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Research Paper

A novel compensating wheat–*Thinopyrum elongatum* Robertsonian translocation line with a positive effect on flour quality

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Wheat flours are used to produce bread, pasta, breakfast cereals, and biscuits; the various properties of these end-products are attributed to the gluten content, produced as seed storage proteins in the wheat endosperm. Thus, genes encoding gluten protein are major targets of wheat breeders aiming to improve the various properties of wheat flour. Here, we describe a novel compensating wheat–*Thinopyrum elongatum* Robertsonian translocation (T1AS.1EL) line involving the short arm of wheat chromosome 1A (1AS) and the long arm of *Th. elongatum* chromosome 1E (1EL); we developed this line through centric breakage-fusion. Compared to the common wheat cultivars Chinese Spring and Norin 61, we detected two additional 1EL-derived high-molecular-weight glutenin subunits (HMW-GSs) in the T1AS.1EL plants. Based on the results of an SDS-sedimentation volume to estimate the gluten strength of T1AS.1EL-derived flour, we predict that T1AS.1EL-derived flour is better suited to bread-making than Chinese Spring- and Norin 61-derived flour and that this is because of its greater gluten diversity. Also, we were able to assign 33 of 121 wheat PCR-based Landmark Unique Gene markers to chromosome 1E of *Th. elongatum*. These markers can now be used for further chromosome engineering of the *Th. elongatum* segment of T1AS.1EL.

Key Words: *Triticum aestivum*, *Thinopyrum elongatum*, Robertsonian translocation, bread-making quality, wheat PLUG markers.

Introduction

There is little genetic diversity in many major crop species. Such narrow genetic diversity is attributed to domestication, with a subsequent bottlenecking of originally large and diverse populations (Reif *et al*. 2005, Tanksley and McCouch 1997). Common wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is an allohexaploid that evolved through natural hybridization and allopolyploid speciation. The number of independent hybridization events between the progenitors of common wheat is unclear, but it is considered limited (Dvořák *et al*. 1998, Talbert *et al*. 1998) and presumably results in a loss of diversity.

Wheat flour produces visco-elastic dough when mixed with water. The elasticity of the dough influences the processing quality of wheat flour end-products, such as bread, noodles, and cookies. Wheat flours derived from different wheat varieties often have distinct properties, and this can be attributed to their diversity of gluten. Gluten is composed of seed storage proteins (SSPs), glutenins and gliadins, which are stored in the wheat endosperm. High-molecular-weight glutenin subunits (HMW-GSs) are the major determinants of gluten elasticity; thus, HMW-GSs are important for the bread-making process (Tatham *et al*. 1985). Although HMW-GSs account for only about 10% of the total SSPs in mature seeds, multiple correlation coefficients have indicated that almost 80% of the variation in the Alveograph *w* value (a combined measure of dough strength and extensibility) can be accounted for by variations in flour HMW-GS composition and protein content (Payne *et al*. 1988). Thus, expanding gluten diversity is likely to facilitate a greater variety of wheat flour end-products.

HMW-GSs in wheat are encoded by the *Glu-A1*, *Glu-B1*, and *Glu-D1* genes (at complex loci on the long arms of the homoeologous group-1 chromosomes 1A, 1B, and 1D

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respectively) (Galili and Feldman 1985, Lawrence and Shepherd 1980, Payne *et al*. 1980, 1981, 1982). The structural features of HMW-GSs differ between common wheat and its wild relatives. The diploid wheatgrass *Thinopyrum elongatum* (Host) D. R. Dewey [=*Agropyron elongatum* (Host) P. Beauv.] (2n = 2x = 14, EE genome) is a wild relative of wheat with many agronomic characters superior to those in domesticate wheat, including biotic and abiotic stress resistance (Dvořák *et al*. 1988, Friebe *et al*. 1994, Jauhar and Peterson 2000, Ma *et al*. 1999, Roundy 1985) and superior flour quality (Feng *et al*. 2004). Work from our group has demonstrated that, among seven disomic addition lines (DALs) of wheat, each containing a homoeologous group-1 chromosome of various wild relatives, the presence of the chromosome 1E from *Th. elongatum* results in the strongest dough (Garg *et al*. 2009b). Thus, this DAL (which contains the *Th. elongatum* chromosome 1E) might produce wheat flour that is better suited to downstream applications, particularly bread-making.

Dvořák *et al*. (1986) reported that the SSP genes of the E-genome are similar to wheat SSP genes and are located on the same chromosomes (mainly homoeologous group-1 chromosomes) as those in *Triticum* species. Homoeologous group-1 chromosomes also contain major clusters of agronomically important genes (McIntosh *et al*. 2003), including glutenins and gliadins. Therefore, homoeologous group-1 chromosomes of modern wheat cultivars contain a large number of superior genes that have been collected during evolution, in breeding programs, or both, whereas the chromosomes of wheat wild relatives carry genes associated with various agronomically undesirable traits, which should be reduced in breeding programs.

Here, we first identified HMW-GSs of the *Th. elongatum* chromosome 1E within a wheat genetic background. Next, we produced a compensating wheat–*Th. elongatum* Robertsonian (centric) translocation (T1AS.1EL) line carrying the HMW-GSs of the long arm of the *Th. elongatum* chromosome 1E (1EL). In small-scale tests, we evaluated the quality of T1AS.1EL-derived wheat flour. Also, to confirm the presence of 1EL in the wheat genetic background, we used the wheat PCR-based Landmark Unique Gene (PLUG) markers reported by Ishikawa *et al*. (2007) to develop *Th. elongatum* chromosome 1E-specific PCR-based markers.

