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SUMMARY OF DOCTORAL THESIS

Name: Ikram Elsadig Suliman Mohamed

Title: Exploiting genetic diversity in *Aegilops tauschii* to improve wheat flour quality under heat stress conditions

（高温ストレス条件下での小麦粉の品質改良に向けたタルホコムギが持つ遺伝的多様性の活用）

Wheat grain quality, a characteristic that affects food processing quality and nutritional value, is crucial for assessing new wheat varieties' market potential and commercial value. One of the most important characteristics that affect the quality of wheat is the unique gluten protein, which gives viscoelastic properties that are harnessed to process wheat dough into different products likes: bread, noodles, pasta, and other food products. The gluten proteins are seeds storage proteins, divided into monomeric gliadins and polymeric glutenins. Gliadin proteins are classified into the four major types, α-, β-, γ-, and ω-gliadins, according to their electrophoretic mobility in acid conditions. Glutenins are classified into high molecular weight (HMW) and low molecular weight (LMW) types. The high-molecular-weight glutenin subunits (HMW-GSs) of the glutenin constitute about 10% of seeds storage proteins. However, they are the decisive factor in wheat flour quality to be processed into different products. This is due to the fact that they are the major factor that determines the gluten elasticity, and thus, they are essential for the bread-making process. Therefore, the expansion in the diversity of HMW-GS alleles possibly leads to increased varieties of choices for wheat flour end-products.

The genes encoding for HMW-GSs are located on the long arms of chromosomes 1A, 1B, and 1D at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively. The literatures stated that alleles at *Glu-D1* locus have a significantly strong effect on dough strength and documented the positive and the negative impact of allele 5+10 and 2+12 on dough strength in common wheat respectively. However, the limitation of allele at *Glu-D1* locus due to the narrow diversity in the common wheat is well documented.

Breeding to obtain a wheat variety combine high yields and good quality is very difficult due to the inverse relationship between quality traits (mainly protein content) and grain yield. This relationship depends on several factors, including genotype, source-sink interactions, and environmental factors such as heat stress.

Heat stress is one of the most important abiotic factors that negatively affect both grain yield and flour quality all over the world, especially if the crop is exposed to heat stress during the grain-filling period. It was documented the huge reduction in yield and decrease in the dough strength if the wheat was exposed to heat during this period.

Therefore, breeding for heat stress-resilience wheat genotypes that combine high yield and good quality is crucial to counteracting or adapting to global warming that is expected to increase severely over time.

Thus, to breed for a heat stress-resilience wheat genotypes, it's essential to understand and determine the impact of high temperature on wheat flour quality and grain yield, understand the genetic basis of the diversity resilience to wheat quality, and evaluate differential genotypic responses.

The studies that evaluated the effect of heat and identified climate-resilient wheat genotypes either used very few genotypes or the investigation was under a controlled environment or only identified climate-resilient genotypes considering grain yield and grain-related traits without addressing the wheat quality aspects.

Since the wheat quality (dough strength) have been documented in several studies to be greatly affected by the HMW-GSs, these studies have been done under normal condition and the effect of HMW-GSs on dough strength under heat stress condition is unclear.

Since both the superior HMW-GS alleles and climate-resilient wheat germplasm is becoming rare due to the narrow genetic diversity of the common wheat, we used in this study (chapter two and three) a diverse panel of wheat multiple synthetic derivatives (MSD). The MSD panel has been developed using 43 *Aegilops tauschii* accessions that represent the existing diversity in the entire natural habitat.

In chapter two, we aimed to explore allelic variation of HMW-GS at the *Glu-D1* locus from *Ae. tauschii* in 392 MSD lines. We aimed to evaluate the impact of the allele at the *Glu-D1* locus from *Ae. tauschii* on dough strength which was under normal condition in Japan to identify good flour quality lines. We also aimed to know the relationship between protein content and grain yield to identify lines that could combine good quality and high grain yield. We observed broad diversity for alleles at the *Glu-D1* locus, reflecting the influence of the different chromosomal inserted segments from *Ae. tauschii*. These alleles at the *Glu-D1* locus may confer different choices in breeding programs for different end-use products. We documented a large variation in dough strength even between lines with the same HMW-GS composition. We document an adverse effect of allele 5^t+10^t and a relatively positive effect of allele $2^t+12.1^t$ from *Ae. tauschii* on dough strength. We identified four superior lines that improved the flour quality; MSD272, MSD363, MSD219, and MSD61 which carried two different alleles at the *Glu-D1* locus (2.1^t+12^t and 2^t+12^t) derived from *Ae. tauschii*. These lines are promising and could serve as a good source to improve wheat flour quality in the breeding programs. The regression between grain yield and protein content for MSD lines revealed no correlation between the two traits. We could identify MSD lines maintained comparable yields and high protein content compared to the backcrossed parent 'Norin 61'. These MSD lines are promising and could serve as a good source to improve wheat flour quality without any concern about the deterioration in grain yields.

In chapter three, we studied the effect of heat stress on flour quality and grain yield under moderate and continuous heat stress in the field condition in Sudan in 129 MSD lines. We aimed identify heat-stress resilience lines which combine both grain yield and good quality traits. We studied the effect of HMW-GS alleles on flour quality under heat stress to identify subunits that has stable performance or could maintain good dough strength under both optimum and heat stress condition. We performed genome wide association study on a panel of 127 MSD lines, to identify marker-trait associations (MTAs) associated with quality traits and grain yield under heat stress conditions that can be used to enhance both grain yield and flour quality under heat stress conditions. We also aimed to evaluate to which extent the *Ae. tauschii* diversity can be utilized to improve wheat quality under heat stress conditions.
The MSD lines exhibited noticeable genetic variation for quality traits and grain yield under heat stress conditions. We identified two lines, MSD159 and MSD65 showed superior performance to the recurrent parent Norin 61 regards dough strength under heat-stressed and severe heat-stressed environments respectively. Thus, those lines could be used in breeding programs to improve dough strength under heat stress and even in severe heat stress environments. We Identified three HMW-GS alleles at the *Glu-D1* locus (2.1^t+12^t, $2^t+12.1^t$, and 5^t+10^t) derived from *Ae. tauschii*, that showed no significant difference in their dough strength across four environments ranging from optimum to severe heat-stressed conditions. These alleles could be used in applications for future improvement of end-use qualities targeting wheat under severe heat stress. We could identify several MSD lines that showed grain yield higher than the recurrent parent Norin 61 under heat stress condition. Thus, the identified lines could be used as a source to improve grain yield under heat stress environments.
We identified 251 markers traits association, the majority of them on the D genome, confirming the power of the MSD panel as a platform for mining and exploring the genes of *Ae. tauschii*. We identified stable markers for dough strength under heat stress conditions, which simultaneously control grain yield under heat stress or optimum conditions. The identified lines, stable and pleiotropic markers explored in this study, are considered a good resource to develop resilient wheat cultivars that combine both good flour quality and grain yield under stress conditions using marker-assisted selection.

Overall, both studies showed that the wheat wild relative (*Ae. tauschii*) is an inexhaustible resource for gene mining to improve common wheat.