Theor Appl Genet (2009) 119:471–481
DOI 10.1007/s00122-009-1053-y

ORIGINAL PAPER

Development of isohomoeoallelic lines within the wheat cv. Courtot for high molecular weight glutenin subunits: transfer of the *Glu-D1* locus to chromosome 1A

J. Dumur · G. Branlard · A.-M. Tanguy · M. Dardevet · O. Coriton · V. Huteau · J. Lemoine · Joseph Jahier

Received: 30 April 2008 / Accepted: 24 April 2009 / Published online: 13 May 2009
© Springer-Verlag 2009

Abstract Wheat quality depends on protein composition and grain protein content. High molecular weight glutenin subunits (HMW-GS) play an important role in determining the viscoelastic properties of gluten. In an attempt to improve the bread-making quality of hexaploid wheat by elaborating novel HMW-GS combinations, a fragment of wheat chromosome 1D containing the *Glu-D1* locus encoding the Dx2+Dy12 subunits was translocated to the long arm of chromosome 1A using the *ph1b* mutation. The partially isohomoeoallelic line selected was characterized using cytogenetical and molecular approaches to assess the amount of chromatin introgressed in the translocated 1A chromosome. Triple-target genomic in situ hybridization indicated that the translocated 1A chromosome had a terminal 1D segment representing 25% of the length of the recombinant long arm. The translocation was also identified on the long arm using molecular markers, and its length was estimated with a minimum of 91 cM. Proteome analysis was performed on total endosperm proteins. Out of the 152 major spots detected, 9 spots were up-regulated and 4 spots were down-regulated. Most of these proteins were identified as α-, β-, γ-gliadins assigned to the chromosomes of homoeologous groups 1 and 6. Quantitative variations in the HMW-GS were only observed in subunit Dy12 in response to duplication of the *Glu-D1* locus.

Communicated by J. Snape.

J. Dumur · A.-M. Tanguy · O. Coriton · V. Huteau · J. Lemoine · J. Jahier (✉)
INRA, UMR 118 INRA-Agrocampus Rennes-Université de Rennes I, Amélioration des Plantes et Biotechnologies Végétales, 35000 Rennes, France
e-mail: joseph.jahier@rennes.inra.fr

J. Dumur · G. Branlard · M. Dardevet
UMR INRA-UBP, Génétique, Diversité et Ecophysiologie des Céréales, 234 Avenue du Brézet, 63039 Clermont-Ferrand Cedex 2, France

Introduction

The bread-making quality of hexaploid wheat has been largely attributed to quality and to the amounts of storage proteins, glutenins and gliadins. The high molecular weight glutenin subunits (HMW-GS), encoded at *Glu-1* loci (Singh and Shepherd 1988a), and the low molecular weight glutenin subunits (LMW-GS), encoded at *Glu-3* loci (Singh and Shepherd 1988b) are essential for the viscoelastic properties of glutens and thus the quality of bread prepared with wheat flour. Much attention has also been focused on the quality of flour tightly linked to allelic variation of the *Glu-1* loci. Selection indices for quality evaluation in wheat breeding have been established for each HMW-GS in the additive context of codominant *Glu-1* alleles (Payne 1987; Branlard et al. 1992). Following these studies, the HMW-GS Dx5+Dy10 encoded by the *Glu-D1d* allele were associated with good bread-making quality and increased dough strength, while subunits Dx2+Dy12 encoded by the *GluD1a* allele were associated with poor bread-making quality. However, subunits Dx2+Dy12 were associated to dough extensibility and higher loaf volume when flour protein content was above 13%.

To improve the baking quality of bread wheat, Rogers et al. (1990) studied the dosage effects of chromosomes of homoeologous groups 1 and 6 on bread-making quality in hexaploid wheat. They suggested that the duplication of *Glu-D1* genes carried by the long arm of 1D could be beneficial for the quality of bread wheat. Conversely, deletion of

Glu-A1 genes on the long arm of 1A would be expected to provide some benefit. Thus, replacement of *Glu-A1* by *Glu-D1*, while retaining the copy on 1D could lead to improvement. So far, this goal has not been achieved in bread wheat. But, Rogers' proposal was used for durum and triticale breeding. In hexaploid triticale, Lukaszewski and Curtis (1992, 1994) translocated a chromosomal segment of wheat chromosome 1DL containing the *Glu-D1d* allele encoding the HMW-GS Dx5+Dy10 subunits, to the long arm of chromosome 1R and to the long arm of chromosome 1A. The translocated wheat chromosome was then introduced into durum wheat (Blanco et al. 2001) and was evaluated by cytogenetics and molecular approaches (Blanco et al. 2002). The mapping data indicated that the 1AL segment was substituted by a 1DL segment with a minimal length of 72 cM, and that the translocation breakpoint was located near the 1A centromeric region. The physical data obtained by genomic in situ hybridization (GISH) showed that the terminal 1DL segment represented about 35–40% of the recombinant arm length. In durum wheat, Vitellozzi et al. (1997) reported the transfer of a chromosomal segment of the 1DL containing the *Glu-D1d* allele onto the 1A chromosome long arm of a tetraploid derivative by means of induced homoeologous recombination in backcross progeny derived from bread wheat × durum wheat cross. Recently, Xu et al. (2005) reported the molecular cytogenetic characterization and storage protein analysis of two durum wheat lines obtained by Joppa et al. (1998) in which, a distal 1DL translocation carrying *Glu-D1d* replaced the distal region of 1AL.

Dosage effect could be obtained when the same gene is transferred onto homoeologous chromosomes. Of particular interest would be the development of a genotype having the same *Glu-1* gene on each of the group 1 chromosomes with alleles of complementary effects designated as an isohomoeoallelic line. An intermediate stage in their development is the extraction of partially isohomoeoallelic genotypes, in which, one *Glu-1* loci is carried by two group 1 chromosomes. The present paper describes the cytogenetic, molecular and storage protein characterization of a partially isohomoeoallelic line with the transfer of the locus *Glu-D1a* encoding HMW-GS Dx2+Dy12 from chromosome 1D onto chromosome 1A.

Materials and methods

Plant material

Experiments were performed with the cultivar Courtot that is a French semi-dwarf winter bread wheat of good bread-making quality. Courtot line monosomic for 1A and Courtot *ph1b* were obtained from INRA (Denis et al. 1982). Monosomic 1A plants were first retained based on their chromosome numbers determined by root tip counting ($2n = 41$) and secondly based on their meiotic behavior [expected 20 bivalents + 1 univalent in most pollen meiotic cells (PMCs)]. The line Courtot *ph1b* is the intervarietal substitution line Courtot (Chinese Spring *ph1b* 5B), Chinese Spring *ph1b* being a mutant line of Chinese Spring for the gene *Ph1* (Sears 1977).

