The wheat and barley vernalization gene VRN3 is an orthologue of FT

L. Yan, D. Fu, C. Li, A. Blechl, G. Tranquilli, M. Bonafede, A. Sanchez, M. Valarik, S. Yasuda, and J. Dubcovsky

PNAS published online Dec 8, 2006; doi:10.1073/pnas.0607142103

This information is current as of December 2006.
The wheat and barley vernalization gene VRN3 is an orthologue of FT

L. Yan*, D. Fu*, C. Li*, A. Blechl†, G. Tranquilli*§, M. Bonafede*§, A. Sanchez*, M. Valarik*, S. Yasuda‡, and J. Dubcovsky*‡

*Department of Plant Sciences, University of California, Davis, CA 95616; †U.S. Department of Agriculture–Agricultural Research Service, Western Regional Research Center, Albany, CA 94710; and ‡Research Institute for Bioresources, Okayama University, Kurashiki 710-046, Japan

Edited by Richard M. Amasino, University of Wisconsin, Madison, WI, and approved October 30, 2006 (received for review August 16, 2006)

Winter wheat and barley varieties require an extended exposure to low temperatures to accelerate flowering (vernalization), whereas spring varieties do not have this requirement. In this study, we show that in these species, the vernalization gene VRN3 is linked completely to a gene similar to Arabidopsis FLOWERING LOCUS T (FT). FT induction in the leaves results in a transmissible signal that promotes flowering. Transcript levels of the barley and wheat orthologues, designated as HvFT and TaFT, respectively, are significantly higher in plants homozygous for the dominant Vrn3 alleles (early flowering) than in plants homozygous for the recessive vrn3 alleles (late flowering). In wheat, the dominant Vrn3 allele is associated with the insertion of a retroelement in the TaFT promoter, whereas in barley, mutations in the HvFT first intron differentiate plants with dominant and recessive VRN3 alleles. Winter wheat plants transformed with the TaFT allele carrying the promoter retroelement insertion flowered significantly earlier than nontransgenic plants, supporting the identity between TaFT and VRN-B3. Statistical analyses of flowering times confirmed the presence of significant interactions between vernalization and FT allelic classes in both wheat and barley (P < 0.0001). These interactions were supported further by the observed up-regulation of HvFT transcript levels by vernalization in barley winter plants (P = 0.002). These results confirmed that the wheat and barley FT genes are responsible for natural allelic variation in vernalization requirement, providing additional sources of adaptive diversity to these economically important crops.

The propagation and survival of a plant species depends critically on its ability to precisely regulate the transition from vegetative to reproductive growth. Consequently, plants have evolved refined mechanisms capable of integrating photoperiod and vernalization (extended exposure to low temperatures) signals associated with seasonal variation to optimize flowering time and seed production. The photoperiod pathway is relatively well conserved among flowering plants, with the gene CONSTANS (CO) playing a central regulatory role (1, 2). In Arabidopsis, a long-day (LD) plant, CO induces the transcription of the FLOWERING LOCUS T (FT) whereas in rice, a short-day (SD) plant, CO represses FT (referred to as Htl1 and Htd3a, respectively, in rice) (2). Overexpression of FT in transgenic plants from several species is associated with early flowering (3–7), suggesting that this gene is a conserved promoter of flowering. FT induction in the leaves results in a transmissible signal that travels through the phloem to the apex, where it induces flowering (8–10).

In contrast with the conserved photoperiod pathway, several aspects of the vernalization pathway vary between Arabidopsis and the temperate grasses (11). In Arabidopsis, the MADS-box gene FLOWERING LOCUS C (FLC) plays a central role in the vernalization pathway (12, 13). FLC delays flowering by repressing the production of FT in the leaves and SOCI in the meristems, which prevents the up-regulation of the FD transcription factor, a partner to FT in the induction of flowering (9, 10, 14). Vernalization permanently down-regulates FLC, thereby releasing FT and SOCI repression to induce the transcription of API, which is responsible for the transition between the vegetative and reproductive meristem (12). FLC is positively regulated by FRI (15) and negatively regulated by genes in the Arabidopsis autonomous pathway (12, 13). Surprisingly, no clear homologues of FRI or FLC have been found in temperate grasses (e.g., wheat and barley).

The VRN2 gene from temperate grasses (different from Arabidopsis VRN2; ref. 15) is a dominant repressor of flowering down-regulated by both vernalization (11) and SDs (16, 17). VRN2 has no close homologues in Arabidopsis, but plays a role in vernalization similar to that of FLC (11). Reduction of VRN2 transcript levels by RNA interference (RNAi) in hexaploid winter wheat variety Jagger significantly accelerates flowering (11). VRN2 has a CCT domain (CO, CO-like, and TOC1) similar to that found in CO (11). Mutations within this domain or deletions of the complete VRN2 gene result in recessive alleles for spring growth habit in diploid wheat and barley that eliminate the vernalization requirement (11, 18).

