Theor Appl Genet (2007) 114:1141–1150
DOI 10.1007/s00122-007-0506-4

ORIGINAL PAPER

Negative effect of chromosome 1A on dough strength shown by modification of 1D addition in durum wheat (*Triticum durum*)

Monika Garg · Harcharan Singh Dhaliwal · Parveen Chhuneja · Deepak Kumar · Quan-Wen Dou · Hiroyuki Tanaka · Hala M. M. Elamein · Hisashi Tsujimoto

Received: 13 June 2006 / Accepted: 13 January 2007 / Published online: 8 February 2007

Abstract A monosomic addition line of *Aegilops tauschii* chromosome 1D in *Triticum durum* cv. PBW114 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 had substitution of chromosome 1D for 1B. One of these lines carried a pair of small chromosomes in addition to the 1D chromosome. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the derived lines showed the presence of high-molecular-weight (HMW) glutenin encoded by the *Glu-D1* locus. The small chromosome found in one of the lines had nearly regular pairing and transmission to daughter nuclei. Fluorescent in situ hybridization (FISH) and analysis of 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 terminal deletions on both the terminal and centromeric sides. The line with the small chromosome showed improvement of the sodium dodecyl sulfate (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*.

Communicated by M. Morell.

M. Garg · Q.-W. Dou · H. Tanaka · H. M. M. Elamein · H. Tsujimoto (✉)
Laboratory of Plant Genetics and Breeding Science, Faculty of Agriculture, Tottori University, 4-101 Minami, Koyama, Tottori 680-8553, Japan
e-mail: tsujim@muses.tottori-u.ac.jp

H. S. Dhaliwal · D. Kumar
Department of Biotechnology, Indian Institute of Technology, Roorkee, Uttranchal, India

P. Chhuneja
Department of Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

Introduction

Durum wheat (*Triticum durum*, $2n = 4x = 28$, AABB) has traditionally been used for pasta products and common wheat (*Triticum aestivum*, $2n = 6x = 42$, AABBDD) for bread, because of the different physical properties of the glutens from the respective flours. In recent years, wheat breeding objectives have included the development of crop varieties with dual-purpose end use. Homoeologous group 1 chromosomes in wheat and related species are known to carry genes of seed storage proteins; high-molecular-

weight (HMW) glutenins, low-molecular-weight (LMW) glutenins, and certain subunits of gliadin storage proteins. These proteins affect processing and nutritional qualities (Shepherd 1968; Wrigley and Shepherd 1973; Bietz et al. 1975; Lawrence and Shepherd 1980, 1981). Most *T. durum* cultivars possess low gluten and poor bread-making quality because of the absence of the D genome and silencing of the *Glu-A1* locus due to the insertion of transposon and/or presence of stop codon within the locus (Lafiandra et al. 1997; Bustos et al. 2000). Several attempts have been made to improve the protein content and quality of *T. durum* cultivars by the transfer of genes of homoeologous group 1 chromosomes through interspecific hybridization and following chromosome manipulations (Lukaszewski and Curtis 1994; Vitellozzi et al. 1997; Joppa et al. 1998; Blanco et al. 2002; Lukaszewski 2003; Dhaliwal et al. 2002). Dhaliwal et al. (1990) developed a complete series of *Aegilops tauschii*-D genome chromosome addition lines in an Indian durum wheat cultivar, 'PBW114.' The plants with monosomic 1D addition could be easily identified by the presence of a dominant allele of red glume color, the *Rg* gene, on the short arm. Selfed seeds of monosomic 1D addition plants with red glume color have been maintained for several generations since 1990 at the Punjab Agricultural University, Ludhiana, India. Modification of nearly true bred derivatives of the monosomic 1D addition line led to the formation of small (midget) chromosomes. Several *Aegilops* species carry gametocidal (*Gc*) genes that lead to chromosome deletions, breakage-fusion-bridges and healing of broken ends (Tsujimoto 2005). These types of gametocidal genes have not been reported in *T. durum* or *Ae. tauschii*. Thus, some other phenomenon might be involved in the formation of small chromosomes in the derivatives of the monosomic 1D addition line. This study deals with the chromosome constitution of derivatives of the monosomic 1D addition line, the origin of a small chromosome and its effect on bread-making quality.

Materials and methods

Plant material

Selfed seeds of three true bred derivatives (3596, 3598, and 3602) from the monosomic 1D addition line of *T. durum* cv. PBW114 were used in this study. These lines were grown in evenly fertilized, well conditioned field with at least four plants per line for two consecutive years, i.e., 2005 and 2006 at Tottori University, Japan. In addition in the year 2006 these lines were also grown in at least four replications in glass house with one plant per pot for morphological analysis.

Analyses of chromosomes

The young spikes were fixed at an appropriate time of heading in fixative (acetic acid + ethanol = 1v/3v), and meiosis in pollen mother cells (PMCs) was observed by the acetocarmine smear method. The images were captured with a digital camera (Olympus DP12). Mitotic chromosomes were prepared from root tips using the acetocarmine squash method and subjected to fluorescent in situ hybridization (FISH) analysis (Kishii et al. 1999). Total genomic DNA of *Ae. tauschii* and pAs1 (D genome specific sequences) were labeled with flouresciene-12-dUTP or tetramethyl-rhodamine-5-dUTP by the random primer labeling method (Gene Images™ Random Prime Labeling Module, Amersham Bioscience, Piscataway, NJ, USA). Total genomic DNA of *T. durum* cv. PBW114 was sonicated by autoclaving for 2 min, and used as the blocking DNA to examine the presence of the 1D chromosome. To determine the origin of the small chromosome, total genomic DNA of *Triticum urartu* and 45S rDNA (specific for 1B, 6B, and 1AS) were labeled similarly. Chromosomes were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI). The chromosomes were observed with a fluorescent microscope (Olympus BX61) and images were captured with a cooled CCD camera Olympus U-CMAD-2 (Photometrix, Kew, VIC, Australia).

