Introduction

There is little genetic diversity in many major crop species. Such narrow genetic diversity is attributed to domestication, with a subsequent bottleneeking 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). Therefore, 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 homoeologous group-1 chromosomes 1A, 1B, and 1D.

Research Paper

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

Hiroyuki Tanaka*1), Chisato Nabeuchi1), Misaki Kurogaki1), Monika Garg2), Mika Saito3), Goro Ishikawa3,4), Toshiki Nakamura3) and Hisashi Tsujimoto5)

1) Laboratory of Plant Genetics, Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan
2) National Agri-food Biotechnology Institute, Punjab 160-071, India
3) NARO Tohoku National Agriculture Research Center, Iwate 020-0198, Japan
4) NARO Institute of Crop Science, Tsukuba, Ibaraki 305-8518, Japan
5) Laboratory of Molecular Breeding, Arid Land Research Center, Tottori University, Tottori 680-0001, Japan

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.

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*Corresponding author (e-mail: htanaka@muses.tottori-u.ac.jp)
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 domesticated 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”, AABBDD-1A1A + 1D1D) (Sears 1966); the chromosome 1E disomic addition line of Th. elongatum in the CS genetic background (CSDLAL1E, 2n = 44 = 22”, AABBDD + 1E1E) (Dvořák and Knott 1974); and a disomic substitution line of chromosome 1E for chromosome 1D of CS [CSDL1E(1D), 2n = 42 = 21”, AABBDD-1D1D + 1E1E] (Garg et al. 2009a). CSDLAL1E 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, %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

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**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 F₁ plants were monosomic for wheat chromosome 1A, as well as for the Th. elongatum chromosome 1E (2n = 42 = 20⁺1', 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.

**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 MgCl₂ (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 Taq 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.

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 α-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 F2, BC1F1, and BC2F1 extracts, we demonstrated that the 1Ex and 1Ey HMW-GSs are completely linked (Fig. 3). How the F2, BC1F1 and BC2F1 extracts segregated for the presence and absence of the 1EL-derived HMW-GSs is shown in Table 1. Among the tested F2 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 F2 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 BC1F1 plants, 15 of 36 (42%) carried the 1EL-derived HMW-GSs. Among the tested BC2F1...
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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.

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 BC1F1 and BC2F1 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 BC1F1 plants and their parents, because BC1F1 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 CSDLAL1E 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 (Garg et al. 2009a). The CS/N61 plants, BC1F1 carrying the chromosome 1EL (1ES) or 1EL by using the T1AS.1EL disomic plant, which was obtained from the F2 generation (Fig. 1, Table 2, Supplemental Table 1). Seventeen of the 74 marker locations (23.0%) are marker locations displaying polymorphism between the common wheat cultivars (CS and N61) and CSDLAL1E: 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). 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 F2 generation, whose HMW-GSs 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 CSDLAL1E. In the PCR products of two that was not identified the marker locations, we also detected band(s) specific for CSDLAL1E. By PCR-RFLP analysis, we identified 49 (22.4%) additional marker locations displaying polymorphism between the common wheat cultivars (CS and N61) and CSDLAL1E: 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 F2 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.
| Marker name | PCR Primer | Detection | Approximate size (bp) of Th. elongatum chromosome 1E-specific band(s) | Chromosome arm assigned |
|-------------|------------|-----------|-------------------------------------------------|------------------------|
| TNAC1009    | CGAACGTGACCATCTACATCA | H | 350, 500, 900 | 1ES                     |
| TNAC1010    | GATGCAACTGCAGGAATGAAG | H | 500 | 1ES                     |
| TNAC1035    | TGCACTGGGATCTAACCTAAA | T | 750 | 1EL                     |
| TNAC1041    | TCACCACCTCTTTCAGTTGCT | T | 230, 250 or 600, 700 | 1EL                     |
| TNAC1048    | ACTGAGGTAGAATCGCCACTG | H | 230, 500 | 1EL                     |
| TNAC1063    | AGCCATTCACAGCTCTTCTTG | T | 600 | 1ES                     |
| TNAC1076    | GGGAGACGATCCTCTTATGATCT | T | 800 | 1EL                     |
| TNAC1085    | CCAGGCCACATGATAACATTC | ND | 900 | 1EL                     |
| TNAC1088    | GGAATCCTTCCTTGTTGAAGA | H | 500 | 1EL                     |
| TNAC4240    | ATGCCAGTGACAGTGTGTGC | D | 450, 500 | 1ES                     |
| TNAC4242    | GGACTGGCAGGAACAAGAAG | C | 180, 300 | 1ES                     |
| TNAC4272    | ACCAACGACAGAGAAGGAA | G | 500 | 1ES                     |
| TNAC4275    | CTGCGCCAGGGAAAGAAGA | D | 50 | 1ES                     |
| TNAC4289    | TGATGGGCAAGTATGGTGAA | R | 250, 300 | 1ES                     |
| TNAC4291    | CTCACGTTCAAGACTCACATG | D | 330, 400 | 1ES                     |
| TNAC4292    | TGAGGTGCTTGCTTTCAAGA | A | 480 | 1ES                     |
| TNAC4293    | GTCCGGCTGTTCAAGATGAT | A | 600, 700, 800 | 1ES                     |
| TNAC4349    | CGAACAGCAATTACTGGAGAGA | R | 380 | 1ES                     |
| TNAC4374    | AACGTTATCATGGGCCTGAGA | ND | 800 or 700, 800 | 1EL                     |
| TNAC4375    | CTGTGCCTCTCCGACAGC | ND | 700, 900 | 1EL                     |
| TNAC4376    | GTGTCGCTGTCTCTCAT | ND | 500, 900 | 1EL                     |
| TNAC4394    | CGAACAGCAATTACTGGAGAGA | R | 380 | 1ES                     |
| TNAC4401    | AGCTGAAGGCTGTTTTCCAC | ND | 300 | 1EL                     |
| TNAC4440    | CACACCAATGGGATGCTTTC | ND | 700 | 1EL                     |
| TNAC4441    | GCTGTGGTGCGTCCACTT | ND | 900 | 1ES                     |
| TNAC4452    | TCACGCAATACTCCTTTGCTT | R | 100, 150 | 1EL                     |
| TNAC4474    | AGGGAACACTCTGGGCATCAT | ND or D | 700 or 200 | 1EL                     |
| TNAC4495    | GAGGCAGGAGCAGGAGTGC | ND | 900 | 1ES                     |
| TNAC4520    | GATCCGAGACATATGCTTCA | ND | 700 | 1EL                     |
| TNAC4562    | AGGACACTACAGAGACAGAC | ND | 900 | 1ES                     |
| TNAC4589    | GAGGAGCACACGAGAGATG | ND | 700 | 1EL                     |

*ND, D, H, R, and T indicate non-digest, DpnII, HaeIII, RsaI and TaqI digestion, respectively.*
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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, Łukaszewski 1993, 1994, 1997, Łukaszewski 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 as associated with the 1EL-derived HMW-GSs, despite a high standard deviation in the T1AS.1EL (BC1F 1) data, which we attribute to the segregation of other seed storage proteins in the BC1F 1 generation as follows. The T1AS.1EL (BC1F 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 BC1F 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 (BC1F 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.

Discussion

Here, we report the development of a novel T1AS.1EL line

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.
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 1E-L. 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 Triticaceae (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 1E-L 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 1E-L chromosome by *phib*-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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