The effect of VRN2 allelic variation on flowering time is reduced or eliminated by mutations in the promoter or first intron of the VRN1 vernalization gene in both wheat and barley (18–21). This dominant promoter of flowering is orthologous to the Arabidopsis meristem identity gene API (22). VRN1 transcripts are up-regulated by vernalization in winter wheat varieties (22), and its down-regulation by RNAi in transgenic wheat plants delays flowering (23).

Two additional vernalization genes have been reported in barley (VRN-H3) and wheat (VRN-B4). VRN-H3 was tentatively assigned to chromosome 1H based on its loose linkage with the morphological marker BLP (24), whereas VRN-B4 was mapped on the short arm of wheat chromosome 7B (25–28). We show here that the VRN-H3 gene actually is located on barley chromosome arm 7HS and is orthologous to the wheat vernalization gene VRN3.
gene VRN-B4, which is referred hereafter as VRN-B3. We also show that VRN3 is an orthologue of the Arabidopsis FT gene.

Results

Genetic Mapping of Wheat VRN-B3. We mapped VRN-B3 on the short arm of chromosome 7B, 1 cM distal to marker ABC158 and 5 cM proximal to microsatellite marker GW569 (Fig. 1A) by using 82 recombinant substitution lines (RSLs) from a cross between Chinese Spring (CS) and chromosome substitution line CS(Hope7B) (Supporting information [SI] Appendix 1). The ABC158 sequence (L43928) is 90% identical to a DNA sequence on rice chromosome 6 coding for protein BAD69198. This sequence is 50 kb proximal to Hd3a, a rice gene responsible for significant differences in flowering time and orthologous to the Arabidopsis FT gene (6).

Because FT was a potential candidate gene for VRN-B3, we developed a marker for the orthologous Triticum aestivum L. gene (TaFT) by using the published sequence of the barley orthologue (HvFT, DQ100327) (29). We developed a second marker from a rice gene located between ABC158 and Hd3a (AK121981), which was designated UCW99 (SI Table 1). Using these markers, we mapped TaFT completely linked to VRN-B3 and UCW99 and 1 cM distal to ABC158 (Fig. 1A). Complete linkage between VRN-B3 and TaFT was confirmed by analyzing flowering time for 10–11 plants from each critical RSL with recombination events between flanking markers ABC158 and GWM569 (SI Table 2).

Genetic Mapping of Barley VRN-H3. We mapped VRN-H3 in an F_{2} population from the cross between BGS213 (spring, Vrn-H3) and Hordeum vulgare subsp. spontaneum (C. Koch) Thell. (winter, vrn-H3). The 3:1 ratio between spring and winter plants found before in this population (30) confirmed segregation for a single dominant gene. This gene was mapped on chromosome 7H linked to microsatellite loci EBMAC0603 and EBMAC0016 (Fig. 1B). To explore the relationship between VRN-H3 and the vernalization gene mapped on the homoeologous chromosome

**Throughout this study flowering time refers to the time of complete emergence of the spike from the leaf sheath, which is usually referred by cereal scientists as heading time.
7B in wheat, we developed barley markers for the same genes mapped in wheat (SI Table 3). Markers UCW99 and HvFT were completely linked to each other and to VRN-H3 and 1 cM distal to ABC158 (Fig. 1B).

Plants carrying the BGS213 UCW99/HvFT allele flowered 36 to 50 days after sowing, whereas those carrying the H. vulgare subsp. spontaneum UCW99/HvFT allele flowered 85 to 111 days after sowing, facilitating the precise mapping of VRN-H3. The complete linkage between HvFT and VRN-H3 was confirmed by using a second barley mapping population from a cross between the spring genetic stock BGS213 and the winter barley variety “Igri” (SI Appendix, section 1).

To rule out the possibility of an error in the BGS213 genetic stock, we tested two additional sets of VRN-H3 isogenic lines in which the spring growth habit from Tammi (Vrn-H3) was introgressed into winter varieties Hayakiso 2 and Dairokkaku 1 by 11 backcrosses. Using the molecular markers developed for HvFT (SI Table 3), we confirmed that the two spring Vrn-H3 isogenic lines have the Tammi allele (same as BGS213), whereas the recurrent winter parents have a different HvFT allele. Our results indicate that Vrn-H3 is on chromosome 7H and linked to HvFT, and not on chromosome 1H as initially suggested by its loose linkage to BLP (24).

Based on the known colinearity between barley and wheat genomes (31) and the close linkage between both barley VRN-H3 and wheat VRN-B4 with the same three molecular markers located on homoeologous group 7 (Fig. 1A and B), we conclude that these two genes are orthologous and propose to rename the wheat vernalization gene as VRN-B3.