Analyses of proteins

The composition of glutenin subunits from the endosperm half of the seeds was determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE), using 10% acrylamide, according to Smith and Payne (1984) with two modifications. The pH of the separating gel was changed from 8.8 to 8.6 and the volume of bisacrylamide was changed from 1.63 to 2.08 ml to increase the resolution. Gliadins were fractionated by acid polyacrylamide-gel electrophoresis (A-PAGE; Tanaka et al. 2003). Protein content (Zaman and Verwilghen 1979) and SDS sedimentation values (Takata et al. 1999), were measured on a small scale in three replications per plant, from at least four plants except for lines 3598 and 3596 under field conditions, which had low yield per plant to do three replications.

Analyses of genes

DNA was isolated from young seedlings by the CTAB method (Murray and Thompson 1980). To amplify genes of LMW glutenins, specific primers on the short arm of homoeologous group 1 were used (Long et al. 2005). Microsatellite markers supplied by Dr. A. Torada, Hokkaido Greenbio Co. (Hokkaido, Japan), were used based on their relative map position. Additional primers specific for the short arm of chromosome 1A were synthesized based on the position on the microsatellite-based deletion bin map (Sourdille et al. 2004). Sequences of the primers were retrieved from *Xgwm* (Röder et al. 1998), *Xcfa* and *Xbarc*: (http://www.wheat.pw.usda.gov/GG2/index.shtml). PCR reaction was performed in a final volume of 20 µl in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA).

To amplify *Ae. tauschii* HMW glutenin genes, specific primers were synthesized and amplified by PCR according to Liu et al. (2003). DNA fragments of the expected size were recovered from the gel and subsequently cloned into pGEM-T Easy Vector (Promega, Madison, WI, USA). The inserts were sequenced by the CREST-Akita Plant Molecular Science Satellite Laboratory at the Life Research Report Centre established at Akita Prefectural University. The sequences were compared with published HMW gene sequences using open reading frame (ORF) finder program (http://www.ncbi.hml.nih.gov/blast).

Results

Morphology of derivative lines

All the progenies of the 1D addition line showed red glume color (Fig. 1). They were full male and female fertile compared to the parent *T. durum* cv. PBW114. In the green house all the lines had similar morphological characters including yield to those of the parent except line 3602, which had stronger stems than the others, and some plants in line 3598 having fewer tillers and compact spikes with more spikelets per spike (Table 1). In the field lines 3596 and 3598 were weak with significantly lower yield than the parent, while line 3602 was not significantly different in morphology including yield from the parent durum.

Fig. 1 Spikes of true breeding lines with chromosome 1D showing red glume color. Yellow colored spike of parent *Triticum durum* cv. PBW114 is shown on the *left* side

Cytological analyses

Fluorescent in situ hybridization probed with total genomic DNA of the *Ae. tauschii* and D-genome-specific repetitive sequence pAs1 revealed the presence of a pair of 1D chromosomes in all of the lines (Fig. 2). Line 3596 carried a pair of small wheat chromosomes in addition to 26 normal durum wheat chromosomes and a pair of chromosome 1D (Fig. 2a). The small chromosomes paired well with each other in meiosis and segregated normally (Fig. 3). Lines 3598 and 3602 had 26 normal durum wheat chromosomes and a pair of 1D chromosomes (Fig. 2b, c). No small chromosome was observed in these lines. To identify the origin of the small chromosome, FISH probed with the total genomic

Table 1 Mean values of yield, number of spikelets per spike and number of tillers of the lines derived from monosomic addition line of durum wheat with chromosome 1D of *Ae. tauschii*

Lines	Yield per plant (gm) 2006(G)	Yield per plant (gm) 2006(F)	Number of spiklets/spike* 2006(G)	Number of tillers* 2006(G)
PBW114	4.60[a]	3.24[a]	11.75[a]	11.75[a]
3596	4.71[a]	1.03[b]	10.50[a]	10.75[a]
3598	5.14[a]	0.22[b]	14.25[b]	4.00[b]
3602	4.08[a]	3.08[a]	12.00[a]	13.67[a]

G glass house, *F* field

*Means followed by the same letter within each trait in the column are not significantly different at the 5% level as determined by LSD