Development of wheat lines with a translocated chromosome carrying *Glu-D1* genes

A Courtot line monosomic for 1A was crossed as female to Courtot *ph1b* (Fig. 1). The monosomic F1 was backcrossed to Courtot *ph1b*. BC1 plants monosomic for 1A and homozygous *ph1bph1b* were selfed and the search for recombinant plants was performed by analyzing the HMW glutenin profile of the progenies. The monosomic 1A condition of F1 and BC1 plants was checked by crossing them with Chinese Spring double ditelosomic 1A then by examining the meiotic pairing in the hybrids with $2n = 40 + 2t$. The homozygosity for *ph1b* of BC1 plants was checked using a molecular marker developed by Gill and Gill (1996).

Courtot has the HMW glutenin profile Ax2*, Bx7+By8, Dx2+Dy12 encoded at *Glu-A1*, *Glu-B1* and *Glu-D1* respectively. Expected recombinants with the profile −, Bx7+By8, Dx2+Dy12 were searched for in the selfed progeny of *ph1bph1b* monosomic 1A BC1 plants (Fig. 2). In order to identify each recombinant line, proteins were extracted from a single half seed using the sequential procedure of Singh et al. (1991). The storage proteins were separated by 1D sodium dodecyl sulphate gel electrophoresis (SDS-PAGE). In a few cases, the protocol of Laemmli (1970) modified by Payne et al. (1979) was used to improve the separation of the HMW-GS subunits Ax2* and Dx2.

Detected recombinant plants were backcrossed three times to Courtot monosomic 1A and, at each BC generation, the meiosis of the monosomic plants was examined allowing selection of the most meiotically regular plant within each progeny. In the selfed progeny of the third backcross with Courtot monosomic 1A, disomic plants were selected, meiotically analyzed, and crossed to Courtot monosomic 1A to evaluate pairing between the translocated 1A and a normal 1D. The disomic plants of the recombinant line RR240 were crossed to Chinese Spring ditelosomic line 1AL to evaluate the interstitial or terminal nature of the introgressed segment.

Cytogenetic methods

Mitotic and meiotic chromosome preparations were made using standard techniques.

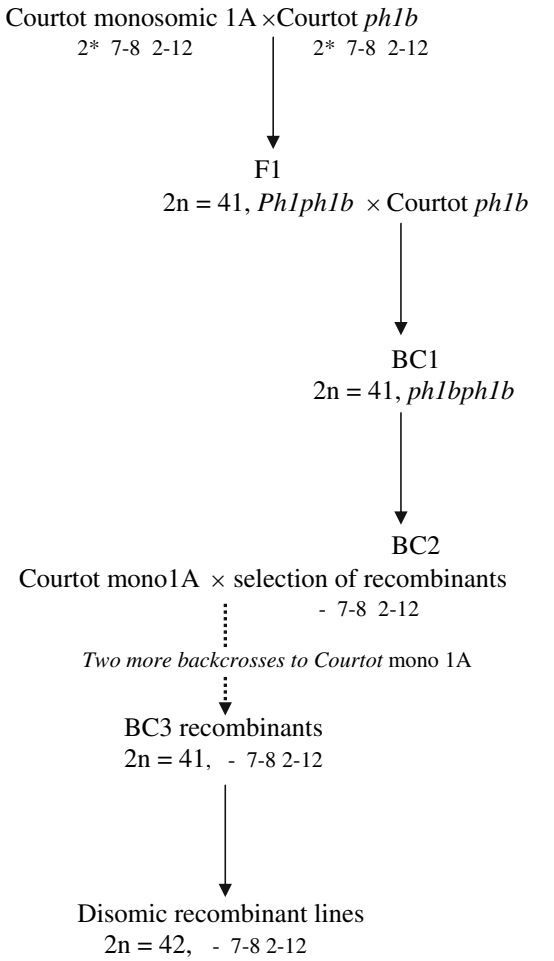

Fig. 1 Breeding procedure to achieve homoeologous recombination between chromosome 1A and chromosomes 1B and 1D, and to select stable disomic recombinant lines in the wheat cv. Courtot

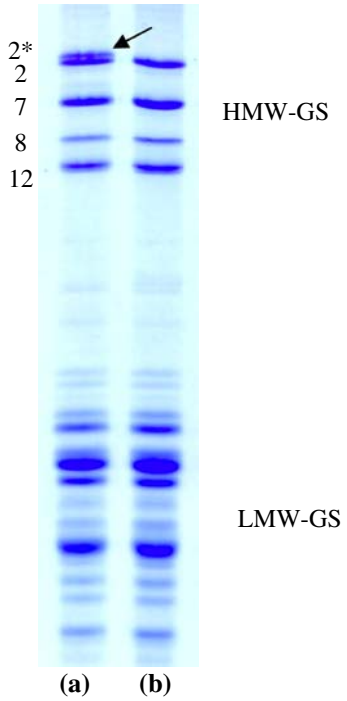

Fig. 2 SDS-PAGE separation of glutenins extracted from the recombinant RR240 line and Courtot. The *arrow* indicates HMW-GS Ax2* encoded by *Glu-A1* absent in the RR240 line (*lane b*) and present in Courtot (*lane a*). *Numbers* indicate HMW-GS controlled by the *Glu-A1* (2*), *Glu-B1* (7 + 8) and *Glu-D1* (2 + 12)

Genomic in situ hybridization

Chromosomal preparation and Genomic in situ hybridization (GISH) were carried out as described in Tanguy et al. (2005) with a few modifications. Total genomic DNAs from *T. urartu* Tum ex Gand (A-genome) and *Ae. tauschii* Coss (D-genome) were used as probes. DNAs were labeled by nick translation with biotin-16-dUTP (Invitrogen Life Technologies) and digoxigenin-11-dUTP (Roche Diagnostics, Mannheim, Germany) respectively. Total genomic DNA isolated from *Ae. speltoides* Tausch (S-genome), the most likely donor of the genome B, was autoclaved to give fragments of 100–300 bp and used as a block. A series of test assessments determined the amount of blocking DNA that was required to discriminate between the three genomes. Optimum results were obtained with a ratio of 1:2:24 for A genome probe, D genome probe, and blocking DNA in the hybridization mixture. Biotinylated probes were immunodetected by Texas-red conjugated with avidin antibodies (Vector Laboratories, Burlingame, CA) and digoxigenin-labeled probes were detected with anti-digoxigenin antibody conjugated with FITC (Roche Diagnostics). The chromosomes were mounted and counterstained in Vectashield (Vector Laboratories) containing 2.5 µg/mL 4′,6-diamidino-2-phenylindole (DAPI). Fluorescence images were captured using a CoolSnap HQ camera (Photometrics, Tucson, Arizona) on an Axioplan 2 microscope (Zeiss, Oberkochen, Germany) and analyzed using MetaVue™ (Universal Imaging Corporation, Downington, PA).