Materials and Methods

Plant materials

We used the common wheat cultivar Chinese Spring (CS, 2n = 42 = 21", genome AABBDD); Japanese commercial common wheat cultivar Norin 61 (N61, 2n = 42 = 21", AABBDD); diploid wheatgrass *Th. elongatum* (2n = 14 = 7", EE); a nullisomic-1A tetrasomic-1D line of CS (N1AT1D, 2n = 42 = 19" + 1^{iv}, AABBDD-1A1A + 1D1D) (Sears 1966); the chromosome 1E disomic addition line of *Th. elongatum* in the CS genetic background (CSDAL1E, 2n = 44 = 22", AABBDD + 1E1E) (Dvořák and Knott 1974); and a disomic substitution line of chromosome 1E for chromosome 1D of CS [CSDSL1E(1D) , 2n = 42 = 21", AABBDD-1D1D + 1E1E] (Garg *et al*. 2009a). CSDAL1E was maintained at Kyoto University and Tottori University as part of the National BioResource Project-Wheat (NBRP ID: TACBOW0038).

HMW-GS composition analysis

At first, the HMW-GS composition of the tested wheat varieties was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), according to Tanaka *et al*. (2003). The separation of molecules within a gel is determined by the relative size of the pores formed within the gel. The pore size of a gel is determined by two factors, the total amount of acrylamide present (designated as %T) and the amount of N N' bisacrylamide, cross-linker (%C). As the total amount of acrylamide increases, the pore size decreases. With cross-linking, 5%C gives the smallest pore size. Any increase or decrease in %C increases the pore size. The decrease of %C is generally known to differentiate among similar band patterns based on the molecular structures. Thus, following the method of Smith and Payne (1984), we increased the total acrylamide content (%T) of the polyacrylamide gel from 10% to 18% and decreased the N N' bisacrylamide cross-linker (%C) content from 3.3% to 0.46%.

Chromosome analysis

Mitotic chromosomes were prepared from root tip cells by using the acetocarmine squash method and used for genomic *in situ* hybridization (GISH). Chromosomes were probed with *Th. elongatum* genomic DNA labeled with fluorescein-12-dUTP (Roche Diagnostics, Mannheim, Germany) by using the nick translation method, according to Ishii *et al*. (2010).

Evaluation of grain quality

To evaluate the grain quality of T1AS.1EL plants, 5 g of seeds from each plant were ground in a UDY cyclone sample mill (UDY Corp., Fort Collins, CO, USA) fitted with 1-mm screens. The protein content of the ground seeds was then measured by near-infrared spectroscopy (Kett, model KJT-270, NIR composition analyzer). The SDS sedimentation volume (SDS-SV), which is highly correlated with the bread loaf volume, is a reflection of gluten quantity and quality (Axford *et al*. 1979); we measured SDS-SV in 1 g of flour, according to the method of Takata *et al*. (1999). For an index of gluten quality, specific sedimentation values (SSVs) were calculated by dividing the SDS-SV by the percentage of protein content, because the protein content of wheat is reported to be highly correlated with the SDS-SV (Moonen *et al*. 1982). Previously, we also studied the relationship between the protein content and the SDS-SV (Tanaka and Tsujimoto 2012). The coefficient of correlation ($R = 0.9989$; $P < 0.01$) showed a significant positive correlation between the protein content and SDS-SV. Thus, the

SSV can be a substantially constant value within the same wheat variety.

Selection of the 1EL-specific PLUG markers

To detect chromosome 1E, we used 121 wheat PLUG markers located on various bins of chromosomes 1A, 1B, and 1D, and include the 64 multilocal markers described by Ishikawa *et al*. (2007). The total number of marker locations was 219 (74 on chromosome 1A, 68 on 1B, and 77 on 1D) (**Supplemental Table 1**).

PCR amplification of the wheat PLUG markers was performed using *TaKaRa Ex Taq*® DNA polymerase (0.5 U, Takara Bio Inc., Japan) in 25 µl of reaction buffer containing 1.5 mM $MgCl_2$ (Takara Bio Inc., Japan), 50–100 ng of genomic DNA, 200 µM of each dNTP, and 10 pmol of each primer. The PCR conditions were 95°C for 5 min; followed by 32 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 2 min; with a final extension time of 7 min at 72°C. PCRs were performed using a C1000™ Thermal Cycler (Bio-Rad). An 8-µl aliquot of the PCR mixture was separated by agarose gel (1% [w/v]) electrophoresis. For PCR-restriction fragment length polymorphism (PCR-RFLP) analysis, an 8-µl aliquot of the product was digested overnight with 1 U of DpnII, HaeIII, or RsaI at 37°C or TaqI at 65°C. Digested fragments were separated by agarose gel (4% [w/v]) electrophoresis. Agarose gels were stained with ethidium bromide and visualized under UV light.

Results

Production and identification of a wheat–Th. elongatum Robertsonian translocation line

Centric fission of univalents in meiosis, followed by subsequent fusions, frequently produce centric translocations that have the breakpoint at the centromere. Such chromosome aberrations are referred to as Robertsonian translocations. Robertsonian translocations that involve the long arm and short arm (e.g., T1AS.1BL and T1BS.1AL) genetically compensate the function of the group-1 chromosome, whereas Robertsonian translocations that involve T1AS.1BS and T1AL.1BL are genetically abnormal.

