

Identification of genetic resources resistant to wheat blast and mapping of the novel resistance genes by exploring tetraploid and hexaploid wheat core collections (四倍体および六倍体コムギコアコレクションの探索による、コムギいもち病抵抗性系統の同定と新規抵抗性遺伝子のマッピング)

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General introduction

Wheat is the most widely cultivated cereal on the globe and serves as a staple food for approximately 35% of the world's population (IDRC, 2010). The genus *Triticum* includes a polyploidy series of species consisting of diploids ($2n = 2x = 14$), tetraploids ($2n = 4x = 14$) and hexaploids ($2n = 6x = 42$). *Triticum aestivum* (bread wheat or common wheat, genome constitution AABBDD) and *T. durum* (pasta wheat, AABB) are commercially most important crops. In statistics, wheat production losses significantly by pathogens, estimated to be about 15-20% of the annual production in the world (Figueroa et al., 2018), and now threatened by an emerging pandemic disease; that is wheat blast. Wheat blast is a devastating disease of wheat, in warm and humid regions, wheat blast has become a serious threat to wheat production since it was first observed in 1985 (Urashima 1993). Wheat blast caused by *Magnaporthe oryzae* (Syn. *Pyricularia oryzae*) pathotype *Triticum* (MoT) has recently been spread to Asia (Bangladesh) and Africa (Zambia) from its emerging region of South America. Wheat varieties with durable resistance are needed to secure wheat production, but very limited resistance resources are currently available. So far, only six genes (*Rmg2*, *Rmg3*, *Rmg7*, *Rmg8*, *RmgGR119* and *Rmg11*) and the 2NS chromosome segment from *Aegilops ventricosa* against MoT are reported (Zhan et al. 2008; Cruppe et al. 2020; Asuke et al. 2024; Islam et al. 2024). However, only *Rmg8*, 2NS, and *Rmg11* are effective at high temperatures and at the heading stage, and unfortunately, they are not resistant to all the MoT isolates. Considering the rareness of the available wheat blast resistance genes in wheat, I plan to use accessions collected last century and *ex-situ* conserved by the National BioResources Project (NBRP)-Wheat, Kyoto University to search for the resistance accessions and try to map the novel resistance genes with the specific focus to the hexaploid and tetraploid germplasms as primary gene pools. The tetraploid and hexaploid core collections used in this study include germplasms collected from a wide range of regions and encompassing various subspecies, offering high potential genetic diversity and the prospect of discovering novel disease-resistance genes.

Chapter 1: Develop the 4x core collection and exploration resistance resource to wheat blast

NBRP-Wheat maintains more than 2,000 wild and landrace accessions of tetraploid wheat. These accessions are divided into two groups, Emmer wheat (*T. turgidum*, AABB) and Timopheevii wheat (*T. timopheevii*, AAGG). To promote the utilization of genetic resources, I investigated the genetic diversity of all the accessions with 300 DArT markers. The population structure analysis showed that the Emmer wheat accessions were divided into three groups. Based on the population structure analysis, I selected 180 accessions as a core collection of tetraploid wheat, which represents a wide range of genetic diversity of all the NBRP-Wheat tetraploid wheat accessions. Through the genotyping by random amplicon sequencing-direct (GRAS-Di) method (Hosoya et al., 2019), I selected 27,618 markers shared by 160 Emmer wheat accessions with a missing rate less than 20% and a minor allele frequency large than 5%. I phenotyped and screened this core collection using the wheat blast isolate Br48 (Zhan et al. 2008) at the seedling stage and found 14 resistance accessions. Through the k-mer based genome wide association study (GWAS) method (Arora et al. 2019), I found a marker-trait association (MTA) on chromosome 2A of the SY-Mattis reference genome, which overlapped with the *Rmg8* locus conferring resistance to wheat blast (Asuke et al. 2024). By inoculating the BW strain Br48ΔA8, an *AVR-Rmg8* disruptant of Br48, I verified that 11 resistance accessions carry the *Rmg8* gene, and the rest three do not, indicated that the three accessions may carry novel resistance genes. The rarity of novel resistant accessions indicated that tetraploid wheat may not be a good resource for wheat blast resistance.