Microsatellite analysis

DNA was extracted from leaves using the method described by Doyle and Doyle (1990). Microsatellite markers mapped in homoeologous group 1 chromosomes were amplified by polymerase chain reaction (PCR) following the protocol described in Röder et al. (1998) and Pestsova et al. (2000) for Gaterleben wheat microsatellites (*gwm* and *gdm* microsatellites), Sourdille et al. (2004) for the *cfa*, *cfd*, and *wmc* microsatellites and Cregan and Song (http://www.scabusa.org) for *barc* microsatellites. Based on the reference maps (Röder et al. 1998; Somers et al. 2004; Sourdille et al. 2004, and theirs updated genetic maps in the

web site Graingenes http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/groupe1v2.xls), microsatellite markers were selected based on their relative positions along chromosomes 1A and 1D. Fifteen microsatellite loci used in the molecular analysis were *Xgdm33*, *Xgwm136*, *Xcfa2153,* *Xgwm33*, *Xcfd15*, *Xgwm164*, *Xgwm497*, *Xwmc59*, *Xbarc158*, *Xbarc17*, *Xbarc145*, *Xcfa2219*, *Xgwm357*, *Xgwm135*, *Xgwm99* mapping on chromosome 1A, and twelve microsatellite loci *Xgdm60*, *Xbarc99*, *Xcfd59*, *Xcfd65*, *Xgwm458*, *Xcfd19*, *Xwmc36*, *Xgdm126*, *Xgwm642*, *Xgwm232*, *Xgdm111*, *Xbarc62* on the chromosome arm 1DL. The Courtot nullisomic lines for chromosomes 1A and 1D were used as positive controls to confirm microsatellite loci assignment. Amplified PCR products were separated on 6% polyacrylamide non-denaturing gels or 5% agarose gels and visualized by ethidium bromide staining.

Proteome analysis

The protocol of two-dimensional (2-D) gel electrophoresis (pH 3–10) was performed as described in Dumur et al. (2004) to reveal the seed storage proteins, glutenins and gliadins. Protein patterns were analyzed using Melanie 3 software (Genebio, Geneva, Switzerland). The volume and volume percentage of each spot was used in statistical analyses using Statgraphics 5.5 software with a statistical significance level of $p < 0.05$ and $p < 0.01$. Spots that differed between the six replicates of the RR240 line and the six replicates of Courtot were identified by statistical analysis. The percentage of variation, calculated for each spot that differed, was expressed as a percentage of the volume of the same spot in Courtot.

Identification of endosperm proteins

In order to identify a large number of gliadins and LMW-GS, specific extractions were performed with two different methods. Using the A-PAGE method (Branlard et al. 1990), the numerous α-, β-, γ- and ω-gliadin bands were clearly identified as was their locus assignment on the chromosomes of homoeologous groups 1 and 6. By means of sequential extraction of endosperm protein, it was possible to isolate the LWS-GS fraction and to identify spots corresponding to known alleles using 1-D SDS-PAGE. The identification of proteins obtained with the two methods was confirmed by mass spectrometer analyses as described by Dumur et al. 2004.

Quality test

Both Courtot and RR240 lines were tested for quality trait. The Zeleny sedimentation test (AFNOR NF V03-704) modified by Branlard et al. (1991) was assessed on white flour (Brabender Quadrumat Senior mill).

Results

The glutenin profiles of 288 plants of the selfed progenies of *ph1bph1b* monosomic 1A plants were analyzed: 231 plants had a Ax2*, Bx7+By8, Dx2+Dy12 profile similar to that of the parental line cv. Courtot and were eliminated, 36 plants had a −, Bx7+By8, Dx2+Dy12 profile (Fig. 2) and 21 others had different modified profiles, the alterations concerned bands Bx7+By8 and Dx2+Dy12 including one plant with a Ax2*, Bx7+By8, − profile, 12 plants with a Ax2*, −, Dx2+Dy12 profile, 7 plants with a faint Ax2*, Bx7+By8, Dx2+Dy12 profile, one plant with a faint Ax2*, Bx7+By8, − profile.

The 36 plants without *Glu-A1* encoded HMW-GS were grown. Their chromosome numbers varied between 39 + t (t = telocentric) and 42. Four of them died and six had no progenies either by selfing or by backcrossing to Courtot mono1A. In the BC1, BC2 and BC3 generations, selection was based on the vigor and fertility of the plants, their glutenin profiles, and their meiotic behavior. It was assumed that if the chromosomal segment introduced into 1A was short, the hemizygous translocated chromosome would behave more or less like a normal 1A, i.e. it would be systematic univalent at MI of meiosis. At each backcrossing generation, the non-germ portion of each half-seed was screened for storage proteins by SDS-PAGE, and the absence of the Ax2* glutenin subunit was checked. In BC3, 15 lines remained. Disomic recombinant plants were selected in the selfed F2BC3 progenies. If two or more disomic plants were obtained in a progeny, the one presenting the most regular meiotic pairing was kept. Further analysis started with the line RR240. The latter with a −, Bx7+By8, Dx2+Dy12 glutenin profile was chosen based on agronomical and technological properties (data not shown).

Visualization of chromosome 1A in the RR240 line using GISH

Genomic in situ hybridization was performed to determine the genomic origin and the physical size of the introgressed segment in the translocated 1A. The chromosomes of the A genome were visualized as red, B genome chromosomes were blue and D genome chromosomes were green (Fig. 3). Two translocations were detected: the first corresponding to the intergenomic translocation 4AL/7BS (Naranjo et al. 1987), present in both durum and bread wheats, and the second translocation where a D-genome segment was introduced into the long arm of an A-genome chromosome pair.

Based on the measurement, its length was about 25% of the long arm of the translocated chromosome.

At this stage, given the glutenin profile (Fig. 2) and the GISH phenotype of RR240 (Fig. 3), we postulated that a 1D-segment carrying *Glu-D1* had replaced its homoeologous counterpart on chromosome 1A and hence that the glutenin phenotype of RR240 could not be due to a deletion. To demonstrate that this result was indisputable, we decided to introduce the 1A chromosome of RR240 into the durum wheat cv. Joyau that has the glutenin profile − Bx6+By8. In the F2 of the cross RR240 × Joyau, three plants with the band Dx2+Dy12 in their glutenin profile were selected. Two of them had 29 chromosomes and the third one had 30 chromosomes. The three plants were backcrossed to the cv. Joyau. Among the BC1 progeny, 28 chromosomes plants with the subunits Dx2+Dy12 (1A) and Bx6+By8 (1B) (or heterozygous Bx6+By8/Bx7+By8) were selected. Their meiotic pairing was regular: most PMCs displayed 14 bivalents at MI of meiosis and in 20% of them, all the 14 bivalents were rings.

