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SUMMARY OF DOCTORAL THESIS

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Title: Harnessing genetic diversity of the wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) for wheat breeding

(コムギ育種のための野生エンマーコムギ (*Triticum turgidum* ssp. *dicoccoides*) の
遺伝的多様性の利用)

During evolution, wheat has gained adaptive mechanisms to overcome different biotic and abiotic stresses. However, the domestication process and high selection pressure applied in modern breeding are associated with the massive erosion of genetic diversity, resulting in the vulnerability and susceptibility of modern cultivars to biotic and abiotic stresses. Wheat is susceptible to a temperature beyond the optimum, the current situation of the world climate involving the warming trend from year to year and the expected climate change scenarios position wheat production in the danger zone and warn about the future food security. The natural variation of wild emmer wheat encompasses important agronomic, physiological, and yield-related traits associated with heat stress tolerance. Thus, the diversity in wild emmer wheat is needed to sustain and improve wheat tolerance against heat stress to cope with climate change.

Substantial research efforts have been devoted to developing a diverse durum wheat population to exploit the wild emmer wheat diversity. However, the combined diversity of both wild emmer wheat lineages in one population has not been fully explored. Also, there is a shortage of genetic information from tetraploid wheat grown under natural field conditions and a lack of knowledge on heat stress tolerance mechanisms. This study used a population of multiple derivative lines (MDL) containing genomic fragments from nine wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) to improve durum and bread wheat. Its suitability for harnessing the diversity of wild emmer wheat for bread and durum wheat improvement was explored. The genomic regions (including candidate genes, QTLs, MTAs, and alleles) regulating heat stress tolerance were highlighted. Genotypes sharing positive alleles from wild emmer wheat under heat stress were identified. Also, the value of utilizing the diversity of the wild emmer wheat for heat stress tolerance adaptation was evaluated.

In chapter 1, a durum wheat population consisting of 178 BC₁F₆ was developed by crossing and backcrossing nine wild emmer wheat (*T. dicoccoides*) with cultivated durum wheat (*T. durum*) cultivar 'Miki 3'. I described the development of this population, which was named multiple derivative lines (MDL), and demonstrated its suitability for durum wheat breeding. I genotyped the MDL population, the parents, and 43 Sudanese durum wheat cultivars on a Diversity Array Technology sequencing platform. I evaluated days to heading and plant height in Dongola (Sudan) and in Tottori (Japan) for MDL validation. The physical map length of the MDL population was 9,939 Mbp, with an average of 1.4 SNP/Mbp. The MDL population had greater diversity than the Sudanese cultivars. I found high gene exchange between the nine wild emmer accessions and the MDL population, indicating that the MDL captured most of the diversity in the wild emmer accessions. Genome-wide association analysis identified three loci for days to heading on chromosomes 1A and 5A in Dongola and one on chromosome 3B in Tottori. For plant height, common genomic loci were found on

chromosomes 4A and 4B in both locations, and one genomic locus on chromosome 7B was found only in Dongola. The results revealed that the MDLs are an attractive resource with which to uncover the genes of wild emmer wheat and facilitate their use for bread and durum wheat improvement.

In chapter 2, the diverse set of the multiple derivative lines (MDL) was evaluated under four environments: two optimum environments at Tottori, Japan, and Dongola, Sudan, and one moderate heat stress environment and one severe heat stress environment at Wad Medani, Sudan. This study aimed to identify germplasm and QTLs/alleles associated with heat stress tolerance from wild emmer wheat diversity. Genome-wide association analysis was conducted with 13,312 SNP markers. Strong marker-trait associations (MTAs) were identified for chlorophyll content at maturity on chromosomes 1A and 5B: these MTAs explained 28.8 and 26.8% of the variation, respectively. A region on chromosome 3A (473.7–638.4 Mbp) contained MTAs controlling grain yield under optimum and severe heat stress. The region on chromosome 1A (358.9–522.6 Mbp) contained MTAs controlled thousand kernel weight under optimum conditions at Dongola, moderate heat, and severe heat stresses with phenotypic variation ranging from 11.2–16.3%, whereas the region on 6B (81.3–146.7 Mbp) harbored MTAs for thousand kernel weight in Dongola and moderate heat stress, explaining 17.9 and 15.6% of the allelic variation, respectively. Under severe heat stress, regions on chromosomes 3A (590.4–713.3 Mbp) controlled grain yield, biomass, days to maturity, and thousand kernel weight, and on 3B (744.0–795.2 Mbp) grain yield and biomass. Heat tolerance efficiency (HTE) was controlled by three MTAs, one each on chromosomes 2A, 2B, and 5A under moderate heat stress and one MTA on chromosome 3A under severe heat stress. Some of the MTAs found here were previously reported, but the new ones originated from the wild emmer wheat genomes. The favorable alleles identified from wild emmer wheat were absent or rare in the elite durum wheat germplasm being bred for heat stress tolerance. Further analysis revealed candidate genes, including serine/threonine-protein kinase, include TaSnRK2.4 from bread wheat regulates hyperosmotic responses and ABA signaling, C4HC3-type zinc finger *TaZnF* regulating heat stress tolerance in bread wheat, and superoxide dismutase type *TaOSD* stimulates antioxidant enzymes in bread wheat. This study provides potential genetic materials, candidate genes, alleles, MTAs, and quantitative trait loci for enhancing wheat adaptation to heat stress. The derivative lines studied here could be investigated to enhance other stress tolerance such as drought and salinity.

Overall, this work illustrates the importance of wild emmer wheat (*T. dicoccoides*) for improving modern durum wheat. It provides potential germplasms and genomic regions (including candidate genes, MTAs, alleles, and QTLs) in response to heat stress adaptation. Stable loci across environments were identified for important agronomic traits such as grain yield and thousand kernel weight. These loci can be further explored for use in marker-assisted selection and gene discovery. Some of the MTAs identified in this study are specific to heat stress and could be targeted in selection to improve heat stress tolerance. Identification of genotypes with favorable alleles and candidate genes from *T. turgidum* ssp. *dicoccoides* for different traits such as biomass, thousand kernel weight, and chlorophyll content at maturity demonstrate that the MDLs are an effective strategy for exploring wild emmer wheat diversity to adapt wheat to heat stress. Such germplasm with positive wild emmer wheat alleles has been used to improve the diversity of bread wheat A and B genomes by intercrossing some MDL lines with some bread wheat lines. Crossing tetraploid wheat and hexaploid wheat, together with appropriate strategies for evaluating and selecting desirable lines under heat and combined heat-drought stress, will aid in the genetic improvement of both durum and bread wheat.

In the future, validation of these findings will enable to serve as effective knowledge for wheat breeding under changing climate.