Fig. 1 shows the crossing scheme used to produce the T1AS.1EL line. To induce a Robertsonian translocation consisting of 1EL and the short arm of wheat chromosome 1A (1AS), we first crossed CSDSL1E(1D) with N1AT1D. The resulting F_1 plants were monosomic for wheat chromosome 1A, as well as for the *Th. elongatum* chromosome 1E (2n = 42 = 20" + 2', AABBDD-1A + 1E). We then crossed this plant with N61, where we would expect the appearance of the compensate Robertsonian translocation chromosome T1AS.1EL in the progeny. In the resulting hybrids, we used protein composition analysis to detect the HMW-GSs encoded on the long arm and the absence of gliadins encoded on the short arm from *Th. elongatum* chromosome 1E (Garg *et al*. 2009a). We obtained self-pollinated seeds from candidate T1AS.1EL plants and twice backcrossed these with N61.

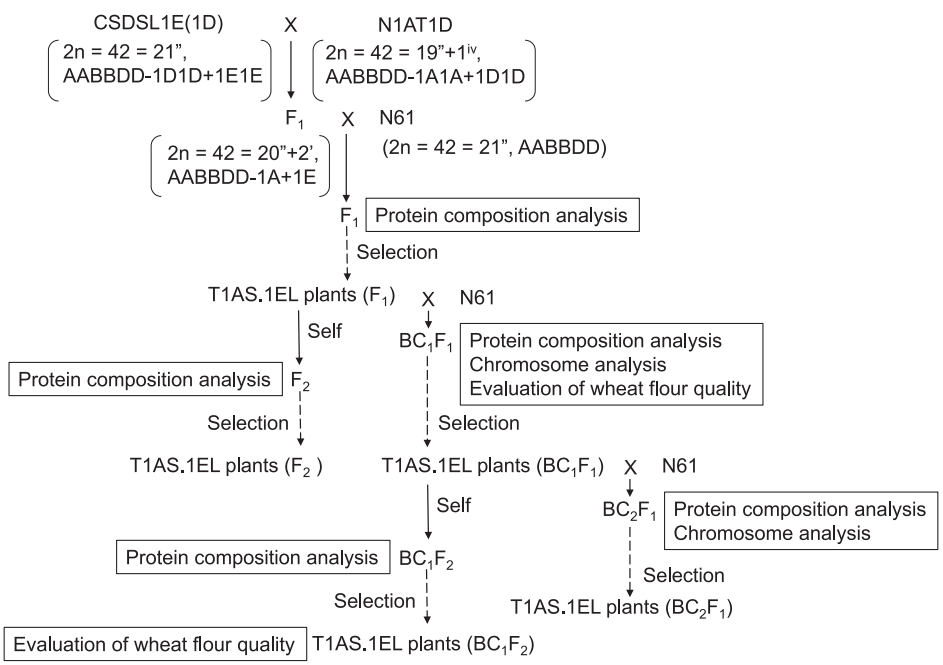


Fig. 1. Crossing scheme for producing the T1AS.1EL line. The wheat varieties used or generated here are as follows: CSDSL1E(1D), a disomic substitution line of *Th. elongatum* chromosome 1E for wheat chromosome 1D of Chinese Spring (Garg *et al*. 2009a); N1AT1D, a nullisomic-1A tetrasomic-1D line of Chinese Spring (Sears 1966); N61, Norin 61; and T1AS.1EL, Robertsonian translocation plants with the chromosome consisting of the long arm of *Th. elongatum* chromosome 1E and the short arm of wheat chromosome 1A. T1AS.1EL plants were selected by using protein composition and chromosome analyses.

Identification of HMW-GSs from Th. elongatum and confirmation of Robertsonian translocation

In an initial SDS-PAGE analysis, following the method of Tanaka *et al*. (2003), we detected one addition of an HMW-GS band (above the 1By8 subunit) from CSDAL1E that is found in neither CS nor N61 (**Fig. 2A**). However, because one HMW-GS locus generally include two tightly linked genes (expressing x- and y-types) on the long arm of group-1 chromosome, we performed further analyses to better discriminate HMW-GSs. Following the method of Smith and Payne (1984), we successfully resolved two additional HMW-GS bands not found in either CS or N61, one slow-moving x-type band below the 1Bx7 subunit and one fast-moving y-type within the ω-gliadin fraction (**Fig. 2B**). Hereafter, we refer to these bands as 1Ex and 1Ey from *Glu-E1* locus, respectively. The remaining HMW-GS bands of the CSDAL1E plants were the same as those of the CS genetic background.

By SDS-PAGE of F_2, BC_1F_1, and BC_2F_1 extracts, we demonstrated that the 1Ex and 1Ey HMW-GSs are completely linked (**Fig. 3**). How the F_2, BC_1F_1 and BC_2F_1 extracts segregated for the presence and absence of the 1EL-derived HMW-GSs is shown in **Table 1**. Among the tested F_2 plants, 22 of 40 (55%) carried the 1EL-derived HMW-GSs (**Table 1**, **Fig. 4**), which better fits a 1:1 than 3:1 ratio for the presence or absence of the 1EL-derived HMW-GSs. Furthermore, almost all tested F_2 plants (37 of 40, 93%) carried the 1Ax2* subunit from the long arm of wheat chromosome 1A (1AL) (**Fig. 4**). These data suggest that, during fertilization, the chromosome T1AS.1EL is more difficult to transmit from pollen to the egg cell than is wheat chromosome 1A.