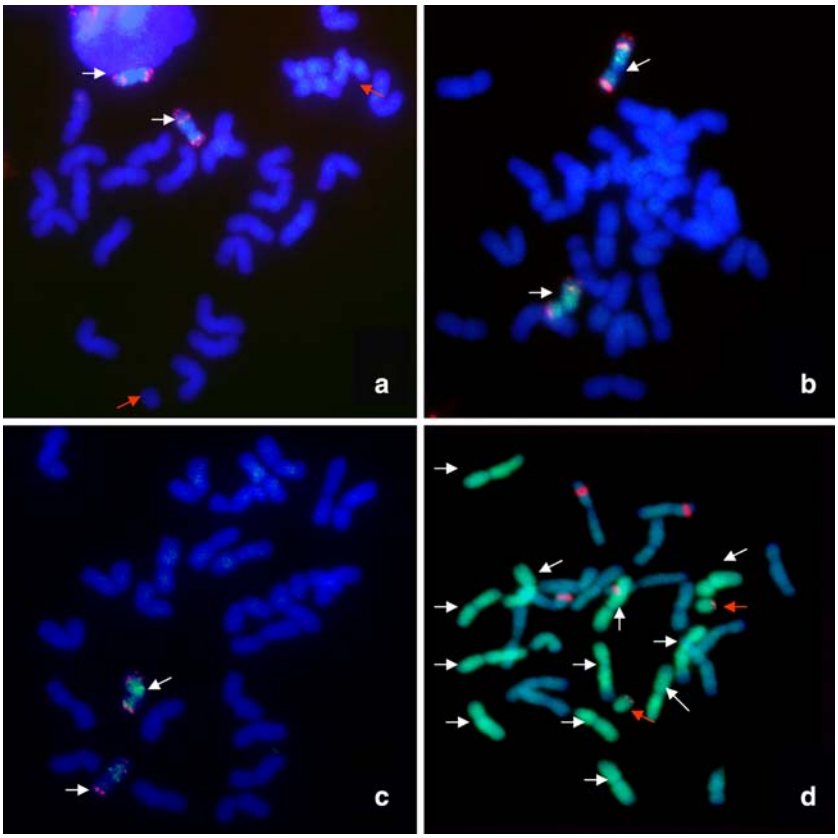

Fig. 2 Fluorescent in situ hybridization of mitotic chromosomes of the lines derived from the monosomic1D addition line of durum wheat. **a–c** Total *Ae. tauschii* genomic DNA was labeled with green color and D-genome specific repetitive sequence (pAs1) with red color. **a** Line 3596 having 26 normal, two small wheat chromosomes (*red arrows*), and a pair of chromosome ID (*white arrows*). **b–c** Lines 3598, and 3602, respectively, carrying 26 normal wheat chromosomes and a pair of 1D (*white arrows*). **d** Total *Triticum urartu* DNA was labeled with green color and 45S rDNA (specific for 1B, 6B, and 1AS) with red color, and hybridized to line 3596. Complete 12 and two small A-genome chromosomes have been indicated by *white and red arrows*, respectively

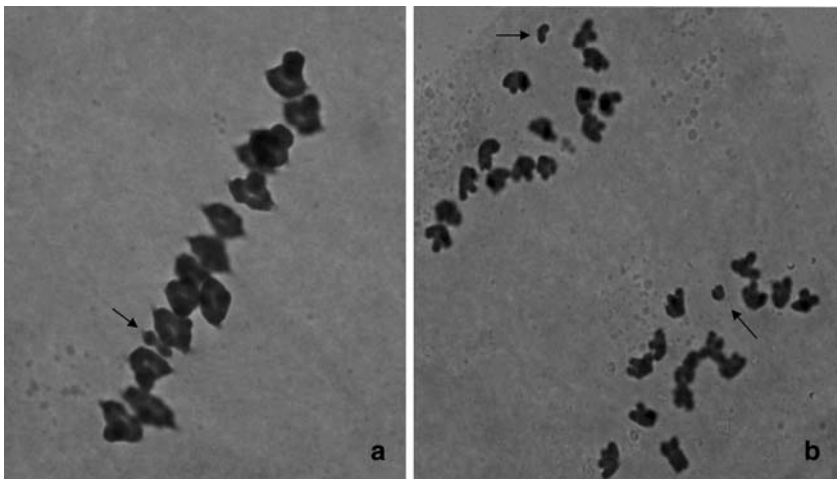

Fig. 3 Meiosis in pollen mother cells of the 1D substitution line 3596 with small wheat chromosomes in disomic state. **a** Normal pairing of small chromosomes at the first metaphase. **b** Normal transmission of the small chromosomes to the daughter cells at the first anaphase

DNA of *T. urartu* and 45S rDNA was conducted in line 3596 (Fig. 2d). It revealed the presence of 12 complete and two small A-genome chromosomes, in addition to 16 other chromosomes. The presence of 45S rDNA signals on the small chromosome indicated that it belonged to the short arm of chromosome 1A.

Composition of seed storage proteins

The SDS-PAGE indicated that the parent durum carried 6 + 8 subunits of HMW glutenin, the genes of which were located on the long arm of chromosome 1B (Fig. 4). Lines 3596 and 3602 carried two extra subunits of HMW glutenin, in addition to the 6 + 8 subunits. Cytological analyses and molecular analyses, which are described below, revealed that the extra subunits were encoded by genes on chromosome 1D. One line, 3598, however, carried only extra subunits of HMW glutenin, and the 6 + 8 subunits of the parent durum were absent. The extra subunits belonged to chromosome 1D, suggesting that chromosome 1D had substituted for 1B in this line. LMW-glutenin subunits are

