Registration of a hard red winter wheat genetic stock homozygous for ph1b for facilitating alien introgression for crop improvement

Bernd Friebe, Lili Qi, Cheng Liu, Wenxuan Liu, and Bikram S. Gill

Published Version Information

Citation: Friebe, B., Qi, L., Liu, C., Liu, W., & Gill, B. S. (2012). Registration of a hard red winter wheat genetic stock homozygous for ph1b for facilitating alien introgression for crop improvement. Journal of Plant Registrations, 6(1), 121-123.

Digital Object Identifier (DOI): doi:10.3198/jpr2011.05.0273crgs

Publisher's Link: https://www.crops.org/publications/jpr/abstracts/6/1/121

CROP SCIENCE

Registration of a hard red winter wheat genetic stock homozygous for *ph1b* for facilitating alien introgression for crop improvement

Journal:	*Crop Science*
Manuscript ID:	Draft
Manuscript Type:	11. Crop Registration-Genetic Stock
Divisions:	C1 crop breeding & genetics
Date Submitted by the Author:	n/a
Complete List of Authors:	Friebe, Bernd; Kansas State University, Department Plant Pathology, Wheat Genetic and Genomic Resources Center Qi, Lili; USDA-ARS, Norther Crop Science Laboratory Liu, Cheng; University of Electronic Science and Technology, School of Life Science and Technology Liu, Wenxuan; Henan Agricultural University, Laboratory of Cell and Chromosome Engineering, College of Life Sciences Gill, Bikram; Kansas State University, Department Plant Pathology, Wheat Genetic and Genomic Resources Center
Keywords:	Crop cytology, Crop genetics, Wheat

Registration of a hard red winter wheat genetic stock homozygous for *ph1b* for facilitating alien introgression for crop improvement

Bernd Friebe, Lili Qi, Cheng Liu, Wenxuan Liu, and Bikram S. Gill

Bernd Friebe, Bikram S. Gill, Wheat Genetic and Genomic Resources Center and Department of Plant Pathology, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS 66506-5502, USA;

Lili Qi, USDA-ARS, Northern Crop Science Laboratory, 1605 Albrecht Blvd N, Fargo, ND 58102-2765, USA;

Cheng Liu, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, Sichuan 610054, PR China;

Wenxuan Liu, Laboratory of Cell and Chromosome Engineering, College of Life Sciences, Henan Agricultural University, Zhengzhou, Henan 450002, PR China.

5585 Guilford Rd., Madison WI 53711

ABSTRACT

Wild relatives of bread wheat, *Triticum aestivum* L. are an important source for disease and pest resistance that can be exploited in wheat improvement. However, in wheat/alien species hybrids the pairing homoeologous gene, *Ph1*, suppresses the pairing and recombination of wheat and alien chromosomes and, thus, no alien genetic transfer can occur. However, in plants nullisomic for the *Ph1* gene, and in the *ph1b* mutant stock, having a large deletion at the *Ph1* locus, homoeologous wheat and alien chromosomes can pair and recombine. The original *ph1b* mutant stock is in Chinese Spring background, which has poor agronomic characteristics and several backcrosses with adapted wheat cultivars are necessary before the agronomic performance of the recombinants can be evaluated. The present report describes the transfer and characterization of the *ph1b* mutant allele into adapted Kansas winter wheat, which will accelerate the evaluation and utilization of wheat alien recombinants in cultivar improvement.

Interspecific hybridization and chromosome engineering have played an important role in wheat crop improvement (Friebe et al. 1996). The diploid-like chromosome pairing behavior of hexaploid wheat, *Triticum aestivum* L. (2n=6x=42, AABBDD) (Riley and Chapman, 1958; Sears and Okamoto, 1958) and the tetraploid wheat species *T. turgidum* (2n=4x=28, AABB) and *T. timopheevii* (2n=4x=28, AAGG) (Dhaliwal 1977; Giorgi 1978) is controlled by *Ph1*, a pairing homoeologous gene. In wheat/alien species hybrids containing genomes other than A, B, D or G, *Ph1* gene suppresses pairing of alien