Among the tested BC_1F_1 plants, 15 of 36 (42%) carried the 1EL-derived HMW-GSs. Among the tested BC_2F_1

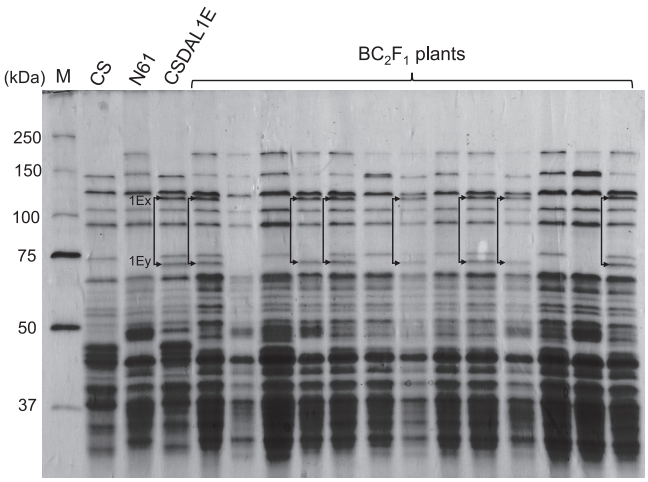


Fig. 3. SDS-PAGE profile of seed storage proteins from BC_2F_1 plants. The lanes of the polyacrylamide gels were loaded with: M, Precision Plus Protein™ Standards (Bio-Rad Laboratories, Inc., USA) or extracts of CS (Chinese Spring), N61 (Norin 61), or CSDAL1E (a homoeologous group-1 disomic addition line of *Th. elongatum* in CS background). The two subunits from the long arm of *Th. elongatum* chromosome 1E are referred to as 1Ex and 1Ey. The gels used for SDS-PAGE consisted of 18% acrylamide and 0.46% N N' bisacrylamide.

Table 1. The segregation ratios of HMW-GSs from the 1EL in each generation

Generation	HMW-GSs from the 1EL			Expected ratio Presence:Absence	χ^2	P
	Number of presence	Number of absence	Total			
F_2	22	18	40	3:1	3.52	0.06
				1:1	0.20	0.65
BC_1F_1	15	21	36	1:1	0.50	0.48
BC_2F_1	39	33	72	1:1	0.25	0.62

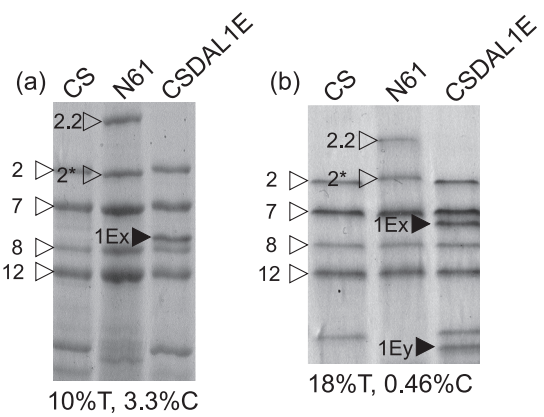


Fig. 2. SDS-PAGE reveals improved protein profiles of HMW-GSs from *Thinopyrum elongatum*. The plants tested were: CS, Chinese Spring; N61, Norin 61; and CSDAL1E, a homoeologous group-1 disomic addition line of *Th. elongatum* in CS background. The open arrowheads indicate HMW-GSs from CS or N61. The subunit numbers of HMW-GSs are also indicated adjacent the open arrowheads. The closed arrowheads indicate HMW-GS from *Th. elongatum*. The x- and y-type subunit of *Th. elongatum* are referred to as 1Ex and 1Ey, respectively. The gels used for SDS-PAGE consisted of 10% acrylamide and 3.3% N N' bisacrylamide (a) or 18% acrylamide and 0.46% N N' bisacrylamide (b).

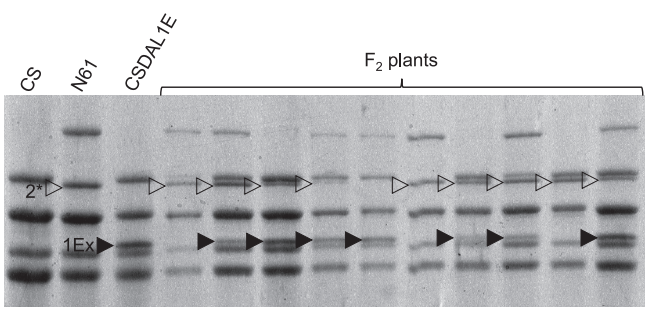


Fig. 4. SDS-PAGE profile of seed storage proteins in F_2 plants. The lanes of the polyacrylamide gels were loaded with: extracts of CS (Chinese Spring), N61 (Norin 61), or CSDAL1E (a homoeologous group-1 disomic addition line of *Th. elongatum* in CS background). The open arrowheads indicate HMW-GS 2* from N61. The closed arrowheads indicate HMW-GS 1Ex from *Th. elongatum*. The gels used for SDS-PAGE consisted of 10% acrylamide and 3.3% N N' bisacrylamide.