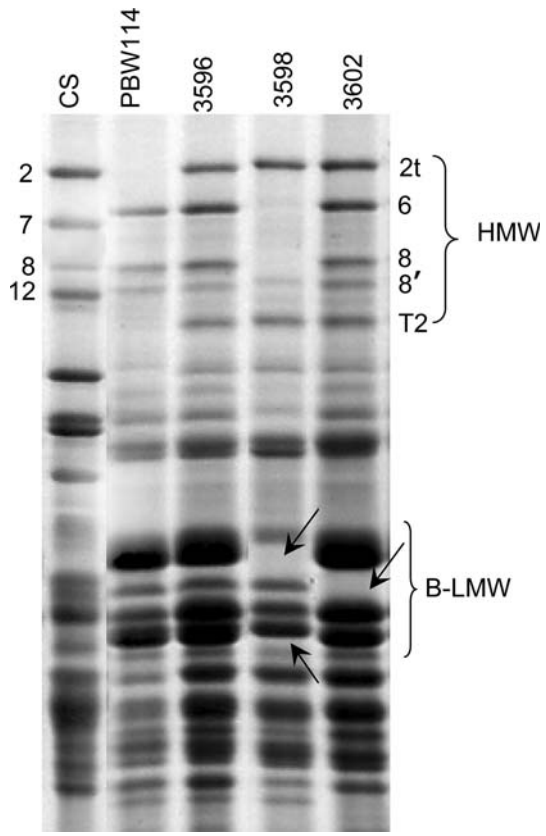

Fig. 4 SDS-PAGE of chromosome 1D addition derivatives showing additional subunits 2^t + T2 encoded by *Glu-D1* locus and LMW-2^- glutenin subunits belonging to *Glu-B3d*. CS is Chinese Spring (2 + 12, 7 + 8). PBW114 has three HMW glutenin subunits (6 +8 + 8′). 8′ is considered to be a byproduct of 1By8

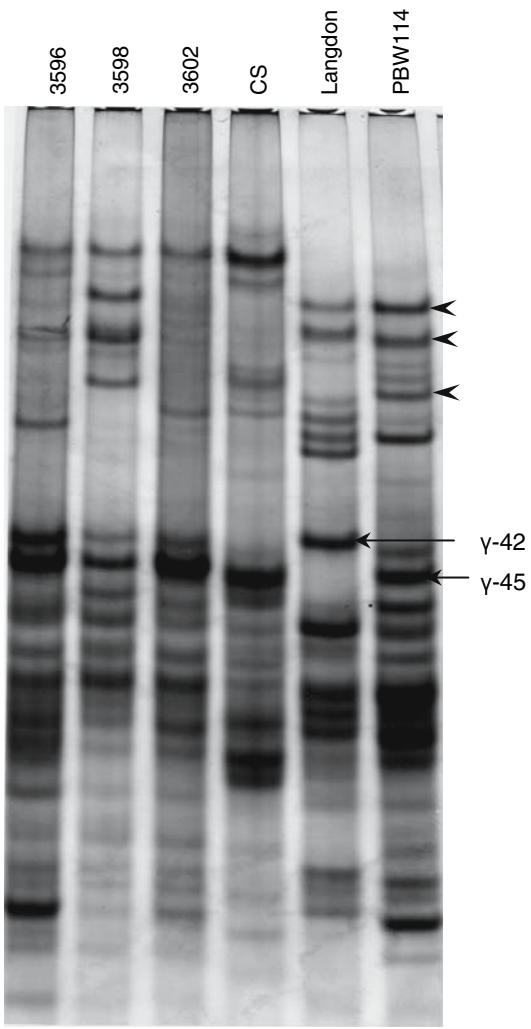

Fig. 5 Acid PAGE of gliadins of chromosome 1D addition derivatives. CS is Chinese Spring wheat and Langdon is durum used as controls. Cultivar PBW114 carries a γ-45 type band (*indicated by arrows*). *Gli1-A1* locus-specific subunits (*indicated by arrowheads*) are absent in lines with (3596) and without (3602) the small 1AS chromosome but present in the substitution line of chromosome 1D for 1B (3598) with full 1A chromosome

classified into three classes, A, B, and C, based on mobility. The B-LMW glutenin subunit of the parent cultivar, PBW114, was most probably *Glu-B3d*, which belongs to the LMW-2^- model (Carrilo et al. 1990) (Fig. 4). In line 3598 second and sixth subunits of the seven of B-LMW glutenin subunits were missing, which most probably belongs to *Glu-B3* locus and further supports that this line is substitution line of chromosome 1D for 1B. In line 3602 third subunit of B-LMW glutenin subunits was missing. This band most probably belongs to *Glu-A3* locus, suggesting that this line is substitution line of chromosome 1D for 1A. Acid-PAGE of gliadin seed storage protein of the parent durum and derived lines indicated that these carried the γ-45 type band encoded by the gene at the *Gli-B1* locus (Fig. 5). Several subunits were missing in the lines with (3596) or without (3602) the small chromosome but present in a substitution line of chromosome 1D for 1B (3598) with the full 1A chromosome (Fig. 5; indicated by arrow heads). This indicates that the *Gli-A1* locus is missing in the small chromosome.

Molecular analyses of the small chromosome

To determine the chromosome constitution of the lines, PCR was conducted using microsatellite markers and primers for LMW glutenins (Fig. 6). Line 3596 carrying the small chromosome exhibited all the molecular markers analyzed except *Xbarc*158 present on the long arm of chromosome 1A. This finding and the FISH results (Fig. 2a, d) indicated that chromosome 1D is substituted for 1A and that the small chromosome originated from a part of chromosome 1A. Line 3598 did not give amplification with *Xwmc* 44 and *GluB3.2* on the long and short arm of chromosome 1B, respectively. This indicates that this line is a substitution line of chromosome 1D for 1B. Line 3602 did not

