene controlled short-styled phenotypes.

It is expected that combining PCR amplification with *S*-linked maker (Y_#3) and phenotyping of flower type on M_2 plants is effective in the screening of *S*-locus-deletion plants. We are planning to screen other sets of M_2 population. In the near future, we will be able to construct a fine deletion map such as that of the Y chromosome of *Silene latifolia*[2].

In this study, we observed a phenotypic change from short-styled to long-styled flowers in the mutant progeny. This makes the creating of *S-del* homozygous (*S-del*/*S-del*) plant possible, and the resulting plants will enable us to estimate the role of *s*-haplotype genes and to narrow down the genomic region harboring these genes.

References

1) Yasui et al.: PLoS ONE **7**, e31264 (2012).
2) Fujita et al.: G3 (Bethesda) **2**, 271 (2012).

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