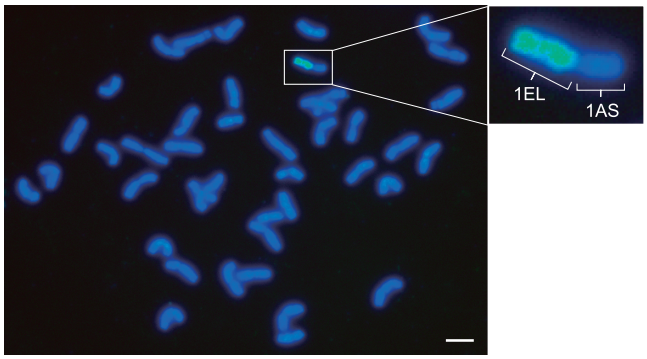
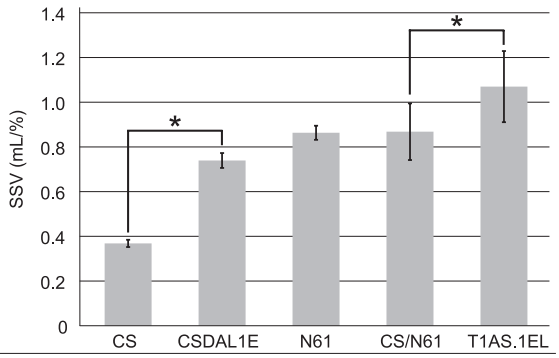


Fig. 5. Cytological analysis of the Robertsonian translocation plants. Genomic *in situ* hybridization performed using genomic DNA of *Thinopyrum elongatum* (green), indicated the presence of the *Th. elongatum* chromosome in the Robertsonian translocation plants. One wheat–*Th. elongatum* Robertsonian translocated chromosome is shown within the rectangle. DNA was counterstained with DAPI (blue). Scale bar = 10 μm.



	CS	CSDAL1E	N61	CS/N61	T1AS.1EL
Number of plants	4	3	3	15	18
PC (%) ± SD	11.63±0.07	13.89±0.09	12.82±0.09	11.76±0.31	12.01±0.55
SDS-SV (mL) ± SD	4.24±0.18	10.27±0.46	11.07±0.40	10.20±0.54	12.79±0.20

Fig. 6. The specific sedimentation values of plants with or without the HMW-GSs from *Th. elongatum*. The plants tested were: CS, Chinese Spring; N61, Norin 61; CSDAL1E, a homoeologous group-1 disomic addition line of *Th. elongatum* in CS background; TAS1.1EL, Robertsonian translocation plants (BC_1F_1) with the long arm of *Th. elongatum* chromosome 1E (1EL) in the CS and N61 background; and CS/N61, BC_1F_1 plants lacking 1EL in the CS and N61 background. Whiskers show standard deviation. Asterisks indicate a significant difference ($p < 0.05$). PC indicates the mean value of the protein content and SDS-SV is the mean value of the SDS sedimentation volume. SSV is the mean value of the specific sedimentation value, and SD is the standard deviation (whiskers).

plants 39 of 72 (54%) carried the 1EL-derived HMW-GSs. Each of these segregation ratios fit a 1:1 ratio, suggesting that the HMW-GS encoding 1EL genes translocate to 1AS and are stably transmitted to the next generation during backcrossing to the recurrent parent, N61.

To confirm the translocation of 1EL to 1AS, those BC_1F_1 and BC_2F_1 plants from which we detected the 1EL-derived HMW-GSs were selected for genomic *in situ* hybridization (GISH) analysis: all of these selected plants carried the chromosome 1EL and had undergone the wheat–*Th. elongatum* Robertsonian translocation (**Fig. 5**).

Evaluation of flour quality of wheat with HMW-GSs from the 1EL

Next, we evaluated the flour quality of the BC_1F_1 plants and their parents, because BC_1F_1 carrying the chromosome T1AS.1EL is the earliest generation close to the genetic background of N61 (**Fig. 6**). If we develop and use a near-isogenic line and/or a recombinant inbred line in the future, we can evaluate the flour quality in detail. In this study, we detected a high SSV by 1Ex and 1Ey HMW-GSs even in this early generation as follows. The mean SSV of CSDAL1E plants (carrying the 1EL-derived HMW-GSs) was significantly higher than that of CS (which lacks those HMW-GSs). These findings are consistent with previous work done by our group (Garg *et al*. 2009a). The CS/N61 plants are equivalent to T1AS.1EL but without 1EL. When comparing the SSVs of wheat flour from the T1AS.1EL (BC_1F_1) and CS/N61 plants, we detected a higher SSV for the T1AS.1EL (BC_1F_1) plants. In next growth season of wheat, we also confirmed a high SSV (mL/%) = 1.24 ± 0.0083 [protein content (%) = 8.04 ± 0.15] for the T1AS.1EL (BC_1F_2) plants, whose HMW-GSs composition were 1Ex + 1Ey, 7 + 8 and 2.2 + 12 from *Glu-E1*, *Glu-B1* and *Glu-D1* loci, respectively.