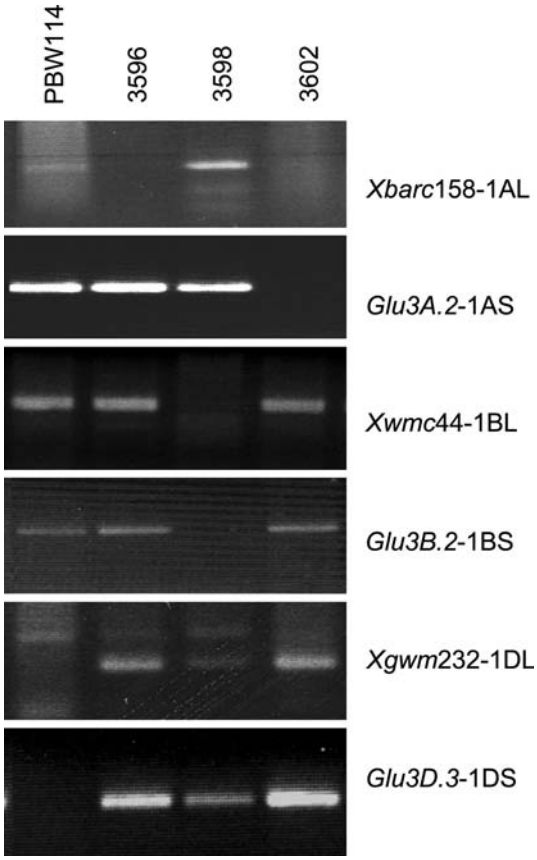

Fig. 6 PCR amplification using *different markers* indicated on the right to determine the chromosome constitution of selected lines

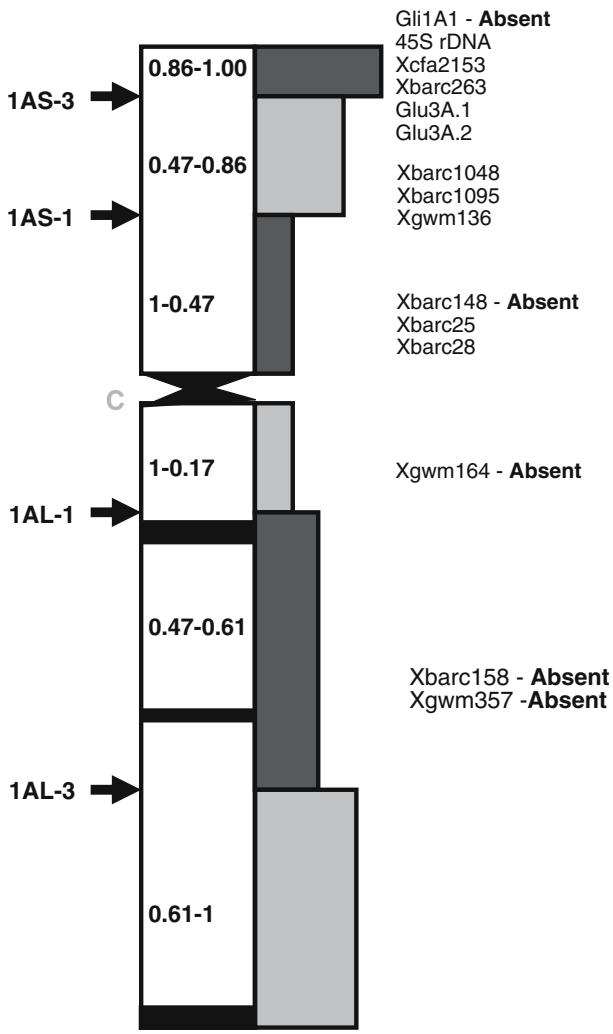

Fig. 7 Estimation of the size of the small 1A chromosome in the 1D substitution lines of *T. durum* using deletion bin map of chromosome 1A. Microsatellites placed on the right according to their more likely position were used for estimating the size of the small chromosome. Primers, which did not give amplification in the lines carrying the small 1A chromosome are indicated. C-bands on the chromosomes are drawn to scale. The breakpoints of the various deletions are indicated with *arrows*. Fraction length measurements are shown inside the chromosome

show amplification with *Xbarc*158 and *GluA3*.2 located on the long and short arm of chromosome 1A, respectively, indicating that this line had disomic substitution of chromosome 1D for 1A.

To determine the construction of the small chromosome in line 3596, the microsatellite-based bin map was amplified. From the mitosis and meiosis images this chromosome appeared small, but microsatellites present in all the three deletion bins of the short arm of chromosome 1A showed amplification in the line carrying the small chromosome (Fig. 7). While none of the markers on the long arm of chromosome 1A showed amplification indicating that small chromosome belongs to the short arm of chromosome 1A. In the first deletion bin (1–0.47) of short arm of chromosome 1A, out of three microsatellite markers studied two were present while the third was missing. This indicates that part of this deletion bin is missing in the small chromosome. All the microsatellite markers in the second deletion bin (0.47–0.86) showed amplification. This indicates that this deletion bin is present in the small chromosome. In the third deletion bin (0.86–1) all molecular markers studied showed amplification. Also, 45S rDNA signals near the tip of the short arm of chromosome 1A were observed in the small chromosome. However, *Gli-A1* encoded subunits were absent in the acid-PAGE of this line. Since *Gli-A1* is present at the tip of this deletion bin, this means that the small chromosome has a short terminal deletion and this deletion is after the 45S rDNA signal. Thus, in the small chromosome, the short arm of chromosome 1A has been deleted from both the terminal and centromeric sides.