Chapter 2: Investigate resistance resource and mapping two resistance genes to wheat blast in 6x core collection

I also utilized a hexaploid wheat core collection (Takenaka, et al, 2018) for a source of resistance resources. In this study, the reactions to the wheat blast pathogen of the 188 hexaploid wheat accessions were investigated, and 13 resistance and 19 moderate resistance accessions were found. Unlike tetraploid wheat, only one resistant hexaploid accession carries the *Rmg8* gene, which was confirmed by the infection assay with Br48ΔA8. Geographic distribution of the resistant accessions and population structures indicate the most resistant accessions are from West and Central Asia and Europe, only a few resistant accessions are from East and South Asia. The accessions of *T. spelta* are rich in resistance (6/13). For GWAS and genetic analysis, this core collection was re-genotyped by GRAS-Di to increase marker number and to improve the marker distribution in the genome, especially near the centromeric regions. I did not find any highly significant MTAs through both SNP and k-mer based GWASs, probably the resistance genes may be rare in the hexaploid core collection and only shared in a few accessions, which makes it hard to be detected by GWAS. To map the resistance genes found in 6x core collection, I selected three accessions (6x028, *T. aestivum* collected from Greece; 6x086, *T. aestivum* collected from Ethiopia; 6x124, *T. spelta* collected from Spain) that showed resistance in an experiment of inoculating spikes at high (28°C) temperatures. I constructed the F_{2:3} populations of the three accessions by crossing them with a susceptible cultivar Chinese Spring. By checking the phenotypes of 200 individuals each of the F₂ population I confirmed that 6x028 carry two resistance genes (15:1 segregation, $\chi^2 = 0.023$), and 6x086 and 6x124 carry one resistance gene (3:1 segregation, $\chi^2 = 0.0008$ and 1.12, respectively). I first focused on the 6x086 and 6x124 populations with the simplest Mendelian inheritance. Through a combination of bulked segregate analysis (BSA), PCR-based genotyping of F₂, and inoculate of F₃ individuals, I could narrow down the locations of the resistance gene of 6x086 and 6x124 to the physical interval of 717 to 724 Mb on chromosome 4A and the interval of 672 to 680 Mb on 5A, respectively. This study not only provides potential resistance resources to wheat blast that may be useful for breeding resistant wheat cultivars, but also provides the basis and guidance for the future exploration of wheat blast resistance resources; namely *T. aestivum* accessions from the Mediterranean region and *T. spelta*, which have different resistance genes to wheat blast would be a good source of resistance to wheat blast.

Chapter 3: Resistance gene mapping in a BIL population crossed by Harukiari and *T. spelta*

In Chapter 2, I conducted an infection assay of the hexaploid core collection and found that the *T. spelta* had a high frequency of resistant accessions. Therefore, I checked six accessions of *T. spelta* that are the parents of BC₁F₆ populations with a common recurrent parent of Japanese cultivar Harukiari. My inoculation results showed that two *T. spelta* accessions, K15013 and K19092A, were resistant to Br48 at the seedling stage, while Harukiari was moderately resistant. I mapped the resistance gene in the K19092A X 'Haruhikari' BC₁F_{6:7} population. I first genotyped 188 BC₁F₆ individuals by GRAS-Di and their BC₁F₇ progeny (15 individuals each) were phenotyped by inoculation. Finally, I established a genetic map with 2,689 markers, with a total map length of 5,743 cM. I performed QLT analysis to locate two loci on the short (40.3 Mb) and long (638 Mb) arms of chromosome 4B, respectively. These two QTLs in total explained 27% of the phenotypic variation. Individuals carrying the combination of two resistance gene alleles showed stronger resistance. This resistance locus is different from that I found previously in 6x124. The accession K19092A was collected from Latvia, different from the collection site of 6x124 in Spain. This shows that *T. spelta* subspecies in different places do not share the same resistance gene. Because of the backcrossing with the elite cultivar 'Haruhikari', and cumulative recombination and homozygosity acquired in the seven generations of selfing, we can expect that in some BC₁F₇ individual, resistance loci and agronomic favorable loci (e.g. early flowering allele and free threshing loci) are pyramided, allowing these ideal resistant individuals may be used for advanced breeding programs.

General Discussion

Core collections are a powerful tool for exploring new genetic resources for crop improvement, as they reduce genetic redundancy and contain genetically and geographically diverse accessions of the entire population. Using the two core collections, I detected three and 31 resistant tetraploid and hexaploid wheat accessions that may carry novel resistance genes. Moreover, by examining the population structure and geographic distribution, I confirmed that the accessions originated from Asia are devoid of resistance genes against wheat blast. The known resistance locus *Rmg8* is found in tetraploid core collection through the k-mer based GWAS in combination with the pan-genome as reference but not by traditional SNP-based GWAS even using the reference genome of resistant cultivar SY-Mattis, showing that the strategy using k-mer GWAS and pan-genome is better detecting MTAs, especially for such genes as *Rmg8* that located in the structure variation region of the genome. Also, I demonstrated that three hexaploid accessions were resistant at the heading stage and under high temperature conditions (28°C), indicating that these resistance genes could be used in practical breeding program of resistant cultivars. Furthermore, I found that *T. spelta* is rich in resistance genes, which may be related to its unique origin and genetic diversity it maintained (Wang et al. 2024). *T. spelta* from Latvia and Spain carry different resistance genes, suggesting the resistant genes might have evolved from different origins.