Molecular characterization of translocated chromosome 1A

The above results showed that Dx2+Dy12 subunits-carrying 1D chromosomal segment replaced the physical 25% distal part of 1AL. However, the resolution level of GISH has some limitations at 10 cM in configurations with terminal translocated segments (Lukaszewski et al. 2005). The amount of chromatin introgressed in the translocated bread wheat line was consequently also evaluated using molecular approaches. In the first step, a total of 15 microsatellite markers genetically mapped on chromosome 1A were used in a presence/absence strategy (Fig. 4). One microsatellite primer that amplifies a locus on chromosome arm 1AL (*Xgwm99*) did not generate any PCR products in the RR240 line. The 14 other microsatellite primers located on the chromosome arms 1AS (*Xgdm33*, *Xgwm136*, *Xcfa2153*, *Xgwm33*, *Xcfd15)* and 1AL (*Xgwm164*, *Xgwm497*, *Xwmc59*, *Xbarc158*, *Xbarc17*, *Xbarc145*, *Xcfa2219*, *Xgwm357*, *Xgwm135*) amplified loci in the translocated line and in Courtot, which was used as positive control. Based on the molecular analysis, the translocation breakpoint on the chromosome 1A would be located between *Xgwm135* and *Xgwm99*. Röder et al. (1998) showed that *Glu-A1* locus lies to the *Xgwm135*, thus the length of the translocation containing the removed *Glu-A1* locus in the long arm was estimated at least 60–120 cM according to the consensus genetic maps of Somers et al. (2004) and Röder et al. (1998), respectively, in Graingenes. In the second step, 12 microsatellite loci genetically mapped around the *Glu-D1* locus on chromosome arm 1DL were tested. Six of them

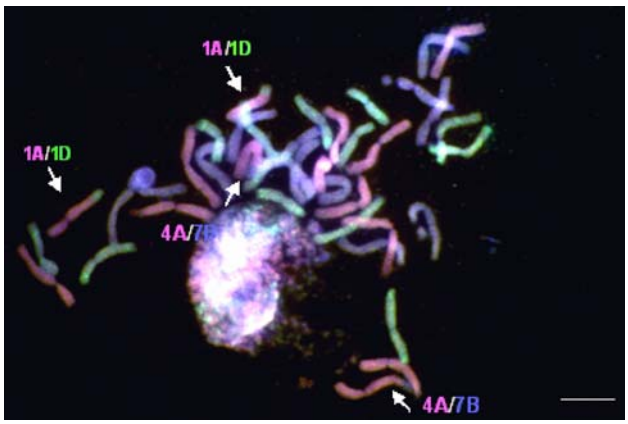

Fig. 3 Mitotic metaphase of the recombinant bread wheat line RR240 after *in situ* hybridization. Total genomic DNAs from *T. urartu* (A-genome) and *Ae. squarrosa* (D-genome) were used as probes and the total genomic DNA isolated from *Ae. speltoides* (S-genome) was used as a block. A genome chromosomes are red (biotin-labeled DNA) and D genome chromosomes are green (digoxigenin-labeled DNA). The length of the chromosomal segment introduced into 1A from 1D (indicated by *arrows*) is about 25 % of the 1AS.1AL-1DL chromosome. *Bar* 10 µm

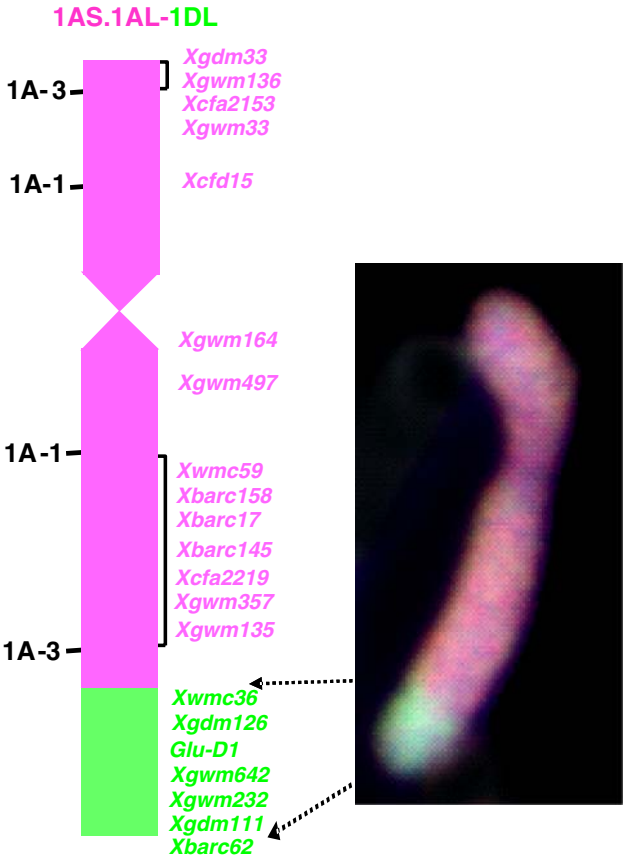

Fig. 4 Physical map of 1AS.1AL-1DL chromosome of the RR240 line. Relative positions of the wheat microsatellite markers are deduced from the genetic-physical maps of Courtot × Chinese Spring (Sourdille et al. 2004) and Synthetic × Opata (Röder et al. 1998; Sourdille et al. 2004). The microsatellite markers transferred into the translocated 1A chromosome from 1DL are shown as *green block*. The deletion bins are placed on the left

(*Xwmc36*, *Xgdm126*, *Xgwm642*, *Xgwm232*, *Xgdm111*, *Xbarc62*) were amplified in the durum material derived from RR240 then indicating the presence of a translocation from 1D (Fig. 4). Based on the position of *Xcfd19,* we estimated the length of the translocated segment between 91 and 180 cM according to the same reference maps as above. Finally, evidence was obtained that *Glu-D1*-carrying 1D chromatin had been translocated to chromosome 1A and the latter was therefore designated as 1AS.1AL-1DL.

Meiotic pairing of the translocated 1A chromosome

Since chromosome 1A (1AS.1AL-1DL) of RR240 contained a significant amount of 1D chromatin in distal position, it was postulated that it could pair with 1D. The meiotic pairing in BC3 F1 monosomic plants was not regular. In 48% of 94 PMCs, 20 bivalents + 1 univalent or rarely 19 bivalents + 3 univalents were observed. In the remaining 52% of PMCs, an open trivalent likely resulting from pairing between 1AS.1AL-1DL and the two 1D chromosomes was observed. In euploid RR240 plants, only bivalents and occasionally univalents were observed in 93% of a total of 150 PMCs. In the remaining cells, a multivalent (quadrivalent or rarely a trivalent) was observed.