Selection and assignment of wheat PLUG markers to the chromosome 1E of Th. elongatum

In the PCR products of eight (3.7%) of the 219 marker locations, we detected band(s) specific for CSDAL1E. In the PCR products of two that was not identified the marker locations, we also detected band(s) specific for CSDAL1E. By PCR-RFLP analysis, we identified 49 (22.4%) additional marker locations displaying polymorphism between the common wheat cultivars (CS and N61) and CSDAL1E: 14, 7, 17 and 12 by DpnII, HaeIII, RsaI, and TaqI digestion, respectively, including one by either HaeIII or TaqI digestion. Thus, we identified 33 (of 121) PLUG markers (at 57 of 219 marker locations, and at two that was not identified the marker locations) that can be used as specific markers for *Th. elongatum* chromosome 1E (**Table 2**, **Supplemental Table 1**). Seventeen of the 74 marker locations (23.0%) are derived from wheat chromosome 1A, 21 of 68 (30.9%) from wheat chromosome 1B, and 19 of 77 (24.7%) from wheat chromosome 1D (**Fig. 7**, **Supplemental Table 1**). Furthermore, we assigned each of the 33 PLUG markers to either the short arm of *Th. elongatum* chromosome 1E (1ES) or 1EL by using the T1AS.1EL disomic plant, which was obtained from the F_2 generation (**Fig. 1**, **Table 2**, **Supplemental Table 1**). When a specific PCR product or unique restriction pattern of a PLUG marker was detected in the T1AS.1EL disomic plant, the PLUG marker was assigned to 1EL, whereas, when a specific PCR product or unique restriction pattern was absent from the T1AS.1EL disomic plant, the PLUG marker was assigned to 1ES

Table 2. Assignment of the wheat homoeologous group-1 PLUG markers to *Th. elongatum* chromosome 1E

Marker name[a]	PCR Primer		Detection[a]	Approximate size (bp) of *Th. elongatum* chromosome 1E-specific band(s)	Chromosome arm assigned
	Forward	Reverse			
TNAC1009	CGAACGTGACCATCTACATCA	CATCTGACTTGGTCTTGGCATA	H	350, 500, 900	1ES
TNAC1010	GATGCAACTGCAGGAATGAAG	TCTCTTCTGAAGCGGTCATGT	T	500	1ES
TNAC1035	TGCACTGGGATCTAACCTAAA	TCCAGTGATCATTTGAAGATTCC	T	750	1EL
TNAC1041	TCACCACCTCTTTCAGTTGCT	GCATCAAGGATGAGGAGTCTG	H or T	230, 250 or 600, 700	1EL
TNAC1048	ACTGAGGTAGAATCGCCACTG	GCCGCTATCGTCTGGTACAT	H	230, 500	1EL
TNAC1063	AGCCATTCACAGCTCTTCTTG	AATATGCTTCCTGGAGTCACG	T	600	1ES
TNAC1076	GGGAGACGATCCTCTTATGATCT	TGCGTGCTTCCTAACTTACCA	T	800	1EL
TNAC1085	CCAGGCCACATGATAACATTC	TCATGGTATTCTGCTCCTCCA	ND	900	1EL
TNAC1088	GGAATCCTTCCTTGTTGAAGA	AACCTCCGAGTGAAACACAAA	H	500	1EL
TNAC4240	ATGCCAGTGACAGTGTGTGC	CCACATTGATGGATTCCTTTG	D	450, 500	1ES
TNAC4242	GGACTGGCAGGAACAAGAAG	CAGGTGGGGCACTTCCTC	R	180, 300	1ES
TNAC4272	ACCAAGGCATTCAAGAAGGA	GGTGTTCCCTGGTGTTGACT	R	500	1ES
TNAC4275	CTTCGGCCGCTTCTTCTT	ACGATCACCAGGTTGGTCTC	D	50	1ES
TNAC4289	TGATGGGCAAGTATGGTGAA	AACATAACGGGCAAATGGAA	R	250, 300	1ES
TNAC4291	CTCACGTTCAAGACTCACATGAT	GCAGAGCTTCATTATACTGTCCA	D	330, 400	1ES
TNAC4292	TGAGGTGCTTGCTTTCAAGA	AAGTGCCATCAGCTTTTGCT	R	480	1ES
TNAC4293	GTCCGGCTGTTCAAGATGAT	AGCCAGTTCAGCTTCCTCAT	D	600, 700, 800	1ES
TNAC4349	GGCAAATGTTATGGCTGCAT	ACAGGGAACTGAATGGCAAC	R	450	1EL
TNAC4374	CCATTCACCATGGTCTTTCC	TTCAGCAGCTCATCGACTTC	R	250	1EL
TNAC4375	GGCAGTGTTGGTGACTGAAG	TGCATCCCTGTTGGGTTTAT	R	350, 400	1EL
TNAC4394	CGAACAGCAATTACTGGAGAGA	GTGAGCCCACTTCTGAGGAA	R	380	1ES
TNAC4401	AACGTTATCATGGGCCTGAG	AGCTCCTTCCCAACGACTTT	ND or D	800 or 700, 800	1EL
TNAC4440	CTGTGCCTCTCCGACAGC	ACTTCCGAATGCATCACAAA	ND	700, 900	1EL
TNAC4441	TCACGAGTGTGGGTTACCTG	AGCCAATTTCTCTGCCACAC	D	480	1EL
TNAC4469	CTGAAGTTGAGGTTGGCAAAA	GCGCTATCTTCACCACGTCT	R	230	1EL
TNAC4474	TTCCCAAAAAGACTGGTTATCAA	TGTCAACAACCTTGGCAAAT	D	450	1EL
TNAC4495	TGAAGGAGTTCTTGGTTGAGC	AGAATGCAGCGAAGAGGAGA	R	480	1EL
TNAC4520	AGCTGAAGGCTGTTTTCCAC	TCACATCGAGCAGCTTGAAA	ND or R	800, 900 or 380	1EL
TNAC4562	GCTGTGGTGCGTCCACTT	CAACGGCATGAAGAATCAAA	ND	1200	1EL
TNAC4585	TCACGCAATACTCCTTTGCTT	GCCATAGCTAGCGTGTGAGAC	R	100, 150	1EL
TNAC4589	TCGCCTGATGACAGTGCTAT	CACAAGGTGACCAACCAACA	D or R	230 or 250, 500	1EL
TNAC4596	GAGGACACTCTGGGCATCAT	CAACATTGTCCCGCAGTATG	ND or D	700 or 200	1EL
TNAC4598	GAGGCAGGAGCAGGAGTG	CCTCATGGTTGACGACCTTT	ND	900	1ES

