Genetic variation of Triticeae species for improvement of wheat end product quality

Monika G, Tanaka H, Tsujimoto H

Laboratory of Plant Genetics and Breeding Science, Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan

ABSTRACT

Wild species of wheat are a useful source of genetic variation for crop improvement. They have been utilized for improving the tolerance of wheat to different biotic and abiotic stresses. However, their potential for wheat quality has not been much investigated. In this study, we used 177 disomic addition lines belonging to 17 wild species of wheat. These lines were screened initially by sodium dodecyl sulphate poly-acrylamide gel electrophoresis (SDS-PAGE) for identification of addition lines carrying seed storage proteins like high-molecular-weight glutenin subunits (HMW-GSs), low-molecular-weight glutenin subunits (LMW-GSs) and gliadins from wild species. The loci of HMW-GSs, LMW-GSs and gliadins were observed on homoeologous group-1 chromosomes of wild species of wheat. Several new alleles of HMW-GSs were identified and named. Dough strength of addition lines was evaluated for 3 consecutive years and 11 addition lines with strong dough were selected. Rheological parameters of 6 addition lines revealed better quality for bread-making. Among these selected addition lines, *Agropyron intermedium* proteins showed best rheological characteristics followed by *Hordeum chilense*, *Ag. elongatum* and rye (*Secale cereale*). Cloning and sequencing of HMW-GS genes of wild species from selected addition lines showed great diversity among these genes. Wild species whose HMW-GS genes aligned with those of the D-genome of wheat showed much better quality characteristics. Substitution lines of chromosome 1D that eventually appeared from addition lines showed very bad characteristics for bread-making quality. Chromosome 1D carries important genes related to bread-making quality. Thus, substitution of chromosome 1D by alien chromosome is not desirable and must be avoided while transferring genes from wild species of wheat.

INTRODUCTION

Studies of the chromosomal location of genes controlling seed storage proteins in hexaploid bread wheat (*Triticum aestivum*) have shown that each of the genomes (A, B, D) possesses one chromosome (1A, 1B, 1D) with genes controlling low-molecular-weight glutenin subunits (LMW-GSs) and gliadins on its short arm and genes controlling high-molecular weight glutenin subunits (HMW-GSs) on its long arm, as well as genes controlling gliadins on the short arm of chromosome 6A, 6B and 6D. . In hexaploid wheat, HMW-GSs are encoded by the x- and y-type genes in the *Glu-1* loci located on the homoeologous group-1 chromosomes. Since the discovery that the composition of HMW glutenin subunits affects the baking properties of bread wheat varieties, considerable efforts have been devoted to understanding genetic regulation, structure and function relationships, and the potential of these proteins to improve the processing properties of wheat varieties (Payne et al. 1981; Shewry et al. 1995). In contrast to such progress, our knowledge of orthologous subunits in wheat-related species is still limited. The present work aimed to study and confirm the chromosomal location of HMW-GSs loci in wheat-related species and their effect on bread making quality, using their addition lines in hexaploid wheat.

MATERIALS AND METHODS

One hundred and seventy-seven disomic addition lines (DALs) belonging to different wild species of wheat obtained from the genebank of National Bioresource Project-Wheat, Japan (NBRP-Wheat) were used for initial screening. The list of addition lines is given in Table 1. Selected addition lines were sown in years 2004/2005 and 2005/2006 in well fertilized fields at Tottori University, Japan for second level screening. Selected addition lines from second screening were sown in the field in four replications in the year 2006/2007 at Tottori University, Japan for rheological analysis. Four plants of the same addition lines with one plant per pot were grown in the greenhouse to study effects of the environment.

The composition of glutenin subunits from the endosperm half of the seeds was determined by SDS-PAGE, using 10% acrylamide, according to Smith and Payne (1984). Gliadins were extracted by 1.5 M dimethyl formamide (DMF) and fractionated by 17.5% SDS-PAGE.

The SDS sedimentation values (SDSS) were measured on a small scale using 1 g flour. Specific sedimentation was calculated by dividing SDSS values by protein content. Rheological tests of selected addition lines and recipient cv. Chinese Spring (CS) in 2006/2007 were carried out at National Agricultural Research Center for Western Region, Fukuyama, Japan. Physical dough properties were determined using a 10 g mixograph (National Mfg. USA) according to AACC method 54-40A. Protein extraction and size-exclusion high-performance liquid chromatography (SE-HPLC) separation of extractable and unextractable proteins from flour were carried out according to Gupta et al. (1993).

The complete open reading frames (ORFs) of the HMW-GS genes were amplified from the genomic DNAs by PCR with degenerate primers. The fragments

were recovered from the gel by QIAquick Gel Extraction Kit (QIAGEN) and cloned into the pGEM-T Easy vector (Promega). The best match was found using the BLAST link of the NCBI. The HMW-GS gene ORFs from the addition lines were translated into deduced amino acid sequences using GENETYX software. These amino acid sequences from N and C-termini were compared to those of previously published HMW-GSs and a phylogenetic tree constructed using the Clustal W program version 1.83.

