

SUMMARY OF DOCTORAL THESIS

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Title: Elucidation of *Aegilops tauschii* contribution to heat and drought tolerance diversity in bread wheat through genomics and metabolomics

(ゲノミクスとメタボロミクスに基づくタルホコムギのパンコムギ耐暑・耐乾性変異への貢献解明)

Many studies have shown the potential of using *A. tauschii* for breeding to enhance bread wheat productivity in drought- and heat-prone areas. However, the diversity in heat and drought resilience traits in bread wheat has not been fully explored. Also, there is a dearth of knowledge on the mechanism of combined heat and drought resilience, and a lack of genetic materials for combined stress resilience breeding. In this research, the heat and drought resilience diversity in bread wheat lines containing *Ae. tauschii* introgressions was explored; the genomic regions (including loci, candidate genes and alleles) regulating the resilience and the underlying physiological and metabolomic dynamics were highlighted. Also, the practicability of utilizing the diversity of *Ae. tauschii* for combined stress resilience breeding was assessed.

In Chapter 1, a wheat diversity panel containing *Ae. tauschii* introgressions was evaluated under heat (H) and combined heat-drought (HD) stress in Sudan to identify QTLs associated with resilience to the combined stress, and to assess the practicability of harnessing *Ae. tauschii* diversity for combined stress resilience breeding. Novel alleles and quantitative trait loci (QTLs) were identified on chromosomes 3D, 5D, and 7A controlling grain yield (GY), kernel number per spike (KPS), and thousand-kernel weight (TKW), and another on 3D (521–549 Mbp) controlling GY alone. A strong marker-trait association for GY stability was identified on chromosome 3D (508.3 Mbp) explaining 20.3% of the variation. Furthermore, leaf traits including canopy temperature (CT), normalized difference vegetative index (NDVI), and carbon-13 composition ($\delta^{13}\text{C}$) were controlled by five QTLs on 2D (23–96, 511–554, and 606–614 Mbp), 3D (155–171 Mbp), and 5D (407–413 Mbp), some of which were pleiotropic for GY and related traits. Most MTAs and QTLs were found in the D genome, indicating the potential of using *Ae. tauschii* diversity for wheat breeding. Further analysis revealed candidate genes, including *GA20ox*, regulating GY stability, and *CaaX prenyl protease 2*, regulating CT at the flowering stage, under H and HD stress. As this is the first such study, our results provide genomic landmarks for wheat breeding to improve adaptation to H and HD conditions under climate change.

In Chapter 2, twenty-four selected wheat lines, were evaluated under a drought-rewatering-drought cycle for two years in a greenhouse in Tottori, Japan. The objective was to validate the lines selected in Chapter 1. Drought was imposed by withholding water during flowering. The results revealed considerable genetic variability in physio-agronomic traits, reflecting the variation in introgressed segments. High heritability estimates (above 47%) were recorded for most traits, including days to 50% heading, plant height, and TKW, indicating the genetic control of these traits which may be useful for cultivar development. The trait-trait correlations within and between water regimes highlighted a strong association among the genetic factors controlling these traits. Some lines exhibited superior performance in terms of stress tolerance index and mean productivity compared with their backcross parent (N61) and elite cultivars commonly grown in hot and dry areas. Graphical genotyping revealed unique introgressed segments on chromosomes 4B, 6B, 2D, and 3D in some drought-resilient lines which

may be linked to drought resilience. Therefore, these lines were recommended for further breeding to develop climate-resilient wheat varieties.

In Chapter 3, I used two classical physiological methods, the fraction of transpirable soil water threshold (FTSW_{Th}) and drought stress response function, to characterize the water conservation traits of two selected wheat lines (MSD53 and MSD345) which both contain introgressed segments from *Ae. tauschii* but differ in drought resilience. The lines and N61 were subjected to dry-down conditions. MSD53 had a higher FTSW_{Th} for transpiration decrease than N61 and MSD345. In terms of drought stress response function, MSD53 had the lowest threshold suction, suggesting a lower drought resilience capacity compared with MSD345. However, MSD53 exhibited an effective-water-use trait whereas MSD345 exhibited a water-saving trait under dry-down conditions. These results are consistent with the reported higher GY of MSD53 in comparison with MSD345 under drought stress in Sudan, and demonstrate that high FTSW_{Th} supports effective water use for improved agricultural productivity in drylands. The differences in water conservation traits between the two MSD lines may be attributed to variation in introgressed segments, which can be further explored for drought resilience breeding. This study validates the results in Chapter 1 and 2.

In Chapter 4, my aim was to gain in-depth understanding of drought effect on wheat metabolism. I exposed wheat N61 plants to progressive drought stress [0 (before drought), 2, 4, 6, 8, and 10 days after withholding water] during the flowering stage and investigated physiological and metabolomic responses. Key abscisic acid-responsive genes, $\delta^{13}\text{C}$ and CT played major roles in wheat response to progressive drought stress. The CT depression was tightly correlated with soil water potential (SWP). Additionally, SWP at -517 kPa was identified as the critical point for increasing CT and inducing reactive oxygen species. Metabolome analysis identified four potential drought-responsive biomarkers, the enhancement of nitrogen recycling through purine and pyrimidine metabolism, drought-induced senescence based on 1-aminocyclopropane-1-carboxylic acid and Asn accumulation, and an anti-senescence response through serotonin accumulation under severe drought stress. These findings provide insights into the molecular, physiological and metabolite changes involved in drought response which are useful for wheat breeding programs developing drought-resilient wheat varieties.

In Chapter 5, the physiological and metabolic plasticity of three drought-resilient wheat lines and N61 were evaluated in response to drought stress at the seedling stage. The results suggested that the D-genome introgressions from *Ae. tauschii* to the lines improved their drought-adaptive traits. Specifically, MNH5 and MSD345 showed higher photosynthesis rates and triose phosphate utilization than N61 under control conditions, resulting in greater accumulation of glucose and sucrose in the shoots. However, under drought stress, MNH5 and MSD345 had higher intrinsic water use efficiency than MSD53 and N61. The total antioxidant capacity and superoxide dismutase activity increased in the three lines, whereas no significant changes were found in N61 in response to drought stress. Metabolome analysis identified six common drought-induced metabolites in all of the investigated genotypes. However, four metabolites (adenine, gamma aminobutyric acid, histidine, and putrescine) each specifically accumulated in one resilient line in response to drought stress, suggesting that these metabolites are important for drought resilience.

Overall, this work has expanded current knowledge on the role of high diversity breeding panels for wheat breeding for heat and drought resilience. It has provided in-depth insights on important genomic regions, and metabolic and physiological dynamics in wheat in response to heat and drought stress. In the future, high-throughput analyses and validation of these findings will allow them to serve as effective tools for climate-resilience breeding.