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 sequenced1). 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.

For obtaining an S-locus-deletion plant, buckwheat seeds were irradiated with accelerated \(^{12}\text{C}^{6+}\) ions in doses ranging from 100 Gy to 125 Gy. The linear energy transfer (LET) range of \(^{12}\text{C}^{6+}\) was from 22.5 keV/\(\mu\)m to 30 keV/\(\mu\)m. 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-ELF31) 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 that of the Y chromosome of Silene latifolia2).

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

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