Analysis of protein content and effect on bread-making quality

To examine the effect of 1D substitution and the small chromosome on bread-making quality, the protein

content, and SDS-sedimentation value of the lines were measured (Table 2). The protein content of all the lines was not significantly different from parent durum in the glass house, but under the field conditions it varied in years 2005 and 2006. Line 3598 had significantly higher protein content than parent durum under field condition for both the years. Lines 3596 and 3602 had significantly higher protein content only in 2006 (Table 2) indicating that protein content is a character controlled environmentally more than genetically. SDS-sedimentation value of line 3602, with disomic substitution of chromosome 1D for 1A, was significantly higher than parent durum PBW114, for both years, in the field as well as glass house. This indicates either a negative effect of chromosome 1A or a positive effect of chromosome 1D on the SDS-sedimentation value related to dough strength. Line 3598, a disomic substitution line of chromosome 1D for 1B, showed a significant increase in protein content under field conditions but its SDS-sedimentation value was similar to the parent durum. But its specific SDS sedimentation was lower than the parent durum PBW114 under field conditions, suggesting lower dough strength. This indicates that chromosome 1D produced a larger amount of protein but the increase did not reflect the SDS-sedimentation value. In glass house this line (3598) showed no difference in protein content as compared to the parent but its SDS-sedimentation value and specific SDS-sedimentation were significantly lower than parent durum. This indicates that chromosome 1B is more important for dough strength of durum wheat than chromosome 1D. The results from these two lines combined conclude that chromosome 1A has a negative effect on bread-making quality. This conclusion is further supported by the result of line 3596. The protein content of line 3596 with the 1D chromosome and a small chromosome from 1A was not significantly different from control in 2005 (field) and 2006 (glass house) but was significantly higher than parent durum in 2006 (field), The SDS-sedimentation value of this line was between those of the durum parent and line 3602 without the small chromosome under field conditions and similar to line 3602 in the glass house. Specific SDS-sedimentation of line 3596 was between those of parent durum and line 3602 for 2005 (field) and 2006 (glass house) but almost similar to parent durum in 2006 (field). In other words overall dough strength of line 3596 was between parent durum and line 3602. Both lines 3596 and 3602 carried chromosome 1D, and only line 3596 carried the small chromosome. The lower SDS-sedimentation value of this line as compared to 3602 confirms the negative effect of the small chromosome on dough strength.

As the HMW-glutenin subunits are major determinants of bread-making quality, we cloned and sequenced the N- and C-terminals of both the 1Dx and 1Dy genes encoded by *Glu-1D* in these lines. Blast search using these sequences indicated that these were equivalent to 2^t + T2 (Anderson et al. 2003).

Discussion

A major objective in durum breeding is to produce varieties with strong, elastic gluten that is satisfactory for bread and pasta. Such dual-purpose durum would be an ideal crop for future markets. A highly efficient approach for durum wheat improvement can be by introducing genes of seed storage proteins from chromosome 1D (Liu et al. 1996). The substitution lines of 1D reported here have arisen spontaneously from a monosomic addition line. The substitution line of chromosome 1D for 1A (3602), showed significant improvement in SDS sedimentation value. Liu et al. (1995) reported twofold increases in the rheological properties including SDS-sedimentation value, mix time and peak resistance values of a 1D (1A) substitution line of durum cv. Langdon. Rogers et al. (1990)

Table 2 Mean values of protein content, sodium dodecyl sulfate (SDS) sedimentation value, and specific SDS sedimentation of the lines derived from monosomic addition line of durum wheat with chromosome 1D of *Ae. tauschii*

Lines	Chromosome constitution	Protein content (%)			SDS sedimentation value (ml)*			Specific SDS sedimentation**		
		2005(F)	2006(F)	2006(G)	2005(F)	2006(F)	2006(G)	2005(F)	2006(F)	2006(G)
PBW114	14A + 14B	9.60^{a}	8.00^{a}	7.48^{a}	5.00^{a}	5.32^{a}	9.42^{a}	0.52	0.66	1.26
3596	12A + 14B +2D + 2***	10.01^{a}	11.75^{ba}	8.63^{a}	7.50^{b}	7.90^{b}	12.77^{b}	0.75	0.67	1.48
3598	14A + 12B + 2D	12.76^{b}	10.83^{b}	8.53^{a}	5.00^{a}	5.5^{a}	5.98^{c}	0.39	0.51	0.7
3602	12A + 14B + 2D	10.28^{a}	10.15^{bc}	7.33^{a}	9.30^{c}	8.22^{c}	12.78^{b}	0.9	0.81	1.74

F field, *G* glass house

*Means followed by the same letter within each trait in the column are not significantly different at the 5% level as determined by LSD