1A-1D chromosome pairing in RR240 was confirmed by meiotic analysis of four hybrid plants between RR240 and Chinese Spring ditelosomic line for the long arm of chromosome 1A. One hundred and twenty PMCs were analyzed. The chromosome arm 1AL as a telocentric chromosome paired with 1A in 5% of PMCs in the hybrid RR240 × CS ditelosomic 1A. In the control hybrids between Courtot and CS ditelosomic 1AL, 1AL paired at frequency of 96%.

Proteome analysis of the RR240 line

Total endosperm proteins were extracted from mature wheat grains, then separated by 2-DE and made visible by Coomassie Blue staining. The 2-DE gels of the Courtot parental line revealed approximately 152 highly reproducible spots whose relative molecular masses ranged from 15 to 110 kDa with a p*I* of between 3 and 10 (Fig. 5). Changes in the composition of the endosperm protein induced by the translocated 1AS.1AL-1DL chromosome were investigated by comparing the protein pattern of the recombinant RR240 line to the pattern of the Courtot reference line.

Wheat polypeptides whose percentage volume varied in the recombinant line are shown in Table 1. Both quantitative and qualitative variations were observed. Out of the 152 major spots detected in the parental line cv. Courtot used as a reference pattern, 9 spots were up-regulated and 4 were down-regulated in the RR240 line. Most of the proteins that varied were identified as α-, β-, and γ-gliadins assigned to the chromosomes of homoeologous groups 1 and 6. No ω-gliadin displayed quantitative variations.

HMW-GS encoded in Courtot were Ax2*, Bx7 and By8, Dx2 and Dy12 located at the loci *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively. Each of these subunits was split into several spots, for example three for subunit Bx7 or two for subunit Dy12, and identified as isoforms of the same protein (Islam et al. 2002) arising from post-translational modifications (Holt et al. 1981). Comparison of quantitative variations in response to translocation revealed that only the amount of subunit Dy12 was increased (between 140 and 200% for the two isoform spots). This quantitative variation, which can be explained by the duplication of the *Glu-D1* locus, was the only variation observed in the D genome. Two spots corresponding to the location of the removed subunit Ax2* also disappeared (Fig. 5).

The Zeleny sedimentation test performed on white flour indicated that the RR240 line duplicated for the *Glu-D1a* locus had higher sedimentation values (51 ml) than the parental line Courtot (37 ml) although any significant difference was found in grain protein content (data not shown).

Discussion

Bread wheat genotypes with a higher dosage of genes have mainly been produced through duplication or triplication of a chromosome pair, in nullisomic–tetrasomic combinations for example. In the literature, no example of increased copy number of a gene of agronomic interest has so far been reported for wheat with normal chromosome constitution. It would be very useful to duplicate or triplicate genes with a quantitative effect. Here, we have demonstrated the feasibility of transferring the distally located *Glu-D1* gene from chromosome 1D to chromosome 1A.

Sears (1972) and Luo et al. (1996) emphasized that in alien introgression mediated in the absence of *Ph1*, it is preferable to construct plants in which the alien chromosome and the wheat chromosome targeted for homoeologous recombination are monosomes. In our experiment, the situation resembled that of an alien introduction even though the alien chromosome was a wheat homoeologue. If Sears' strategy had been fully adopted, recombinants would have been expected in the progenies of double monosomics 1A-1D, homozygous *ph1bph1b*. The advantage over our procedure would be that, in addition to a possibly higher number of recombinants, reciprocal recombinants would probably also be obtained. The main disadvantage would be that it is far more difficult to produce a *ph1bph1b* double monosomic than a *ph1bph1b* monosomic. Our results demonstrated that from *ph1bph1b* mono1A, it was at least possible to obtain 1A recombinants. Taking different