Mitotic chromosomes were prepared from root tips using the acetocarmine squash method and subjected to fluorescent *in situ* hybridization (FISH) analysis.

Table 1. List of chromosome recipients, donors and source of addition lines used in this study

Recipient	Chromosome donor	Source
Triticum aestivum cv. Chinese Spring	*Leymus racemosus*	Kishii et al. (2004)
Triticum aestivum cv. Chinese Spring	*Secale cereale*	Driscoll and Sears (1971), Nakata et al. (1979), Lukaszewaski (unpublished)
Triticum aestivum cv. Vilmorin 27	*Agropyron intermedium*	Cauderon et al. (1973)
Triticum aestivum cv. Chinese Spring	*Agropyron intermedium*	Tsujimoto et al. (1987)
Triticum aestivum cv. Chinese Spring	*Agropyron elongatum*	Dvorak and Knott (1974)
Triticum aestivum cv. Chinese Spring	*Aegilops umbellulata*	Kimber (1967)
Triticum aestivum cv. Chinese Spring	*Hordeum chilense*	Miller et al. (1982)
Triticum aestivum cv. Chinese Spring	*Haynaldia villosa*	Sears (unpublished)
Triticum aestivum cv. Chinese Spring	*Leymus mollis*	Tsujimoto et al. (unpublished)
Triticum aestivum cv. Chinese Spring	*Psathyrostachys huashanica*	Tsujimoto et al. (unpublished)
Triticum aestivum cv. Alcedo	*Aegilops caudata*	Blüthner et al. (1988)
Triticum aestivum cv. Chinese Spring	*Aegilops longissima*	Hart and Tuleen (1983), Maan (unpublished), Waines (unpublished)
Triticum aestivum cv. Chinese Spring	*Aegilops searsii*	Pietro et al. (1988)
Triticum aestivum cv. Chinese Spring	*Elymus trachycaulus*	Gill (unpublished)
Triticum aestivum cv. Chinese Spring	*Elymus ciliaris*	Wang et al. (2001)
Triticum aestivum cv. Chinese Spring	*Aegilops peregrina*	Yang et al. (1996)
Triticum aestivum cv. Chinese Spring	*Aegilops geniculata*	Friebe et al. (1999)
Triticum durum cv. PBW114	*Aegilops tauschii*	Dhaliwal et al. (1990)

RESULTS AND DISCUSSION

Protein electrophoresis

Among the addition lines studied here, none of the *Leymus racemosus* and *L. mollis* addition lines carried, *Leymus*-specific HMW-GSs. Therefore none of them probably belong to homoeologous group 1. Rye addition lines indicated the presence of the HMW-GSs locus on chromosome 1R. In *Agropyron elongatum, Aegilops umbellulata* and *Hordeum chilense,* HMW-GSs were found to be encoded by genes located on chromosomes 1E, 1U and $1H^{ch}$, respectively, as previously reported. In *Ae. longissima, Ag. intermedium* and *Haynaldia villosa* HMW-GSs were found to be encoded by genes located on chromosomes $1S^{l}$, 1Agi and 1V, as previously reported. From our observations on chromosome addition lines of polyploid *Elymus trachycaulus* (StStHtHt) in common wheat (*T. aestivum*), the HMW-GS locus can be assigned to the long arm of chromosome 1Ht, as previously reported, and no HMW glutenin locus, or null allele of the HMW-GS locus is present on the 1St chromosome. *Psathyrostachys haushanica* addition line B and *Ae. caudata* addition line D carry the HMW glutenin locus and thus are homoeologous to group 1 wheat chromosome. These loci were assigned *Glu-Nx1* and *Glu-C1* loci respectively in this study. Chromosome location of HMW-GSs gene in *Ae. searsii, Ae. geniculata, E. ciliaris and Ae. peregrina* was reported in this study and are hereby assigned loci *Glu-*S^{s}*1* (*Ae. searsii*), *Glu-*M^{g}*1, Glu-*U^{g}*1* (*Ae. geniculata)*, *Glu-*S^{v}*1 and Glu-*U^{v}*1* (*Ae. peregrina*). *E. ciliaris* has no HMW-GS locus, neither on $1S^{c}$ nor $1Y^{c}$ chromosomes. LMW-GS and gliadin profiles of addition lines revealed location of respective genes on the homoeologous group 1 chromosomes of wild species. This finding suggests that there is a genetic locus on the homoeologous group 1 chromosomes (designated as *Glu-3* and *Gli-3*) that contains varying number of genes in different wild species and at least one is expressed in all the wild species, leading to synthesis of LMW-GSs and gliadins in the seeds.

Grain and flour characteristics

By studying the HMW-GS, LMW-GS and gliadin profile of disomic addition lines, we identified 24 addition lines belonging to homoeologous group 1. Selected addition lines were sown in the field in years 2004/2005. These addition lines and many more addition lines were sown in field in 2005/2006. In most of the cases specific sedimentation of addition lines was similar to the recipient parent CS. But in some cases we reported significant and very high increase in specific sedimentation of addition lines in comparison to recipient cultivar (Fig. 1). These six addition lines along with five other addition lines were selected for detailed

rheological testing. *H. chilense* $1H^{ch}$ addition line performed better than CS in all the years and environments studied. An increase in the Mixograph peak for height, width and time indicated that proteins of *H. chilense* have good effect on quality. Increased quality performance was also reported for chromosome $1S^{l}$ and $1S^{s}$ addition lines of *Ae. longissima* and *Ae. searsii,* respectively.