5585 Guilford Rd., Madison WI 53711

chromosomes with wheat chromosomes. As a practical consequence, no alien genetic transfers can occur in the presence of *Ph1* gene. However, in plants nullisomic for *Ph1* gene (Riley and Chapman 1958) or in hybrids containing genes that are epistatic to *Ph1* gene (Dover and Riley, 1972; Dvorak et al. 2006), homoeologous chromosomes can pair and recombine. Sears (1977) used radiation treatment to produce a *ph1b* mutant stock in Chinese Spring (CS) wheat. The CS*ph1b* mutant stock has a large deletion at the *Ph1* locus spanning about 70 Mbp (Gill and Gill, 1991; Dunfort et al., 1995). In homozygous *ph1b* plants, meiotic metaphase I pairing is no longer restricted to homologous chromosomes but also can occur among homoeologous wheat chromosomes and between homoeologous wheat and alien chromosomes. The CS*ph1b* mutant stock, has been widely used in inducing homoeologous recombination between wheat and homoeologous alien chromosomes and in wheat crop improvement (Friebe et al. 1996; Qi et al. 2007).

The original *ph1b* mutant stock (TA3809) was isolated in Chinese Spring background, an old land race from China that has poor agronomic characteristics. The Chinese Spring stocks must be used for producing desirable wheat-alien chromosome recombinants and several backcrosses with adapted wheat cultivars are necessary before the agronomic performance of the recombinants can be evaluated. Thus, it would be desirable to have the *ph1b* mutant allele in an advanced wheat background and the present report describes the transfer of *ph1b* into an adapted Kansas wheat cultivar.

Methods

5585 Guilford Rd., Madison WI 53711

Previously, several markers were reported that tag the *ph1b* deletion (Roberts et al. 1999; Segal et al. 1997). Two PCR-based markers WGP90 and PSR2120 were used to detect the homozygous *ph1b* mutant in the first two generations of F_2 and BC_1F_2. In BC_2F_2, we used a pair of barley chromosome 5H-specific STS-PCR primers ABC302.3, (forward primer: 5'-ATAAAGGAGAAGATTGAGTC-3'; reverse primer: 5'-ATAAGGAACAGGAACAGAGT-3') to identify plants that were homozygous for *ph1b* (Blake et al., 1996; Wang et al., 2002). The primers amplify two fragments in Chinese Spring (CS). The top fragment is 5B-specific and bottom one is 5A-specific (Wang et al., 2002). The top fragment of about 920 bp (designated as ABC_{920}) is the *Ph1*-specific band that is absent in the CS*ph1b* mutant stock TA3809 and 5BL deletion stocks, but 5A-specific fragment is present in both lines (Fig. 1). The 5A-fragment serves as an internal control to rule out the possibility of PCR errors leading to the missing 5B-fragment in *ph1b* mutant.

STS-PCR reactions were performed in 15 µL of reaction mixture containing 1x PCR buffer (Bioline USA Inc., Taunton, MA, USA); 2 mM $MgCl_2$, 0.25 mM dNTPs; 5 pmol forward primer and reverse primer, respectively; 0.02 unit/µl of Taq DNA polymerase (Bioline USA Inc., Taunton, MA, USA); and 90 ng of genomic DNA. Genomic DNA was isolated using a BioSprint work station following the protocol as described in the BioSprint DNA Plant Handbook (Cat. no. 941558, QIAGEN Inc., Valencia, CA, USA). PCR reaction cocktails were initially denatured at 95°C for 5 min, and then amplified 35 cycles of 1 min at 95°C, 1 min at 48°-55°C dependant primers), and 2 min at 72°C followed by a final extension reaction of 8 min at 72°C. PCR products

5585 Guilford Rd., Madison WI 53711

were resolved on 1.5% agarose gels in 1x TBE and visualized by Ethidium bromide staining under UV light.

Characteristics

The *ph1b* mutant stock TA3809 was crossed with the Kansas hard red winter wheat Overley, and the F_1 was selfed and screened by molecular markers to identify plants homozygous for *ph1b*. These plants were again crossed with Overley, selfed, and screened to identify plants homozygous for *ph1b*. Homozygous *ph1b* plants were crossed with Overley/Amadina (O/A) derived lines, selfed, and screened by molecular markers to identify plants homozygous for *ph1b*. The genotype of these plants, which are about 88% in adapted winter wheat background, were verified by the primer ABC302 (Fig. 1).

Meiotic metaphase I pairing was analyzed in some of these plants to verify that they are high pairing, and the data are summarized in Table 1. The O/A*ph1b* plants had multivalents in about 46% of their pollen mother cells, which is slightly higher than the level in the original CS*ph1b* stock (Table 1, Fig. 2). The O/Ap*h1b* stock had a reduced fertility with 38 seeds per spike compared to 54 seeds per spike of Chinese Spring wheat. The O/Aph1b stock was released as KS12WGGRC55 (TA5092).

