

Genetic studies on spike and grain morphologies
and on recombination frequency in common wheat
by whole genome genotyping

Motohiro Yoshioka

Common wheat (*Triticum aestivum* L.) is the most widely cultivated crop in the world. To tackle a growing population and changing climate, wheat genetics and genomics leading to identification of genes that control valuable agricultural characters, such as stress tolerance and high yield, are in great demand. The genome of common wheat (genome constitution AABBDD, $2n = 6x = 42$) is characterized by its large size, sequence homology among the A, B and D genomes, and an abundance of transposable elements, all of which have hampered genetic analysis of traits of interests, especially of complex traits. During the last decade, the emergence of second-generation sequencing technology has enabled genome-wide genotyping for each individual in a segregation population or in a genetically diverse stock.

In this dissertation, I applied RAD-seq, a genotyping method based on second-generation sequencing of a representative portion of genomic sequence defined by restriction sites, to common wheat for the purpose of studying the following three themes. In Chapter 2, I examined the spike morphology with an emphasis on awn morphology that is related to drought tolerance. Although three inhibitor genes, *Hd*, *B1*, and *B2*, have been identified by classical genetics, we still do not know how much of the variation in awn length observed in common wheat cultivars can be explained by them. Moreover, the possibility that other loci could influence awn lengths had not yet been extensively studied. I performed a genome-wide association study (GWAS) on 185 accessions of common wheat for awn lengths measured at the top and the middle of the spikes. I found that a single-nucleotide polymorphism (SNP) that affects awn length at the middle of the spike resides in the promoter region of the *B1* gene, recently cloned awn inhibiting gene, which accounted for as much as 25% of the variation within the core collection. In contrast, no significant marker-trait association (MTA) with awn length was detected in the vicinity of the *Hd* and *B2* loci, suggesting that these loci only contribute to a fraction of the overall variation of awn length observed in common wheat varieties. I did not

detect any increase in linkage disequilibrium around the *B1* locus within accessions carrying the awn-inhibiting *B1* allele. This result suggests that the *B1* locus has not been under intense selection pressure. I detected four QTLs other than *Hd*, *B1* and *B2*. To my knowledge, none of these are homologous to any known genes identified in relation to awn morphogenesis in rice and barley, nor in the region of previously reported QTL for awn elongation in wheat.

In Chapter 3, I searched for genomic regions associated with grain size and shape, which can affect total yield and milling yield. I investigated the genetic diversity in terms of grain morphology within the core collection of hexaploid wheat accessions of NBRP-Wheat, which consists of 190 accessions. I scanned images of grains of each accession and recorded six metric characters: area size, perimeter length, grain length, grain width, length-to-width ratio, and circularity. Additionally, I measured hundred-grain weight. Principal component analysis of these seven characters demonstrated that two principal components (PC_{core1} and PC_{core2}) explain more than 96% of the variation in the core collection. The correlation coefficients between the principal components and characters indicate that PC_{core1} is related to grain size and PC_{core2} to grain shape. From a GWAS, I detected a total of 17 significant MTAs for five traits: two for area size, three for perimeter length, four for grain length, six for grain width, and one for hundred-grain weight, as well as one MTA for PC_{core2}. No predominant peaks of MTAs were found for any characters of grain morphology, and the number of significant MTAs was fewer than those found for awn morphology, an indication that grain morphology in common wheat is not regulated by a few genes with a strong effect but by the cumulative effects of many genes.

In Chapter 4, the effect of an alien chromosome segment on recombination frequency was evaluated to confirm if the introduction of alien chromatin increases the recombination frequency of an innate genome as reported in other plants. The low recombination frequency in the common wheat genome presents a challenge when attempting to breed new varieties and genetically map genes of interests. While an alien chromosome addition in the *Brassica* species leads to a genome-wide elevation in recombination frequency, the general effect of alien chromosomes on meiotic recombination remains poorly understood in other crop species. I investigated whether an alien chromosome segment translocated from *Aegilops sharonensis* Eig would affect the recombination frequency in the A and B genomes of common wheat. I detected a 5%

increase in total map length of the A and B genomes in the cross with the alien segment compared to the control. On the chromosome base, chromosomes 7A had a significantly larger number of crossovers. The recombination landscape was similar between the populations, indicating that the differences in the chromosome-scale recombination rate should be attributed to accumulations of slight differences in the recombination rate between markers.

In this dissertation, I successfully applied whole genome genotyping with RAD markers for a wide range of genetic analyses of complex characters in common wheat. I would like to conclude that my dissertation demonstrates feasibility to identify genes of interest in wheat without relying on model plants.