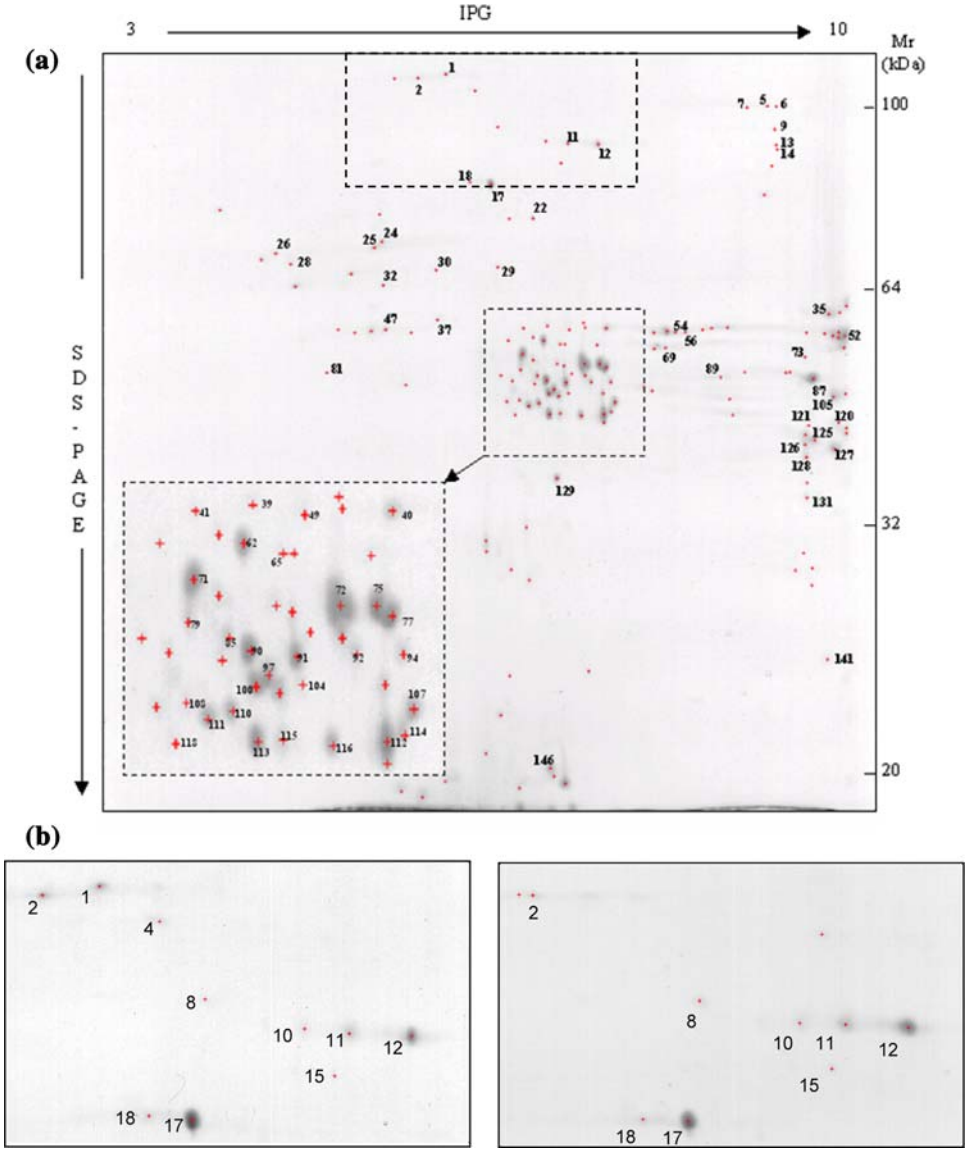

Fig. 5 **a** IPG × SDS-PAGE of endosperm proteins of Courtot using 3–10 immobilized gradient strips. Proteins were Coomassie Blue stained. Expended windows highlight differences between Courtot and RR240 for (**b**) region where some Ax-type HMW-GS were located and removed in the translocated line

parameters into account, we thus consider that the strategy chosen for the production of isohomoeoallelic lines was acceptable.

The GISH technique allowed one 1D segment to be visualized that represented approximately 25% of the long arm of 1AS.1AL-1DL. The introduction of the translocated chromosome 1AS.1AL-1DL into durum wheat enabled us to demonstrate that it did carry the *Glu-D1a* locus. Later, molecular marker analyses confirmed that through homoeologous recombination, the distal 1DL chromatin was transferred to 1A and the translocation breakpoint lies between *Xgwm135* and *Glu-A1* locus on 1AL. Xu et al (2005) reported the development of similar 1AL-1DL translocation lines of durum wheat. In their material, a 1DL segment spans approximately 31% of the long arm of the translocated chromosome 1AS.1AL-1DL and the breakpoint was determined to be distal to *Xgwm357* and proximal to *Xgwm135*. That last result corroborates that the length of the translocated segment of RR240 was found shorter through its visualization using GISH.

The genetic length of the 1D translocated segment in RR240 was estimated, according to the updated consensus genetic maps of Somers et al. (2004) and Röder et al. (1998) between 91 and 180 cM, respectively. Discrepancies between the figures are due to the fact that position of markers depends on the density in molecular markers, the crosses and the analysed population size. According to the updated genetic maps in Graingenes, the *Glu-D1* and *Glu-A1* loci are positioned 147 and 129 cM, respectively, from the distal ends of 1DL and 1AL. These distances correspond to 80 and 87% of the genetic maps of 1DL and 1AL. The translocated 1DL segment into the chromosome 1AS.1AL-1DL represents 25% of the physical length of 1AL-1DL. As chromosome arm 1DL is about 30% shorter

Table 1 Characteristics and chromosome assignment of wheat polypeptides whose percentage volume varied significantly between RR240 and Courtot

Spot no.	Identification	p*I*	Mr (kDa)	Chromosome assignation	Variation (%)
Up-regulated					
11	HMW-GS Dy12	6.6	85	1D	140
12	HMW-GS Dy12	6.7	85	1D	200
49	γ-gliadin	6.6	44	1B	225
66	γ-gliadin	6.7	43	1A	163
73	β-gliadin	9.0	41	6A or 6B	270
77	β-gliadin	6.8	40	6A or 6B	148
101	α-gliadin	6.7	39	6A	134
110	LMW-GS	6.3	38	1B	115
117	α-gliadin	8.0	37	nd	168
Down-regulated					
34	LMW-GS	9.6	46	1B	43
65	γ-gliadin	6.6	43	1A	21
92	β-gliadin	6.7	39	6B	49
146	nd	6.5	18	nd	49
Spots that diseappared					
1	HMW-GS Ax2*	5.7	103	1A	
4	HMW-GS Ax2*	5.8	102	1A	

than 1AL (Gill et al. 1991), the 1AL-removed segment should represent between 30 and 35% of 1AL under the hypothesis the translocation is genetically balanced. That would mean that 30–35% distal portion of 1AL correspond to 87% of the genetic length. In the in situ comparative mapping of *Glu-1* loci in *Triticum* and *Hordeum*, Cabrera et al. (2002) mapped physically the *Glu-A1* and *Glu-D1* loci at the same relative position from the centromere 0.76 ± 0.01 in bread wheat. This latter result is in agreement with our GISH observation (Fig. 3) and with the physical BIN location of the markers from 1D into 1A chromosome. Our observations confirm other studies showing that recombination frequency increased exponentially with the proximity towards the telomere (Kota et al. 1993; Lukaszewski and Curtis 1993; Lukaszewski 1995).

Meiotic analysis of hybrids between RR240 and CS ditelosomic for 1AL corroborated our conclusions based on molecular data for the presence of the 1D chromatin on long arm of chromosome 1A. Pairing between 1AL and 1AS.1AL.1DL was drastically reduced. The amount of translocated 1D chromatin resulted in occasional pairing between 1AS.1AL.1DL and 1D chromosomes in RR240 since a quadrivalent or trivalent was observed in 7% of its PMCs. Meiotic pairing between 1A and 1AS.1AL-1DL in the derived durum wheat line was found higher to that between 1AL and 1AS.1AL-1DL in bread wheat. There is no reason to believe that the difference might be due to homoeologous pairing occurring between the 1D-translocated segment and 1AL. Our explanation was the observed difference should be due to a higher ability to initiate synapsis that leads to pairing between the 1AL portion shared by the complete 1A and the recombinant chromosome.

Our objective was to select isohomoeoallelic or partially isohomoeoallelic lines with regular meiotic pairing, i.e. without pairing between 1D and 1AS.1AL.1DL that could result in lower fertility. In the present case, this requires to reduce the translocated segment. As *Glu-D1* is located in its proximal part, reduction should concern the distal part. We plan to achieve that goal in durum wheat. The *ph1c* mutation is being introduced in the durum line with Dx2+Dy12. Selection of the desired line will be made using GISH and the six markers in the vicinity of the *Glu-D1*. When produced, the novel 1AS.1AL-1DL chromosome will be reintroduced in bread wheat. As in wheat and related species, pairing and crossover events are concentrated in small distal regions of chromosomes (Jones et al. 2002) it therefore can be expected that pairing between 1D and 1AS.1AL-1DL will not occur.