Availability

5585 Guilford Rd., Madison WI 53711

The O/A*ph1b* mutant stock is maintained at the Wheat Genetic and Genomic Resources Center housed at the Department of Plant Pathology at Kansas State University. Seeds of KS12WGGRC55 (TA5092) are available upon request and we request that appropriate recognition to be made of the source when this genetic stock contributes to the development and release of a new germplasm, breeding line, or a cultivar.

Acknowledgment

This research was supported by grants from the Kansas Wheat Commission, the Kansas Crop Improvement Association and a special USDA-NIFA grant to Wheat Genetic and Genomic Resources Center at Kansas State University. This paper is contribution number 11-337-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, Kansas, 66506-5502.

5585 Guilford Rd., Madison WI 53711

References

Dhaliwal, H.S. 1977. The *Ph* gene and the origin of tetraploid wheats. Genetics 47:177-182.

Dvorak, J., K.R. Deal, and M.C. Luo. 2006. Discovery and mapping of wheat *Ph1* suppressors. Genetics 147:17-27.

Blake, T.K., D. Kadyrzhanova, K.W. Shepherd, A.K.M.R. Islam, D.L. Langridge, C.L. McDonald, J. Erpelding, N.K. Blake, S. Larson, and L.E. Talbert. 1996. STS-PCR markers appropriate for wheat-barley introgression. Theor. Appl. Genet. 93:826–832.

Dover, G.A., and R. Riley. 1972. Prevention of pairing of homoeologous meiotic chromosomes of wheat by an activity of supernumerary chromosomes of *Aegilops*. Nature 240:1159-161.

Dunford, R.P., N. Kurata, D.A. Laurie, T.A. Money, Y. Minobe, and G. Moore. 1995. Conservation of fine-scale DNA marker order in the genome of rice and the Triticeae. Nucleic Acids Res. 23: 2724-2728.

Friebe, B., J. Jiang, W.J. Raupp, R.A. McIntosh, and B.S. Gill. 1996. Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91:59-87.

5585 Guilford Rd., Madison WI 53711

Gill, K.S., and B.S. Gill. 1991. A DNA fragment mapped within the submicroscopic deletion of *Ph1*, a chromosome pairing regulator gene in polyploidy wheat. Genetics 129:257-259.

Giorgi, B. 1978. A homoeologous pairing mutant isolated in *Triticum durum* cv. Cappelli. Mut. Breed. Newsl. 11:4-5.

Qi, L.L., B. Friebe, P. Zhang, and B.S. Gill. 2007. Homoeologous recombination, chromosome engineering and crop improvement. Chromosome Res. 15:5-19.

Riley, R., and V. Chapman. 1958. Genetic control of cytogenetically diploid behavior in hexaploid wheat. Nature 182:713-715.

Roberts, M.A., S.M. Reader, C. Dalgliesh, T.E. Miller, T.N. Foote, L.J. Fish, J.W. Snape, and G.Moore. 1999. Induction and characterization of *Ph1* wheat mutants. Genetics 153:1909-1918.

Sears, E.R. 1977. An induced mutant with homomoeologous pairing in common wheat. Can. J. Genet. Cytol. 19:585-593.

Sears, E.R., and M. Okamoto, 1958. Intergenomic chromosome relationships in hexaploid wheat. Proc. 10th Int. Congress of Genetics, Montreal, 2:258-259.

Segal, G., B. Liu, J.M. Vega, S. Abbo, M. Rodova, and M. Feldman. 1997. Identification of a chromosome-specific probe that maps within the *Ph1* deletion in common and durum wheat. Theor. Appl. Genet. 94:968-970.

Wang, X., J. Lai, G. Liu, and F. Chen F. 2002. Development of a scar marker for the *Ph1* locus in common wheat and its application. Crop Sci. 42:1365-1368.

5585 Guilford Rd., Madison WI 53711

Table 1: Meiotic metaphase I pairing configurations in pollen mother cells (PMCs) homozygous *ph1b* genotypes in Chinese Spring CS) and Overley/Amadina (O/A) backgrounds.

