

Inferring genetic basis of short-style homostyly and self-compatibility in a KK8 mutant of common buckwheat

S.R. Prokopchuk^{1,2*}, M.D. Logacheva^{1,2}, M.I. Schelkunov^{1,2}, A.A. Penin², N.N. Fesenko³, I.N. Fesenko³

¹ Center for Molecular and Cellular Biology, Skolkovo Institute of Science and Technology, Moscow, Russia

² Vavilov Institute of General Genetics, Moscow, Russia

³ Breeding Centre, Laboratory of Buckwheat Breeding, Federal Scientific Center of Legumes and Groat Crops, Orel, Russia

*psr1653@gmail.com

Motivation and aim: Buckwheat (*Fagopyrum esculentum*) is an obligate cross-pollinated agricultural crop with heterostyly defense mechanism against self-pollination. It is known that heterostyly, manifested as the division of flowers in buckwheat populations into two types: those with a short pistil style and long stamens (short-styled type, SS) and those with a long pistil style and short stamens (long-styled type, LS) is controlled by a region in the genome called the S locus. Currently, only one gene, S-ELF3, is known to be part of the S locus, which regulates style length and pistil self-incompatibility. According to the current S locus model, it is expected that in addition to S-ELF3, gene P (pollen function), gene I^p (pollen incompatibility) and A (stamen length) should be present. The S locus is hemizygous. It is present in the short-styled form in one of two homologous chromosomes and absent in the long-styled form. For buckwheat, several homostyle mutants are known; for long-style homostyle mutants the study of the genetic basis of this phenotype shows that it is caused by the disruption of S-ELF3 gene. Short-styled homostyle mutants are much rarer and less studied. Earlier we have shown that in short-styled homostyle self-pollinated accession KK8 S-ELF is intact; currently we report the results of a follow-up study based on whole genome sequences. This knowledge may enhance the understanding of the S locus structure and will also provide an opportunity to utilize this knowledge in the development of self-pollinated varieties.

Materials and methods: We have sequenced the genomes of the short-styled homostyle self-pollinated mutant KK8 and the genome of short-styled cross-pollinated *F. esculentum* ssp. *ancestrale* (further *Fea*), the wild ancestor of cultivated buckwheat using long-read sequencing on Oxford Nanopore Technologies platform. Assembly was performed using Mabs-hifiasm. S locus structure was inferred by applying local and global sequence alignment algorithms. Structural annotations were generated for the assemblies using Braker3. RNA-seq data from flowers and inflorescences of short-styled buckwheat, long-styled buckwheat of the cultivar Shinano, and RNA-seq of the homostyle line KK8 were also used in the analysis. DESeq2 was used to analyze differential gene expression in inflorescences and flowers. In addition, DNA-seq data collected in two pools for short-styled and long-styled plants were used to further perform QTL-seq analysis and identify potential signal at the S-locus region.

Results: We assembled the genomes up to a chromosome level. Their comparative analysis revealed a translocation of a region from chromosome 2 to chromosome 1 in the genome of KK8. This translocation encompasses 1 Mb including the S-ELF gene. In *ancestrale* genome, the entire S locus is located in chromosome 2. We hypothesize that this translocation is one of the reasons for the homostyly of KK8. Interestingly, that KK8 behaves as typical short-style plant in one aspect – successfully sets seeds when pollinated by the pollen of long-type, but as long-style in other aspect – it successfully pollinates short-style plants. This observation allowed us to assume that the P gene was disrupted in KK8 during translocation. To confirm our assumption, we analysed gene set in KK8 and *Fea*. During the analysis we found the S-locus associated polygalacturonase gene present in *Fea* but absent in KK8. According to the literature, polygalacturonases are involved in pollen function. Gene expression profiles and DE gene analysis also reveals the similarity between KK8 flowers and both long-style cross-pollinated flowers as well as with the short-style cross-pollinated flowers and supports the key role of polygalacturonase.

Conclusions: The analysis revealed a partial translocation of the S locus from chromosome 2 to chromosome 1 in the genome of short homostyle accession KK8. Candidate genes for the role of a gene with P function in the genome of the wild ancestor of cultivated buckwheat were identified. We also analyzed gene expression profiles in the context of the uncommon pollen traits that exhibit the same specificities as pollen in long-styled plants.