[a] ND, D, H, R, and T indicate non-digest, DpnII, HaeIII, RsaI and TaqI digestion, respectively.

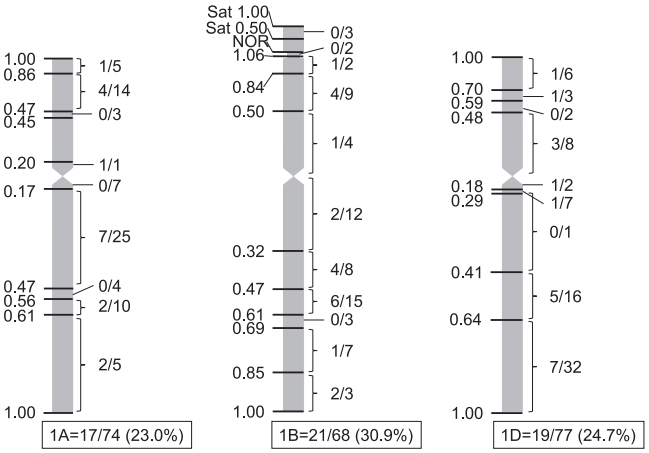


Fig. 7. The number and location of the wheat homoeologous group-1 PLUG markers assigned to *Th. elongatum* chromosome 1E. The fraction length (FL) value is indicated on the left of each chromosome. The FL identifies the position of the breakpoint from the centromere relative to the length of the complete arm. How FL values are calculated is described in detail by Endo and Gill (1996). The number of markers assigned to the *Th. elongatum* chromosome 1E/the total number of markers used in this study is indicated on the right side of each chromosome.

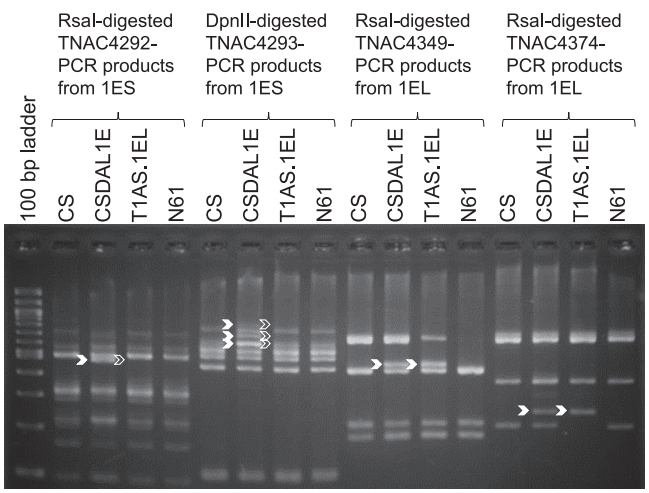


Fig. 8. Representative examples of the PCR analyses used to assign the wheat homoeologous group-1 PLUG markers to the *Th. elongatum* chromosome 1E. The plants tested were: CS, Chinese Spring; N61, Norin 61; CSDAL1E, a homoeologous group-1 disomic addition line of *Th. elongatum* in CS background; and TAS1.1EL, the TAS1.1EL disomic plant in the CS and N61 background. The open and closed arrowheads indicate the presence or absence of the PCR product on chromosome 1E, respectively.

(**Fig. 8**). This resulted in 20 of 33 PLUG markers being assigned to 1EL. Nineteen of 20 PLUG markers were also localized to the long arm of wheat group-1 chromosomes.

Discussion

Here, we report the development of a novel T1AS.1EL line by centric translocation, verified by HMW-GS, chromosome analysis, and molecular marker assays. We also evaluated the flour quality of the T1AS.1EL line. Among spontaneous translocation types, centric fusion (also referred to as Robertsonian translocation) is most common. In double monosomic plants, the desired compensating wheat–alien Robertsonian translocations are known to occur at fairly high frequencies, ranging from 4% to almost 20%, depending on the chromosomes involved (Davies *et al*. 1985, Lukaszewski 1993, 1994, 1997, Lukaszewski and Gustafson 1983, Marais and Marais 1994). In wheat improvement, the T1BL.1RS and T1AL.1RS Robertsonian translocations are most often used (Mettin *et al*. 1973, Zeller 1973). In these cases, genes conferring disease resistance are frequently clustered in the genome; therefore, Robertsonian translocations often confer multiple desirable resistance traits. In the case of SSPs, glutenins and gliadins also exist as multigene families on the homoeologous group-1 and -6 chromosomes, respectively. Thus, Robertsonian translocation is likely an effective strategy for introducing the clustered SSP genes from wheat wild relatives into common wheat.