**SDS-sedimentation value divided by protein content

***Small chromosome derived from chromosome 1A

also reported twofold increase in the SDS sedimentation value in the nulli 1A tetra 1D lines of Chinese Spring. However, the use of these lines for varietal improvement was not recommended because of the unacceptable yield of the former and meiotic instability of the later. Although detailed studies on the agronomical traits of the present lines are still needed, preliminary studies in the glass house indicated them to be full male and female fertile and to have similar yields as compared to the parent cultivar. Substitution line of chromosome 1D for 1B (3598) had rather higher number of spikelets per spike as compared to the parent durum. This line also had a lower number of tillers as compared to the parent durum. Although lines 3596 and 3598 did not perform well in the field. The SDS sedimentation value of line 3598, with substitution for chromosome 1B, was similar or less than that of the parent durum. This is in contrast to a previous report by Joppa et al. (1983) of the improved gluten quality of 1D (1B) substitution line of durum cv. Langdon. This might be because of the presence of poor quality, γ-42 type gliadin and LMW-1, on the short arm of chromosome 1B in Langdon. Indian cultivar PBW114 studied here had γ-45 type gliadin and LMW-2^{-}. The HMW glutenin subunits located on 1BL are the same in both cultivars, i.e., 6 + 8. γ-45 type gliadin allele and LMW-2^{-} on the short arm of chromosome 1B have been found to be associated with good pasta quality in durum wheats in contrast to, γ-42 type gliadin allele and LMW-1 (Carrilo et al. 1990). Moreover, PBW114 is a relatively recent cultivar, because of selection pressure toward better end use quality; it has better alleles at the gliadin and glutenin loci as compared to Langdon. Line 3596 carried a small chromosome derived from chromosome 1A, and thus the *Glu-A3* locus. This line showed an SDS-sedimentation value between those of the parent line and 3602 (1D(1A)). This suggested that gene(s) on the small chromosome, most probably *Glu-3* of chromosome 1A, had a negative effect on the SDS-sedimentation value. Rogers et al. (1990) reported that Chinese Spring ditelosomic lines of the short and long arms of chromosome 1A, 1DT1AS, and 1DT1AL, had improved quality traits and postulated that this might be because of the presence of a negative quality allele at the *Glu-A3/Gli-A1* locus on the short arm and a null allele at the *Glu-A1* locus on the long arm of chromosome 1A of Chinese Spring. Chinese Spring is a hexaploid cultivar, with inferior quality, and ditelosomic lines are meiotically unstable. Line 3596 is durum wheat with a small chromosome, is stable and has improved quality.

The small chromosome present in line 3596 has arisen spontaneously from a monosomic 1D addition line over more than 15 years. The mechanism of its origin is unclear. Several species of the genus *Aegilops* carry *Gc* gene that kill gametes lacking them in the monosomic addition state. Using the action of gametocidal genes, many chromosome deletions and translocations have been produced in wheat chromosomes, and alien chromosomes added to wheat (Tsujimoto 2005). This type of *Gc* gene has not been reported in *T. durum* or *Ae. tauschii*. This suggests that some phenomenon other than *Gc* genes might be involved in the formation of the small chromosome reported here. The small chromosomes paired well in meiosis and segregated normally to the tetrad. This indicates that they are stable and might have acquired telomere repetitive sequences. This type of small chromosome of 1A in *T. durum* without the effect of *Gc* genes has been reported for the first time.

Bread-making quality is dependent on grain protein content and type of the grain proteins (glutenin and gliadins) (Finney and Barmore 1948). Higher protein content, in the case of reduced yield, results in increased SDS-sedimentation value. However, the overall correlation between protein content and SDS-sedimentation value of the derivative lines was −0.11. This indicates that the increase in SDS-sedimentation value of derivative lines is not a function of protein content but rather of protein type, i.e., glutenins and gliadins.

The *Glu-1* locus has been found to have a determinant effect on bread-making quality, with the 5 + 10 subunits of the *Glu-D1d* locus having the most significant effect. As a result, many workers tried to express this allele in hexaploid wheat. Klindworth et al. (2005) studied the agronomic and quality characteristics of 1AS.1AL-1DL translocations line of durum wheat carrying *Glu-D1d* (5 + 10). The loaf volume of translocated lines did not differ from that of the parent cultivar Renville, indicating that HMW glutenin subunits 5 + 10 alone are not sufficient to improve quality. They showed that loaf volume was related to different alleles at the *Glu-B3* locus of the recipient durum wheat.

The *Glu-D1* allele transferred in this line was 2^{t} + T2. Hsam et al. (2001) and Weiser et al. (2003) found that HMW glutenin subunits 2^{t} + T2 were associated with poor rheological properties and bread loaf volume in synthetic hexaploid wheat. Since the HMW glutenin subunits in this case are 2^{t} + T2, this indicates that the approximate twofold increase in the SDS-sedimentation value of the disomic substitution line of chromosome1D for 1A, 3602, cannot be because of chromosome 1D. Furthermore, the lack of increase in the SDS-sedimentation value of the disomic substitution line of chromosome 1D for 1B (3598) supports the notion that chromosome 1D does not have a significant

positive effect on quality characters of durum wheat. Genes on 1B are equally important as genes on 1D for bread-making quality.

The dough strength of line 3596 with small 1A-derived chromosomes was lower than that of line 3602 without the small chromosome, although both had the 1D chromosome. This indicates a negative effect of the short arm of chromosome 1A on quality-related traits rather than a positive effect of chromosome 1D. Thus, the significant improvement in the SDS-sedimentation value of the substitution line of 1D for 1A suggests that the negative effect of chromosome 1A on quality is more important than the allele present at the *Glu-D1* locus and that this negative effect is contributed by both the long arm and short arm of chromosome 1A.

Acknowledgments The first author was supported by a Monbusho (Ministry of Education, Science, Sports, and Culture, Government of Japan) scholarship.

