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SUMMARY OF DOCTORAL THESIS

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Title: Accelerating wheat-alien introgression breeding and genome analysis using genome-wide markers
（ゲノムワイドマーカーを用いたコムギの異種遺伝子導入およびゲノム分析の促進）

SUMMARY

Over time, wild relatives of wheat have played important roles in enhancing improved yield and adaptability of cultivars of wheat. Regardless of their value as potential gene sources for wheat breeding, the current status of their genomic resources is not enough to aid proper understanding of their genomes and support maximal utilization of their genes. This is obvious in the relatively far-related species grouped into the tertiary gene pool. This inadequate genomic database needs to be continually enriched to enhance aggressive utilization of wild genetic resources to manage biotic and abiotic stresses which pose threats to the general performance of elite cultivars of wheat.

The species in the genus *Leymus* are known to be resistant to salinity and economically important diseases of wheat. *Leymus racemosus* is particularly reported to exhibit biological nitrogen inhibition (BNI) activity, a valuable trait of agronomic and environmental importance. To optimally harness these genes for the improvement of wheat, there should be efficient cytological and molecular markers to unmistakably map the alien chromatins in the genetic background of wheat. Before this study, variable cytological markers enabled molecular cytogenetic characterization of *L. racemosus* chromosomes in wheat, but there were no DNA markers to conduct detailed characterization of wheat–*L. racemosus* translocation and recombination lines. Worse still, the lack of both cytological and DNA markers from the genome of *L. mollis* greatly delayed the production of wheat–*L. mollis* chromosome addition lines (CALs). Earlier attempts to apply *L. racemosus* cytological markers to characterize the chromosomes of *L. mollis* in wheat failed to produce satisfactory hybridization signals. This necessitated the development of an alternative strategy to characterize wheat–*L. mollis* lines without necessarily applying *in situ* hybridization for alien identification.

Although there is an appreciable volume of data aiming at elucidating the genomic and phylogeny of the species of tribe Triticeae, there are still some controversies regarding the genomic relationships among *Aegilops* and *Triticum* species. Some of such controversies include the opposing views regarding the primary donor(s) of B/G genomes of polyploid *Triticum* species, including bread wheat, and the speculated unidentified diploid genomes believed to have participated in the evolution of *Ae. crassa*, *Ae. vavilovii*, *Ae. juvenalis*, *Ae. columnaris* and *Ae. triaristata*. Resolving these issues using the ‘genome analyzer’ method, which relies on meiotic chromosome pairing in hybrids of distant crosses, although quite informative, had since come under heavy criticism. Therefore, the application of molecular methods to accumulate useful data that would aid to clarify the evolutionary relationships among these species remains the focus of contemporary studies. Such markers have helped to provide some explanations on *Aegilops-Triticum* relationships, the origin and differentiation of *Aegilops* species, and intra- and inter-specific variations in the D and U genome clusters of *Aegilops* species. However, the numbers of markers in most of the cases are hardly enough to satisfactorily assure genomic coverage.

Consequently, this study was basically designed to develop and validate DNA markers from the genomes of *L. racemosus* and *L. mollis*, develop a molecular marker-based strategy for production of wheat–*L. mollis* CALs and assess the suitability of DArTseq genotyping, an efficient genotyping-by-sequencing platform, in wheat–alien characterization and analysis of genomes of selected species in tribe Triticeae.

Using genome sequence information of *L. racemosus* and DArTseq genotyping, thousands of polymorphic markers were developed from the genomes of *L. racemosus* and *L. mollis*. Additionally, unique SNP markers were identified in the genomes of 19 wheat-*Leymus* CALs, absent in the genomes of the parents. Polymorphic PCR markers were successfully transferred to other distant relatives of wheat, indicating their suitability for mapping alien chromatins from other wild relatives of wheat. A good number of *L. racemosus* markers were applied to clearly characterize 22 wheat–*L. racemosus* chromosome introgression lines, while the PCR markers transferred to *L. mollis* genome, in combination with *L. mollis* genome-based SNP markers, aided selection and characterization of 10 new wheat–*L. mollis* CALs. Genomic *in situ* hybridization confirmed the presence of the alien chromosomes in the 10 CALs. This study has therefore demonstrated that CALs can be produced without relying on *in situ* hybridization for alien identification, resulting in great savings in time and efforts. DArTseq genotyping particularly aided identification of the homoeologous groups of all the *Leymus* chromosomes introgressed into wheat and comparison of the chromosomes of *L. racemosus* and *L. mollis.* The similarity between the two species and the association of their chromosomes were applied to propose, for the first time, a nomenclature system for *Leymus* chromosomes.

Similarly, with DArTseq analysis, the genomes of 34 species in tribe Triticeae were clearly differentiated, and the phylogenetic relationships among the diploid and polyploid *Aegilops* and *Triticum* species were estimated. The SNP markers among *Aegilops* species enabled reliable reconstruction of their phylogeny: diploid species clustered based on their known sections, while the polyploid species formed two main clusters following the presence of two common diploid genomes, *Ae. tauschii* or *Ae. umbellulata*. Also, using species-specific SilicoDArT markers in diploid species as 'analyzers', the putative diploid progenitors of the polyploid species were elucidated. While confirming the genomic constitutions of seven polyploid species of *Aegilops*, this analysis traced the hitherto unidentified diploid progenitors of five polyploid *Aegilops* species to two genomically proximal diploid species, *Ae. speltoides* and *Ae. mutica*.

The analysis also assisted a satisfactory determination of the primary donors of A, B/G and D genomes in polyploid *Triticum* species, and provided evidence-based information clarifying the complex nature of the B/G genomes of polyploid *Triticum* species. The findings suggest that the B genomes either evolved from the hybridization of *Ae. speltoides* and *Ae. searsii* or from a common ancestral species which later differentiated into the present day *Ae. speltoides* and *Ae. searsii*. On the other hand, the A and D genomes substantially matched the genomes of *T. urartu* and *Ae. tauschii*, respectively. However, the significant homology between the A genomes of the polyploid species and the genome of *T. boeoticum*, another A genome species, is an indication of the likelihood of common ancestry of the two A genome species. Therefore, like the B/G genomes, the A genomes of polyploid *Triticum* species may have arisen from an ancestral species that differentiated into *T. urartu* and *T. boeoticum* after the evolution of the A genomes of the polyploid species. The A genomes of all the polyploid *Triticum* species were also proven to derive from the same primary A genome species, most likely *T. urartu*, invalidating earlier claims that the A genomes of polyploid wheats in Emmer and Timopheevi lineages were donated by different A genome species, *T. urartu* and *T. boeoticum*, respectively.

This study has shown the efficacy of applying genome-based markers of relevant wild genetic resources for speedy chromosome introgression breeding of wheat. The value of DArTseq genotyping in characterizing wheat–alien CILs and analyzing evolutionary relationships among plant species has also been proven.