Mature wheat-grain endosperm proteome

In the present study, the proteomic approach used to analyze the composition of endosperm protein in mature wheat grains provided extensive information on modifications in gene expression in response to the transfer of the *Glu-D1* locus to chromosome 1A. Statistical analyses showed that a small number of storage proteins were quantitatively affected. Most were assigned to one chromosome of homoeologous groups 1 or 6 and more rarely to two different chromosomes. The majority of spots whose volume

changed significantly were associated with α-, β- and γ-gliadins, but never with ω-gliadins.

Several studies have highlighted the regulatory effects of chromosome 1D on the expression of seed storage protein genes, and in particular on gliadins encoded on chromosome 6A (Bittel et al. 1991) and 1B (Galili et al. 1986). In these studies, the presence of the whole 1D chromosome reduced amounts of gliadins but no information was obtained on the type of gliadins affected or on the localization of regulator genes in the long or short arm. The same quantitative variations were observed in the group 1 aneuploid lines of the cv. Courtot (Dumur et al. 2004). Endosperm proteins encoded at *Gli-2* (α-, β-gliadins) and *Gli-1* (γ-gliadins) displayed the greatest quantitative variations in comparison with ω-gliadins when one chromosome or a chromosome pair was removed.

Quantitative variation in the *Glu-D1* locus

As revealed by 2-DE patterns in many studies (Holt et al. 1981; Islam et al. 2002), most HMW-GS are split into several spots. Quantitative variations in the HMW-GS were only observed in subunit Dy12 in response to the duplication of the *Glu-D1* locus. Non-significant quantitative variations were observed in subunits Bx7 and By8 (*Glu-B1*) and in duplicated subunit Dx2 (*Glu-D1*). These results can be explained by the complexity of regulatory phenomena in hexaploid wheat such as gene-dosage compensation (Galili et al. 1986) and the average number of regulatory effects found per HMW-GS i.e. an average of 19.8 dosage-sensitive regulators per gene (Wanous et al. 2003). This may still be underestimated as one chromosome arm may have several regulatory loci whose overall effect is averaged when varying the dosage of the whole arm. But results of a recent investigation suggest that there were, for each HMW glutenin, on average, 8 chromosomes arms with a up-regulatory effect and one with a down-regulatory effect (Storlie et al. 2009). Some progress has already been made in understanding the regulatory system controlling the transcription of the HMW glutenin in wheat (Ravel et al. 2006a, b) but the structural gene-dosage effects of each chromosome arm on the expression of HMW glutenin genes are not yet well understood and the precise quantitative variations of each subunit have not yet been fully explained.

In the case of the partially isohomoeoallelic RR240 line, the chromosomal segment introduced into 1A from 1D represented physically 25% of the long arm. The absence of quantitative variations for the duplicated subunit Dx2 could be explained by the loss of the positive regulatory effects of 1AL on the expression of the Dx2 subunit (Wanous et al. 2003) and its low intrinsic ability to be modified in proportion to the response to dosage variation (Bosakova et al. 1998; Dumur et al. 2004).

In bread wheat, Wieser and Zimmermann (2000) reported in quantitative analysis by reverse phase HPLC (RP-HPLC) that contents of HMW subunits varied within a broad range, the proportions of subunits Dx2, Bx7 and Dy12 being major components and subunits, Ax2* and By8 the minors in flour. In the proteomic analysis of the reference line Courtot, the relative amounts of HMW-GS were of 13, 36 and 51% for subunits encoded at *Glu-A1* (Ax2*), *Glu-B1* (Bx7+By8) and *Glu-D1* (Dx2+Dy12) loci, respectively (Dumur et al. 2004). No significant statistical variation was found for the protein content in flour between both lines. Substituting the *Glu-A1* locus by *Glu-D1* should induce only a small compensation phenomenon by the duplicated subunit Dy12 known as being the most reactive subunit in the aneuploid context. Such over-expression of the subunit Dy12 gives rise to changes in storage protein composition and consequently to a higher sedimentation volume in RR240. Such variation indicates a better quality of storage proteins and offers a promising effect on both the rheological and technological dough properties of RR240.

Concluding remarks

The present work shows that genes from chromosome 1D can be transferred onto chromosome 1A. It also demonstrates that chromosome engineering, at least at the level of homoeologous group 1, enables the development of novel gene combinations in bread wheat. It should be stressed that transfers into A and/or B genomes can be exploited for durum and triticale breeding. Plants that are homozygous for the recombinant chromosome displayed normal plant vigor and a meiotic pairing that was slightly less regular than in the control line cv. Courtot. The latter trait could be restored if the distal part of the 1D translocated segment on 1AL could be reduced. Complementary research based on both agronomical and rheological analysis is now needed to better understand the effect of the increased dosage of the *Glu-D1a* gene.

References

Bittel DC, Hueros G, Jouve N, Gustafson JP (1991) Changes in expression of seed storage protein genes effected by chromosome 1D of wheat. Genome 34:845–848

Blanco A, Cenci A, Simeone R, Pignone D, Galasso I (2001) Characterization and transfer of a recombinant chromosome 1AS.1AL-1DL with the *Glu-D1* gene from hexaploid triticale to durum wheat. In: Proceedings 4th International Triticeae Symposium, Cordoba (Spain), 10–12 September 2001

Blanco A, Cenci A, Simeone R, Gadaleta A, Pignone D, Galasso I (2002) The cytogenetics and molecular characteristics of a translocated chromosome 1AS.1AL-1DL with a *Glu-D1* locus in durum wheat. Cell Mol Biol Lett 7:559–567

Bosakova M, Gregova E, Kraic J (1998) Identification of wheat (*Triticum aestivum* L.) aneuploids by protein markers. Hereditas 128:53–58

Branlard G, Picard B, Courvoisier C (1990) Electrophoresis of gliadins in long acrylamide gels: methods and nomenclature. Electrophoresis 11:310–314

Branlard G, Rousset M, Loisel W, Autran JC (1991) Comparison of 46 technological breeding criteria used bread wheat quality evaluation. J Genet Breed 45:263–280

Branlard G, Pierre J, Rousset M (1992) Selection indices for quality evaluation in wheat breeding. Theor Appl Genet 84:57–64

Cabrera A, Martin A, Barro F (2002) In-situ comparative mapping (ISCM) of *Glu-1* loci in *Triticum* and *Hordeum*. Chromosome Res 10:49–54

Denis JB, Bernard M, Arnoux J, Cauderon Y (1982) Analyse statistique des configurations méiotiques lors de la création d'une série monosomique du blé tendre "Courtot". Agronomie 2:701–708

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Dumur J, Jahier J, Bancel E, Laurière M, Bernard M, Branlard G (2004) Proteomic analysis of aneuploid lines in the homoeologous group 1 of the hexaploid wheat cultivar Courtot. Proteomics 4:2685–2695

