Studies on utilization of tetraploid wheat (*Triticum turgidum* L.) as genetic resources and improvement of breeding efficiency by novel techniques for detecting nucleotide polymorphisms

（四倍体コムギの遺伝資源の活用と新規の塩基多型取得技術による育種の効率化に関する研究）

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Abstract

Since flowering time is an important trait for yield in wheat, it is essential to identify various flowering genes for genetic control of flowering time. Although several genes related to the flowering time have been identified so far, it is necessary to identify additional types of flowering-related genes to optimize the flowering time in response to future climate change. It has long been proven that bread wheat is a species that has undergone multiple rounds of ploidy evolution. It is known that tetraploid wheat (AABB; *Triticum turgidum* L.) and Tausch's goatgrass (DD; *Aegilops tauschii* L.) were crossed during this ploidy evolution, resulting in the accidental formation of a non-reducing gametophyte that gave rise to hexaploid common wheat (AABBDD; *Triticum aestivum* L.) (Kihara 1946; Mcfadden and Sears 1946). It is also known that common wheat has less diversity in its D genome than in its A and B genomes (Zhou et al. 2020), which is thought to be due to the fact that few lineages of Tausch's goatgrass were involved in its evolution (Akhunov et al. 2010). Therefore, Tausch's goatgrass is considered to be an important genetic resource to compensate for the low diversity of D genome in common wheat, and there has been a lot of research on the use of artificially produced synthetic hexaploid wheats as a genetic resource (Li et al. 2018).

On the other hand, because recessive mutations are more likely to appear as phenotypes in diploid wheat than in tetraploid and hexaploid wheat, the introduction of useful genes identified in analyses using diploid wild species into tetraploid and hexaploid cultivars may be difficult because of the influence of the homoeologous genes. In tetraploid wheat, the presence of homoeologous genes in the genome suggests that phenotypic diversity is often caused by dominant mutations rather than recessive mutations. Furthermore, while tetraploid wheat is recognized to have genetic exchange with common wheat on several occasions, there are lines that are underutilized in bread wheat due to its genetic diversity, including a variety of subspecies. Thus, the search for

useful genes from tetraploid wheat groups may be advantageous over the diploid wheat in breeding tetraploid and hexaploid wheat. I hypothesized that I could efficiently identify dominant flowering genes that could be easily used for breeding ploidy wheat.

In Chapter 1, I identified a novel early heading allele of *VRN-A3* from an emmer wheat derived from Ethiopia. The early heading allele of *VRN-A3* identified affected flowering time in lines that possessed *VRN-B3* which is thought to be the functional type. This supports the hypothesis that tetraploid wheat can be used to efficiently identify dominant genes that can be easily used for breeding wheat, thus achieving the one of the objectives of this study. There is also the fact that the photoperiod insensitive allele of *Ppd1* (Wilhelm et al. 2009) and the spring-type allele of *VRN1* (Yan et al. 2003; Fu et al. 2005) used in breeding tetraploid and hexaploid wheat are dominant over the wild type as mutant alleles, supporting my hypothesis. These suggest that useful genes can be efficiently identified in gene identification for polyploid crops, including wheat, by analyzing the diversity that occurs in the polyploid relatives.

Besides, for efficient breeding using the genes related flowering time, it is also necessary to improve more high-throughput polymorphism detection methods using NGS. In Chapter 2, focusing on the MIG-seq (Suyama and Matsuki, 2015), which has never been used for crop genetic analysis before, I applied this method to wheat and improved this method to develop a more efficient polymorphism detection technique using NGS with reduced genome complexity. I not only showed that MIG-seq is effective for wheat, but also developed a method, dpMIG-seq, that overcomes the disadvantage of MIG-seq in that the number of polymorphisms that can be detected cannot be changed. In dpMIG-seq, the advantage of MIG-seq over ddRAD-seq and GRAS-di remains that it does not depend on DNA quality (Suyama and Matsuki 2015). Therefore, this method can handle a large number of samples while reducing the costs associated with DNA extraction and purification. Furthermore, since dpMIG-seq is a PCR-based method, it can be applied to crops other than wheat, animals, and bacteria, suggesting the findings obtained in this study will contribute to a wide range of biology beyond crop breeding.

In Chapter 3, I aimed to demonstrate the practicality of the above results. The only durum wheat cultivar in Japan is Setodur, which is suitable for cultivation only in the Setouchi region. Thus, there is a need to develop durum wheat cultivars that possess more early heading traits and can be cultivated in a wide range of regions in Japan. Therefore, I attempted to efficiently construct NILs in which all chromosomal regions except the *VRN-A3* flanking region are Setodur type by applying MIG-seq and dpMIG-seq. As a result, I have successfully bred a durum wheat line that possesses the *VRN-A3* novel early flowering allele. However, since linkage drag was not considered when

constructing the NILs, several TN26-derived genes remained in the *VRN-A3* region. To make this scheme more sophisticated, it is a future task to develop an efficient method to eliminate the remaining regions with genes other than *VRN-A3*.

In this study, I analyzed the trait related with flowering time, but there are also potentially useful genes for important traits such as resistance of fusarium head bright and salinity tolerance in tetraploid wheat (Munns and James 2003; Prat et al. 2014). As many of these studies using tetraploid wheat have not used PCR-based markers such as SSR markers, integration with recently sequenced and assembled wheat genomic information is time consuming. On the other hand, the NGS library construction method developed in Chapter 2 of this study is PCR-based, so it is possible, for example, to create an NGS library using DNA that has been cryopreserved after being used in previous studies and then reanalyzed by creating linkage maps corresponding to the genomic information. This may allow more efficient identification of candidate genes.

While we demonstrated the breeding of durum wheat lines via normal crossing technique in Chapter 3, the introduction of tetraploid wheat-derived genes into bread wheat, which is grown in a wider area, will require the techniques described below. Because bread wheat is a different ploidy than tetraploid wheat, its F_1 are pentaploid and must undergo the time-comsuming procedure of repeated backcrossing until the progenies become hexaploid. Alternatively, synthetic hexaploid wheats can be artificially produced from intergeneric hybrids of these two species. This phenomenon could be exploited to create synthetic hexaploid wheat accessions possessing diverse tetraploid wheat derived AABB genomes. On the other hand, variation among tetraploid wheat accessions has been observed with respect to the hybridization efficiency with tausch's goatgrass and the ability to purify non-reduced gametes (i.e., the ability to naturally produce seeds of synthetic hexaploid wheat) (Matsuoka and Mori 2020). The tetraploid wheat cultivar, 'Langdon' possesses a trait that facilitates the formation of non-reducing gametes in the triploid generation crossed with tausch's goatgrass (Matsuoka and Nasuda 2004; Matsuoka and Mori 2020). If the genetic mechanism for this Langdon trait can be elucidated, it will be possible to turn tetraploid wheat, which is not easily turned into synthetic hexaploid wheat, into synthetic hexaploid wheat.

It is also known that when tetraploid wheat is converted to synthetic hexaploid, epigenetic changes occur in the AB genomes, resulting in variation of multiple gene expression (Yuan et al. 2020). When this phenomenon is considered, it is essential to analyze tetraploid wheat after it has been converted to synthetic hexaploid if genes from tetraploid wheat are to be used for hexaploid wheat breeding with certainty. This is a subject for future research of more efficient use of tetraploid wheat for hexaploid wheat

breeding. Overall, the results obtained by this study provided a new starting point for wheat breeding.