(Format No. 3)

SUMMARY OF DOCTORAL THESIS

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Title: Seed storage proteins of wild species of wheat- Variation in structure and its effect on bread making quality

コムギ近縁種の種子貯蔵タンパク質：構造の変異および製パン性への影響

Wheat proteins, especially the high molecular weight-glutenin subunits (HMW-GS) are important for wheat processing quality; however, the number of HMW-GS alleles with good quality is very limited in wheat cultivars. Wild and related species of wheat are tremendous source of variation. This study involved screening of very large number of disomic addition lines of wild species of wheat for their positive effect on wheat end product quality.

In experiment 1, I used a monosomic addition line of *Aegilops tauschii* chromosome 1D in durum wheat (*T. durum*) cv. PBW114 that was produced in 1990. This line was self-pollinated and maintained for several generations while following the presence of chromosome 1D carrying the gene for red glume color. Cytological analysis indicated that two of the three derivative lines had substitution of chromosome 1D for 1A and another chromosome 1D for 1B. One of these lines carried a pair of small chromosomes in addition to the 1D chromosome. The small chromosome found in one of the lines had nearly regular pairing and transmission to the daughter nuclei. Analyses by FISH and molecular markers indicated that the small chromosome was derived from the short arm of chromosome 1A and carried the *Glu-A3* locus. Microsatellite mapping based on the deletion bin map revealed that the small chromosome had deletions on both the terminal and centromeric sides. The line with the small chromosome showed improvement of the sodium dodecyl sulphate (SDS)-sedimentation value as compared to parent durum. However the increase in SDS-sedimentation value was more significant in the substitution line of chromosome 1D for 1A without the small chromosome. These facts suggest a negative effect of the *Glu-A3* locus on dough strength. The sequence of the *Glu-D1* locus from these lines showed that the HMW glutenin subunits were *Ae. tauschii* specific 2^{t}+T2 which were previously found to be associated with poor rheological properties and bread loaf volume in synthetic hexaploid wheat by other workers. Thus, the significant improvement in the SDS-sedimentation value of the substitution line of 1D for 1A suggests that the absence of the negative effect of chromosome 1A on quality is more important than the presence of *Glu-D1* of *Ae. tauschii.*

In experiment 2, I reported preferential removal of chromosome 1D rather than the alien chromosome from homoeologous group-1 addition lines. The *Agropyron intermedium* chromosome 1Agi (1E) addition line, created in the background of 'Vilmorin 27', showed loss of a part of chromosome 1D, thereby losing its HMW glutenin locus. Even in the case of *Ae. longissima* and *Ae. peregrina,* the genomes of which are closer to the B genome than D genome, chromosome 1D was lost from chromosome $1S^{l}$ and $1S^{v}$ addition lines in cv. 'Chinese Spring' (CS) rather than chromosome 1B during transfer from one generation to another. A similar observation was also observed in the case of a chromosome 1E disomic addition line of *Ag. elongatum* and alloplasmic common wheat

line with *Ag. intermedium* ssp. *trichophorum* cytoplasm. The reason for this strange observation is thought to lie in the history of wheat evolution, the size of chromosome 1D compared to 1A and 1B, or differing pollen competition abilities.

In experiment 3, I selected homoeologous group 1 addition line of *Ae. searsii* ($1S^s$) by screening disomic addition lines of different wild species of wheat for effect on bread making quality. Genetic loci of actively expressed HMW-GS gene and gliadin genes were found on the $1S^s$ chromosome and have been designated as *Glu-S^s1* and *Gli-S^s1*, respectively. Chromosome $1S^s$ showed improved dough strength in different environments and generations as compared to CS. Grains of $1S^s$ addition line were small in size, lesser in weight, harder and higher in protein content than CS. Flour yield and milling score of $1S^s$ addition line was lower, particle size, its flour protein content and SDS sedimentation value were higher than CS. Mixograph peak height and band width were higher, with no difference in mixing peak time than CS. All these factors indicate positive effect of quantity as well as quality of gluten proteins of *Ae. searsii*. HMW-GS genes of *Ae. searsii* were cloned and sequenced from the $1S^s$ addition line. Amino acid sequences comparisons showed that $1S^s$xA.D. (x subunit from $1S^s$ addition line) was more similar to previously published *Ae. searsii* HMW glutenin genes $1S^s$x1 followed by $1S^s$x2 and wheat 1Dx5 subunits. $1S^s$yA.D. was more similar to 1Dy10 followed by $1S^s$y1 and $1S^s$y2 subunits. As chromosome 1D is major determinant of wheat bread making quality, close homology of $1S^s$xA.D. and $1S^s$yA.D. HMW-GS genes with those of D genome of wheat support improvement of quality characters of $1S^s$ addition line of *Ae. searsii*.

In experiment 4, I analyzed HMW-GSs, low molecular glutenin subunits (LMW-GSs) and gliadins of 177 disomic addition lines of different wild species of wheat by SDS-poly acrylamide gel electrophoresis (PAGE). HMW-GSs loci were observed on homoeologous group 1 chromosomes of wild species of wheat. I identified several new HMW-GS loci and assigned name to those loci. LMW-GSs and gliadins were also found on homoeologous group 1 chromosomes of wild species of wheat. Minor *Gli-2* locus was not observed in all the cases. Homoeologous group 1 addition lines were studied for effect on bread making quality by SDS sedimentation test. Eleven addition lines were selected based on improved specific sedimentation. Rheological parameters of selected addition lines revealed better quality of most of addition lines studied. I selected 8 addition lines from 11 addition lines showing better quality characters. Among these selected addition lines *Ag. intermedium* proteins rank the best followed by *Hordeum chilense* and *Ag. elongatum.* Proteins of *Sec-2* locus of rye (Secale cereale) cv. 'Blanco' also showed better properties. Substitution lines of chromosome 1D that eventually appeared from addition lines showed very bad characteristic for bread making quality.

Chromosome 1D carries important genes related to bread-making quality, i. e., encoding HMW- and LMW-glutenins and gliadins. Thus, substitution of chromosome 1D by an alien chromosome and loss of many of these useful genes is not desirable. Alien chromosome carrying useful genes for bread-making character should be replaced by chromosome 1A that possesses small or even negative effect. So now I am trying to make substitution lines of selected alien chromosomes and chromosome 1A of wheat. These lines will be utilized to make translocation lines carrying only a small portion of alien chromosome on chromosome 1A and thus useful, quality related genes while eliminating the negative effects associated with the full chromosomes.