Galili G, Levy A, Feldman M (1986) Gene-dosage compensation of endosperm proteins in hexaploid wheat *Triticum aestivum*. Proc Natl Acad Sci USA 83:6524–6528

Gill KS, Gill BS (1996) A PCR-based screening assay of *Ph1*, the chromosome pairing regulator gene of wheat. Crop Sci 36:719–722

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosom bands and structural aberrations in wheat (*Triticum aestivum*). Genome 34:830–839

Holt LM, Astin R, Payne PI (1981) Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Part 2: Relative isoelectric points determined by two-dimensional fractionation in polyacrylamide gels. Theor Appl Genet 60:237–243

Islam N, Woo S-H, Tsujimoto H, Kawasaki H, Hirano H (2002) Proteome approaches to characterize seed storage proteins related to ditelocentric chromosomes in common wheat (*Triticum aestivum* L.). Proteomics 2:1146–1155

Jones LE, Rybka K, Lukaszewski A (2002) The effects of a deficiency and a deletion on recombination in chromosome 1BL in wheat. Theor Appl Genet 104:1204–1208

Joppa LR, Klindworth DL, Hareland GA (1998) Transfer of high molecular weigh glutenins from spring wheat to durum wheat. In: Slinkard AE (ed) Proceedings of 9th International Wheat Genet Symposium, Saskatoon, Canada, pp 257–260

Kota RS, Gill KS, Gill BS (1993) A cytogenetically based physical map of chromosome 1B in common wheat. Genome 36:548–554

Laemmli UK (1970) Cleavage of structural protein during the assembly of head of bacteriophage T4. Nature 227:680–684

Lukaszewski AJ (1995) Physical distribution of translocation breakpoints in homoeologous recombinants induced by the absence of the *Ph1* gene in wheat and triticale. Theor Appl Genet 90:714–719

Lukaszewski AJ, Curtis CA (1992) Transfer of the *Glu-D1* gene from chromosome 1D of bread wheat to chromosome 1R in hexaploid triticale. Plant Breeding 109:203–210

Lukaszewski AJ, Curtis CA (1993) Physical distribution of recombination in B-genome chromosomes of tetraploid wheat. Theor Appl Genet 86:121–127

Lukaszewski AJ, Curtis CA (1994) Transfer of the *Glu-D1* gene from chromosome 1D to chromosome 1A in hexaploid triticale. Plant Breeding 112:177–182

Lukaszewski AJ, Lapinski B, Rybka K (2005) Limitations of *in situ* hybridization with total genomic DNA in routine screening for alien introgressions in wheat. Cytogenet Genome Res 109:373–377

Luo MC, Dubcovsky J, Goyal S, Dvorak J (1996) Engineering of interstitial foreign chromosome segments containing the K^+/NA^+ selectivity gene *Kna1* by sequential homoeologous recombination in durum wheat. Theor Appl Genet 93:1180–1184

Naranjo T, Roca A, Goicoechea PG, Giraldez R (1987) Arm homeology of wheat and rye chromosomes. Genome 29:873–882

Payne P (1987) Genetics of wheat storage proteins and the effect of allelic variation on breadmaking quality. Annu Rev Plant Physiol 38:141–153

Payne P, Corfield KG, Blackman JA (1979) Identification of a high-molecular-weight subunit of glutenin whose presence correlates with bread-making quality in wheats of related pedigree. Theor Appl Genet 55:153–159

Pestsova E, Ganal M, Röder M (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Ravel C, Praud S, Murigneux A, Linossier L, Dardevet M, Balfourier F, Dufour P, Brunel D, Charmet G (2006a) Identification of Glu-B1–1 as a candidate gene for the quantity of high-molecular-weight glutenin in bread wheat (Triticum aestivum L.) by means of an association study. Theor Appl Genet 112:738–743

Ravel C, Nagy IJ, Martre P, Sourdille P, Dardevet M, Balfourier F, Pont C, Giancola S, Praud S, Charmet G (2006b) Single nucleotide polymorphism, genetic mapping and expression of genes coding for the DOF wheat prolamin-box binding factor. Funct Int Genomics 6:310–321

Röder M, Korzun V, Wendahake K, Plaschke J, Tixier M-H, Leroy P, Ganal M (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rogers W, Rickatson J, Sayers E, Law C (1990) Dosage effects of chromosomes of homoeologous groups 1 and 6 upon bread-making quality in hexaploid wheat. Theor Appl Genet 80:281–287

Sears ER (1972) Reduced proximal crossing over in telocentric chromosomes of wheat. Genet Iber 24:233–239

Sears E (1977) An induced mutant with homoeologous pairing in common wheat. Can J Genet Cytol 19:585–593

Singh N, Shepherd K (1988a) Linkage mapping of genes controlling endosperm storage proteins in wheat. 2. Genes on the long arms of group 1 chromosomes. Theor Appl Genet 75:642–650

Singh N, Shepherd K (1988b) Linkage mapping of genes controlling endosperm storage proteins in wheat. 1. Genes on the short arms of group 1 chromosomes. Theor Appl Genet 75:628–641

Singh N, Shepherd K, Cornish G (1991) A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. J Cereal Sci 14:203–208

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Sourdille P, Singh S, Cadalen T, Brown-Guidera GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25

Storlie EW, Ihry RJ, Baehr LL, Tieszen KA, Engbers JH, Anderson-Daniels JM, Davis EM, Gilbertson AG, Harden NR, Harris KA, Johnson AJ, Kerkvleit AM, Moldan MM, Bell ME, Wanous MK (2009) Genomic regions influencing gene expression of the HMW glutenins in wheat. Theor Appl Genet 118:295–303

Tanguy A-M, Coriton O, Abelard P, Dedryver F, Jahier J (2005) Structure of *Aegilops ventricosa* chromosome $6N^v$, the donor of wheat genes *Yr17*, *Lr17*, *Sr38*, and *Cre5*. Genome 48:541–546

Vitellozzi F, Ciaffi M, Dominici L, Ceoloni C (1997) Isolation of a chromosomally engineered durum wheat line carrying the common wheat *Glu-D1d* allele. Agronomie 17:413–419

Wanous M, Munkvold J, Kruse J, Brachman E, Klawiter M, Fuehrer K (2003) Identification of chromosome arms influencing expression of the HMW glutenins in wheat. Theor Appl Genet 106:213–220

Wieser H, Zimmermann G (2000) Importance of amounts and proportions of high molecular weight subunits of glutenin for wheat quality. Eur Food Res Technol 210:324–330

Xu SS, Faris JD, Cai X, Klindworth DL (2005) Molecular cytogenetic characterization and seed storage protein analysis of 1A/1D translocation lines of durum wheat. Chromosome Res 13:559–568