Ag. elongatum 1E addition line and *Ae. geniculata* $1M^{g}$ addition line showed a significant increase in specific sedimentation in years 2005 and 2006 but dropped down in 2007. FISH analysis indicated that it is due to preferential elimination of chromosome 1D from 1E and $1M^{g}$ addition lines. But *Ag. intermedium* 1Agi addition line retained good quality performance in spite of missing the *Glu-D1* locus. Inbred rye (*Secale cereale*) IR130 substitution line 1R(1A) showed better bread making quality characteristics. Improved bread making quality was also observed in 2R rye cv. Blanco addition line. In general, *Ag. intermedium* proteins showed best rheological characteristics followed by *Hordeum chilense*, *Ag. elongatum* and rye.

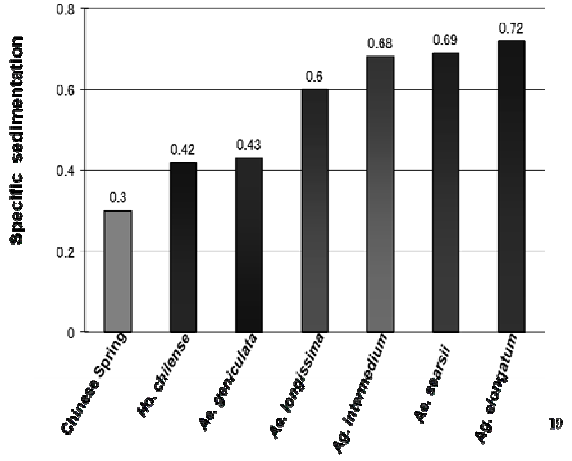

Fig. 1 Specific sedimentation of selected addition lines.

Cloning and sequencing of HMW-GS gene

HMW-GSs gene from selected addition lines were cloned and sequenced. Analysis of derived amino acid sequences from partial ORFs of these genes indicated a primary structure similar to HMW-GSs genes of wheat. In each case, a conserved structure consisting of a signal peptide, an N-terminal region, a central repetitive domain and a C-terminal region was observed. Sequence alignment of the signal peptide, N terminal and C terminal regions of x-type HMW-GS gene of *Ag. elongatum,* 1E addition line (DAL1Ex), revealed its close similarity with those of the B genome of wheat (Fig. 2). It aligned with 1Bx7 and 1Ax2* of wheat. DAL1Ey (y-type) didn't align with any HMW-GS gene, and came between x-type and y-type HMW-GS genes in the phylogenetic tree, but was closer to y-type genes. In *Ag. intermedium, Ae. longissima*, *Ae. searsii* and *Ae. geniculata,* x-type HMW-GSs from the respective addition lines grouped with 1Dx, the HMW-GSs of wheat. In *Ae. longissima* and *Ae. searsii,* y-type genes aligned with those of 1Dy of wheat. *Ae. geniculata* (DAL1M^{g}y) aligned with 1Ay of wheat.

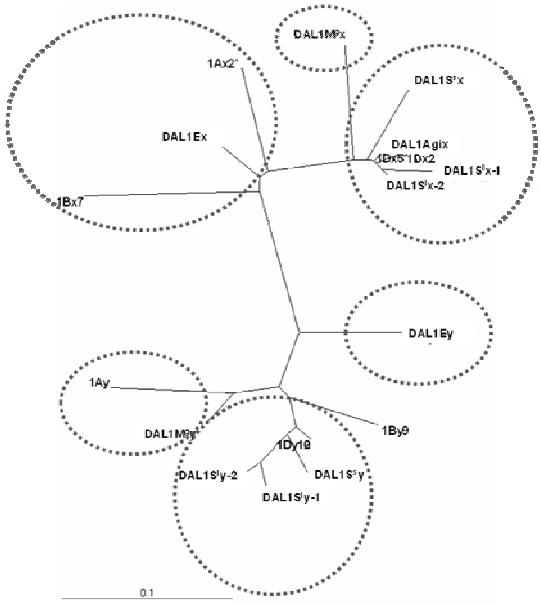

Fig. 2 Maximum likelihood tree obtained by Clustal W program 1.83 with the alignment of the signal peptide, N-terminal and C-terminal amino acid sequences of HMW glutenin subunit genes sequenced from some of selected addition lines and wheat.

Wild species whose HMW-GSs gene aligned with those of the D-genome of wheat showed much better quality characteristics. 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. Thus, substitution of chromosome 1D by alien chromosome is not desirable and must be avoided while transferring genes from wild species of wheat. We are now preparing substitution lines of alien chromosome with chromosome 1A of wheat which have been found to have a rather negative effect on dough strength.

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