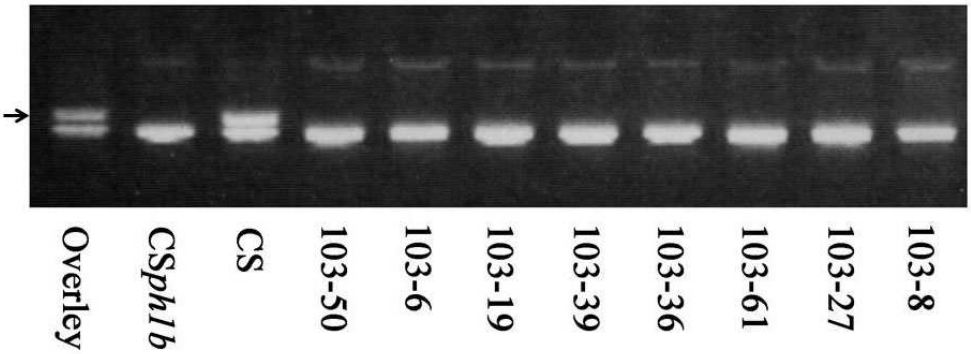
Genoytpe	Number of normal PMCs	Number of PMCs with multivalents
CS*ph1b*	34	21 (38%)
O/A*ph1b*	50	43 (46%)

Legends of Figures

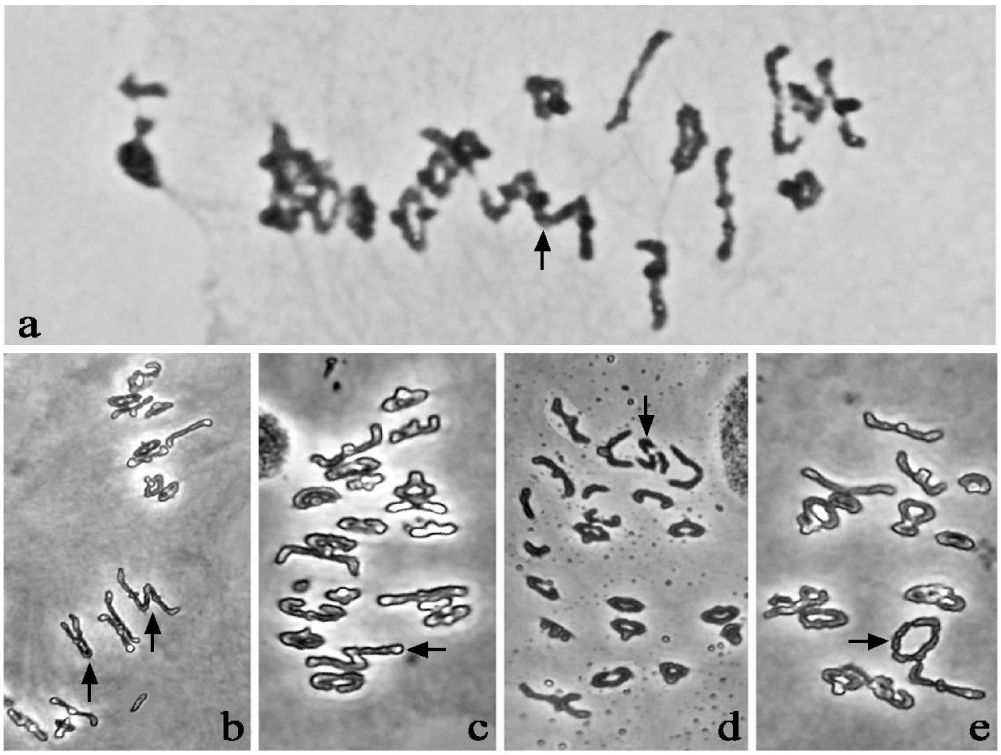
Figure 1: STS-PCR amplicons with ABC302.3 primers. The arrow indicates the *Ph1*-specific ABC920 fragment, which is absent in the *ph1b* mutant and the derived O/A*ph1b* plants 103-6, -8, -19, -27, -36, -39, -50, -61.

Figure 2: Meiotic metaphase I pairing in pollen mother cells of the CS*ph1b* (a) and A/O*ph1b* (b-e) stock; multivalents are marked by arrowheads: a) one hexavalent, b) one trivalent plus one quadrivalent, c) one quadrivalent, d) one trivalent, e) one quadrivalent.

5585 Guilford Rd., Madison WI 53711



246x94mm (96 x 96 DPI)



104x78mm (300 x 300 DPI)