Previously, we reported that the presence of the fraction length (FL) = 0.61–1.00 region of 1AL, which contains the *Glu-A1c* gene, significantly decreased the SSV of wheat flour (Tanaka and Tsujimoto 2012). In T1AS.1EL plants, the absence of 1AL is compensated for by the 1EL component. Therefore, we successfully introduced 1EL into the Japanese commercial cultivar N61. Our data suggest that the translocated 1EL is stably transmitted to progeny. We also confirmed that the T1AS.1EL line retains the high SSV associated with the 1EL-derived HMW-GSs, despite a high standard deviation in the T1AS.1EL (BC_1F_1) data, which we attribute to the segregation of other seed storage proteins in the BC_1F_1 generation as follows. The T1AS.1EL (BC_1F_1) and CS/N61 plants carried 2*/1Ex + 1Ey and 2* from *Glu-A1*/*Glu-E1* and *Glu-A1* loci, respectively. The other HMW-GS compositions in both of BC_1F_1 plants were 7 + 8 and 2 + 12/2.2 + 12 from *Glu-B1* and *Glu-D1*/*Glu-D1* loci, respectively. Therefore, the presence of 1Ex + 1Ey in wheat flour might be responsible for the higher SSV for the T1AS.1EL (BC_1F_1) plants. Furthermore, the protein contents tended to increase in plants carrying the chromosome 1E (**Fig. 6**). Since wheat flour with a high protein content produces strong dough, it is a trait required for bread-making. Generally, the protein content and yield are negatively correlated. In this study, although we did not investigate the yield, if the presence of the T1AS.1EL chromosome does not effect on the yield, this chromosome might have a useful gene associated with the high protein content derived from 1E chromosome. The gene may not exist in common wheat, and could lead to the expansion of the genetic diversity in common wheat using the wild relative. Thus, we propose that the T1AS.1EL line developed here will facilitate breeding of wheat varieties with a positive effect on flour quality, although we need to analyze more detailed wheat flour quality, such as bread-making tests of this line.

Also, we describe a set of molecular markers that can be used to expedite the screening of large numbers of wheat progeny carrying the chromosome T1AS.1EL. Previously, our group investigated the applicability of 1165 barley expressed sequence tag (EST) primer sets to amplify markers capable of revealing polymorphisms between wheat and ten alien species, covering a wide range of variation in Triticeae (Hagras *et al*. 2005). In the case of *Th. elongatum*, only 78 (6.7%) of these markers showed polymorphisms with wheat. Also, based on the co-amplification frequency, we demonstrated that *Th. elongatum* is more closely related to wheat than to barley; therefore, the transferability of the barley EST primer sets for *Th. elongatum* was low. Thus, in this study, we used wheat PLUG markers (Ishikawa *et al*. 2007), which are better suited to *Th. elongatum* than the barley EST primer sets. Indeed, we found that the wheat PLUG markers had better transferability to *Th. elongatum* than the barley EST primer sets (27.3% *vs*. 6.7%).

We also found almost identical syntenic relationships between the chromosome 1E and wheat group-1 chromosomes: 19 of the 20 PLUG markers assigned to 1EL were also localized to the long arm of wheat group-1 chromosomes. Based on these findings, together with previous data from our group showing that the *Glu-A1*, *Glu-B1* and *Glu-D1* loci are located on FL = 0.61–1.00, 0.69–0.85, and 0.64–1.00 of the long arm of chromosome 1A, 1B and 1D, respectively, we propose that the 1Ex and 1Ey subunit encoding genes are likely located on the distal region of 1EL (Tanaka and Tsujimoto 2012).

We assigned two PLUG markers (TNAC1035 and TNAC4469) to FL = 0.61–1.00 of 1AL. TNAC1035 is also located on FL = 0.47–0.61 and 0.64–1.00 of the long arm of wheat chromosomes 1B (1BL) and 1D, respectively. The FL = 0.47–0.61 region of 1BL is closer to the centromere than to the *Glu-B1* locus (**Supplemental Table 1**, Tanaka and Tsujimoto 2012). TNAC4469 is also located on the FL = 0.85–1.00 region of 1BL, which is more terminal than is the *Glu-B1* locus (**Supplemental Table 1**, Tanaka and Tsujimoto 2012). Therefore, on 1EL, TNAC1035 and TNAC4469 might also be located on regions proximal and distal of the 1Ex and 1Ey subunit encoding genes, respectively; therefore, TNAC1035 and TNAC4469 might be useful for introducing the 1Ex and 1Ey subunit encoding genes into commercial common wheat cultivars.

In summary, here we describe a novel wheat line that is likely to be useful for breeding programs aimed at improving flour quality. Although we have not yet identified genes carried by 1EL that negatively affect wheat agronomic traits, if such harmful genes are later identified, these will need to be removed by shortening the *Th. elongatum* segment of the T1AS.1EL chromosome by *ph1b*-induced homoeologous recombination. The PLUG markers that we have assigned to 1EL will be useful for detecting the chromosome regions not only removed the harmful genes, but introduced valuable genes, such as the high protein content genes by future chromosome engineering of these wheat lines.

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