References

Anderson OD, Rausch C, Moullet O, Lagudah ES (2003) The wheat D-genome HMW-glutenin locus: BAC sequencing, gene distribution, and retrotransposon clusters. Funct Integr Genomics 3:56–68

Bietz JA, Shepherd KW, Wall JS (1975) Cereal single-kernel analysis of glutenin: use in wheat genetics and breeding. Cereal Chem 52:513–532

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

Bustos AD, Rubio P, Jouve N (2000) Molecular characterization of the inactive allele of the gene *Glu-A1* and the development of a set of AS-PCR markers for HMW glutenins of wheat. Theor Appl Genet 100:1085–1094

Carrilo JM, Vazquez JF, Orellana J (1990) Relationship between gluten strength and glutenin proteins in durum wheat cultivars. Plant Breed 104:325–333

Dhaliwal HS, Friebe B, Gill KS, Gill BS (1990) Cytogenetic identification of *Aegilops squarrosa* chromosome additions in durum wheat. Theor Appl Genet 79:769–774

Dhaliwal HS, Garg M, Singh H, Chhuneja P, Kaur H (2002) Transfer of HMW-glutenin subunits from wild wheats into *Triticum durum* and improvement of quality. Cereal Res Commun 30:173–180

Finney KF, Barmore MA (1948) Loaf volume and protein content of hard winter and spring wheats. Cereal Chem 25:291–312

Hsam SLK, Kieffer R, Zeller FJ (2001) Significance of *Aegilops tauschii* glutenin genes on breadmaking properties of wheat. Cereal Chem 78:521–525

Joppa LR, Josephides C, Youngs VL (1983) Chromosomal location of genes affecting quality in durum wheat. In: Sakamoto S (ed) Proceedings of the 6th international wheat genetic symposium. Plant Germplasm Institute, Kyoto University, Kyoto, Japan, pp 297–301

Joppa LR, Klindworth DL, Hareland GA (1998) Transfer of high molecular weight glutenins from spring wheat to durum wheat. In: Slinkard AE (ed) Proceedings of the 9 th international wheat genetic symposium. University of Saskatchewan Ext. Press, Saskatoon, SK, Canada, pp 257–260

Kishii M, Nagaki K, Tsujimoto H, Sasakuma T (1999) Exclusive localization of tandem repetitive sequences in subtelomeric heterochromatin regions of *Leymus racemosus* (Poaceae, Triticeae). Chromosome Res 7:519–529

Klindworth DL, Hareland GA, Elias EM, Xu SS (2005) Agronomical and quality characteristics of 1AS.1AL-1DL translocation lines of Durum wheat carrying glutenin allele *Glu-D1d*. Crop Sci 45:77–84

Lafiandra D, Tucci GF, Pavoni A, Turchetta T (1997) PCR analysis of x- and y-type genes present at the complex *Glu-A1* locus in durum and bread wheat. Theor Appl Genet 94:235–240

Lawrence GJ, Shepherd KW (1980) Variation in glutenin protein subunits in wheat. Aust J Biol Sci 3:221–233

Lawrence GJ, Shepherd KW (1981) Chromosomal locations of genes controlling seed proteins in species related to wheat. Theor Appl Genet 59:25–31

Liu C-Y, Rathjen AJ, Shepherd KW, Gras PW, Giles LC (1995) Grain quality and yield characteristics of D-genome disomic substitution lines in Langdon (*Triticum turgidum* var. *durum*). Plant Breed 114:34–39

Liu C-Y, Shepherd KW, Rathjen AJ (1996) Improvement of durum wheat pastamaking and breadmaking qualities. Cereal Chem 73:155–166

Liu Z, Yan Z, Wan Y, Liu K, Zheng Y, Wang D (2003) Analysis of HMW glutenin subunits and their coding sequences in two diploid *Aegilops* species. Theor Appl Genet 106:1368–1378

Long H, Wei Y-M, Yan Z-H, Baum B, Nevo E, Zheng Y-L (2005) Classification of wheat low-molecular-weight glutenin subunit genes and its chromosome assignment by developing LMW-GS group-specific primers. Theor Appl Genet 111:1251–1259

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

Lukaszewski AJ (2003) Registration of six germplasms of durum wheat with introgressions of the *Glu-D1* locus. Crop Sci 43:1138–1139

Murray HG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Röder MS, Korsun V, Gill BS, Ganal MW (1998) The physical mapping of microsatellite markers in wheat. Genome 41:278–283

Rogers WJ, Rickatson JM, Sayers EJ, Law CN (1990) Dosage effect of chromosomes of homoeologous groups 1 and 6 upon bread-making quality in hexaploid wheat. Theor Appl Genet 80:281–287

Shepherd KW (1968) Chromosomal control of endosperm proteins in wheat and rye. In: Findlay KW, Shepherd KW (eds) Proceedings of the 3rd international wheat genetic symposium. Australian Academy of Science, Canberra, pp 86–96

Smith DB, Payne PI (1984) A procedure for the routine determination of electrophoretic band patterns of barley and malt endosperm proteins. J Nat Inst Agric Bot 16:487–498

Sourdille P, Singh S, Cadalen T, Brown-Guedira 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

Tanaka H, Tomita M, Tsujimoto H, Yasumuro Y (2003) Limited but specific variations of seed storage proteins in Japanese common wheat (*Triticum aestivum* L). Euphytica 132:167–174

Takata K, Yamauchi H, Iriki N, Kuwabara T (1999) Prediction of bread-making quality by prolonged swelling SDS-sedimentation test. Breed Sci 49:221–223

Tsujimoto H (2005) Gametocidal genes in wheat as the inducer of chromosome breakage. Wheat Inform Serv 100:33–48

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

Wieser H, Hsam SLK, Zeller FJ (2003) Relationship between the qualitative and quantitative compositions of gluten protein types and technological properties of synthetic hexaploid wheat derived from *Triticum durum* and *Aegilops tauschii*. Cereal Chem 80:247–251

Wrigley CW, Shepherd KW (1973) Electrofocusing of grain proteins from wheat genotypes. Ann NY Acad Sci 209:154–162

Zaman Z, Verwilghen RL (1979) Quantitation of proteins solubilized in sodium dodecyl sulfate-mercaptoethanol-Tris electrophoresis buffer. Anal Biochem 100:64–69