

(Format No. 13)

## SUMMARY OF DOCTORAL THESIS

Name: MAZIN MAHJOOB MOHAMED MAHJOOB

Title: Genetic studies on intraspecific variations of *Aegilops tauschii* to enhance bread wheat diversity

(パンコムギの多様性を拡大するためのタルホコムギの種内変異に関する遺伝学的研究)

The germplasm of related wild species attracts increasing attention because they can provide characters related to adaptation to cultivated species by breeding. The genus *Aegilops* L. (Poaceae) has been intensively studied because of its close relationship with cultivated wheats. The phylogenetic relationship between genera *Aegilops* and *Triticum* L. is widely reported, and on a world scale, the genus *Aegilops* includes 23 wild annual species, of which 11 are diploids and 12 are allopolyploids.

About 8000 to 10,000 years ago, the ancestor of the current bread wheat appeared as a result of natural hybridization between cultivated tetraploid wheat (*Triticum turgidum* L.,  $2n = 4x = 28$ , AABB) and *Ae. tauschii*. Inside this last species, two subspecies were first described by Eig (1929) as *Ae. squarrosa* ssp. *eusquarrosa* and ssp. *strangulata* and their nomenclature was revised by Hammer (1980) as *Ae. tauschii* ssp. *tauschii* and ssp. *strangulata*. *Ae. tauschii* is genetically and morphologically diverse, and the ssp. *tauschii* has elongated cylindrical spikelets, whereas ssp. *strangulata* has quadrate spikelets and empty glumes. The ssp. *tauschii* has a wide distribution throughout the species range, whereas ssp. *strangulata* is limited to the south-eastern Caspian coastal region and the Caucasus.

The genetic diversity in *Ae. tauschii* has been studied at the molecular level including isozymes, random amplified polymorphic DNA (RAPD), chloroplast DNA, amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs) and DArT array markers. Most of these studies classified *Ae. tauschii* into three lineages, TauL1, TauL2 and TauL3: TauL1 including only ssp. *tauschii*, TauL2 including both ssp. *tauschii* and ssp. *strangulata* and TauL3 with intermediate forms.

*Ae. tauschii* is the easiest species in this genus to utilize in wheat breeding, because there is little to no inhibition to meiotic chromosome pairing with the D genome chromosomes of bread wheat. There is a few research proposed that TauL2 is closer to the D genome of bread wheat compare to TauL1. Furthermore, a few studies have assumed that the D genome of bread wheat was donated to common wheat by the ssp. *strangulata*. This was revealed by several isozyme polymorphism such as NADP-dependent aromatic alcohol dehydrogenase and alcohol dehydrogenase isoenzymes.

It has been confirmed that *Ae. tauschii* have useful traits widely used over the past 60 years for wheat breeding for biotic and abiotic stresses tolerance. It has been utilized via synthetic hexaploid wheat as bridge crossing and direct crossing however, both of these methods have limitation. To use the genetic diversity in *Ae. tauschii* effectively in wheat breeding, a precise genomic and morpho-physiological analysis is needed.

In the first part of this dissertation, I clarified the phylogeny of *Ae. tauschii* using 5,880

high-quality SNPs derived from DArTseq and further measured the traits that significantly different between TauL1, TauL2 and TauL3, or between ssp. *tauschii* and ssp. *strangulata*. Genetic and 11 morpho-physiological diversity was examined in 293 accessions covering the entire range of *Ae. tauschii*, including lines that previously lacked passport data. As a result, we were able to allocate 175, 113 and 5 to TauL1, TauL2 and TauL3, respectively. Of 124 lines lacking passport data were assigned 66 to TauL1, 57 to TauL2 and 1 to TauL3. To study the morpho-physiological variation, I measured two leaf parameters (flag leaf length; flag leaf width), four spike parameters (spike length; spike width; seed number per spike; spike weight), days to heading biomass weight and three physiological traits (Normalized Difference Vegetative Index; canopy temperature; and chlorophyll content).

As a result, I observed wide morpho-physiological variation in each lineage and subspecies. Although some of the spike related traits examined differed significantly between the lineages and subspecies, the range of the variation was overlapped. These similarities may resulted due to adaptive convergence which possibly have originated between the different lineages after the geographical isolation under similar environmental condition

In the second part of this dissertation, I identified allelic diversity in *Ae. tauschii* which is of utmost importance for efficient breeding and widening of the genetic base of wheat. Here I identified markers or genes associated with morpho-physiological traits in *Ae. tauschii*, and understood the difference in genetic diversity between the two main lineages. I performed genome-wide association studies of the same 11 morpho-physiological traits used the first part of this dissertation for 293 *Ae. tauschii* accessions representing the entire range of natural species range to cover most genetic diversity that existed in *Ae. tauschii* using 34,920 DArTseq markers. I observed a wide range of morpho-physiological variation among all accessions. I identified 79 marker-trait associations (MTAs) in all accessions, 14 specific to TauL1 and 17 specific to TauL2, suggesting independent evolution in each lineage. Some of the MTAs are novel and have not been reported in bread wheat. The MTAs identified in each lineage are different from each other, which mean both lineages have highly adopted different genes. This should be considered when we use *Ae. tauschii* in wheat breeding. The markers or genes identified in this study will help to reveal the genes controlling the morpho-physiological traits in *Ae. tauschii*, and thus in bread wheat even if the plant morphology is different.

From the phylogenetic study, I have revealed the most traits to discriminate between and subspecies are spike-related traits (spike shape). Also, it is difficult to discriminate between lineage with plant phenology, and the easiest and accurate method is genomic analysis. Also, I have confirmed that genotyping by the DArTseq platform is an accurate platform to study genomic analysis of plant species. Using this platform, I could give an accurate taxonomy for 124 accessions lacked data on their lineages and subspecies. Furthermore, I could propose the geographical origin of these accessions. These findings will largely facilitate the utilization of *Ae. tauschii* in wheat breeding.

From and GWAS analysis, I have revealed that there is intraspecific lineages variation excited in both lineages. This variation must be utilized efficiently to transfer most genetic variation to bread wheat. Furthermore, I have discovered that each lineage has contributed differently to enhance specific traits. This mean breeder must utilize the specific lineage according to the breeding targets. In another word, developing new germplasm from both lineages could transfer a large variation of D-genome diversity compare with using one lineage. A number of studies on different traits and in different environments are needed to gain a better understanding of the genetic diversity present in *Ae. tauschii*. It is also necessary to employ advanced genome analysis so that the large and complex genomes can be easily analyzed and a large number of genetic markers can be generated.