High-Density Genetic Map and Physical Map of Barley VRN-H3. We selected the barley-mapping population BGS213 × H. vulgare subsp. spontaneum for the VRN3 high-density mapping because of its simpler diploid inheritance and higher level of polymorphism relative to the wheat population. To generate additional markers in the region, we developed several UCW markers corresponding to the rice genes flanking Hd3a (Fig. 1C and SI Table 3). Despite a 70-kb inversion detected between barley and rice in this region, the colinearity of the genes within the inversion facilitated the development of barley markers tightly linked to HvFT (Fig. 1C).

We first used HvFT flanking markers UCW98-UCW99 to screen 1,600 gametes from this population and found 12 lines with recombination events within the targeted region (SI Fig. 5). Progeny tests of these 12 lines were used to map VRN-H3 0.3 cM distal to UCW98, 0.4 cM proximal to UCW99, and completely linked to HvFT (Fig. 1C).

Barley probes for HvFT and its flanking markers UCW98, UCW99, UCW116, and UCW117 (Fig. 1C) were used to screen a "Morex" barley BAC library (32). Nineteen BACs were recovered and assembled via fingerprinting and hybridization into three contigs separated by two gaps (SI Fig. 6). The sequencing of barley BACs 440G4 (DQ900086), 761F4 (DQ900085), and 455J22 (DQ900687) revealed the presence of the noncolinear barley gene UCW118 (Fig. 1D) and of the putative gene UCW120 in both rice and barley (SI Fig. 7). The mapping of these two barley markers further delimited the location of VRN-H3 to a 0.2-cM interval flanked by UCW120 and UCW118 (Fig. 1C and SI Table 3). The only annotated genes (excluding hypothetical genes and repetitive elements) found in the colinear 28-kb region in rice were Hd3a and Hd3b, the rice orthologues of FT.

Similarly, no other known gene was found between UCW120 and UCW118 in the three barley BACs except for HvFT (Fig. 1D). To test whether additional genes were closely linked to FT, we also sequenced Aegeilops tauschii Cos. BAC HI41111 (DQ899784), which includes an orthologue of HvFT (coverase 2.8× at PHRED ≥20). Eighty percent of this 170-kb BAC showed similarity to repetitive elements, whereas the rest showed no similarity to known genes outside of FT (data not shown). Based on these results, HvFT is our only candidate gene for VRN-H3.

Southern blot analyses by using HvFT as a probe resulted in a single hybridization band with the barley genomic DNA, suggesting that the Hd3a-Hd3b duplication on rice chromosome 6 occurred after the divergence with the Triticeae, a hypothesis also supported by the phylogenetic analysis of FT-like genes in wheat, barley, rice, and Arabidopsis (SI Fig. 8).

FT Allelic Differences. The TaFT and HvFT genes have three exons encoding for a protein of 177 aa (Fig. 1 E and F). In contrast, all other FT and FT-like genes included in the phylogenetic analysis (SI Fig. 8) have four exons. This difference was generated by the fusion of exons 3 and 4 in TaFT and HvFT.

Wheat. We subcloned and sequenced the wheat TaFT genes and their flanking 5' and 3' regions from CS(Hope7B) (DQ890165) and CS (DQ890162). The CS(Hope7B) allele associated with early flowering (Vrn-B3) has a 5,295-bp repetitive element inserted 591-bp upstream from the start codon, an insertion that is absent in the CS allele associated with late flowering (vrn-B3) (Fig. 1F). Six additional SNPs were detected in the promoter region and three within a foldback element present in intron 1. No differences were detected between the two TaFT alleles in the coding region or in the first 628 bp downstream from the stop codon.

The retrotransposon inserted in the TaFT promoter has identical LTRs, suggesting a recent insertion. This is further supported by the low frequency of this insertion in the wheat germplasm. With the exception of the variety Hope, we did not find this retrotransposon insertion in a collection of 19 tetraploid spring wheats, 29 hexaploid winter wheats, and 77 hexaploid spring wheats (SI Table 4). These results indicate that this mutation has not yet been used extensively in commercial varieties and, therefore, represents a potentially valuable source of genetic diversity to modulate wheat flowering time.

Barley. The HvFT allele from BGS213 (DQ898515) associated with Vrn-H3 differs from both Igri (DQ898517) and H. vulgare subsp. spontaneum (DQ898516) alleles associated with vrn-H3 by nine linked polymorphisms (seven SNPs and two indels) in the first 550 bp upstream from the start codon and two linked polymorphisms in the first intron (SI Fig. 9).

We sequenced HvFT from seven additional winter varieties (recessive vrn-H3) and found heterogeneity for the promoter haplotypes. The promoter haplotypes were similar to Igri in two varieties and similar to BGS213 in the other five varieties (SI Fig. 9). These results indicate that the BGS213 promoter haplotype is not sufficient to determine a dominant spring growth habit. However, we cannot rule out an effect of this promoter polymorphism on flowering time when alleles for spring growth habit from the other vernalization genes are present. A germplasm survey at Okayama University showed that all of the varieties carrying the Vrn-H3 allele also have the dominant Vrn-H1 allele (33).

The haplotypes found in the first intron were more consistent with the observed differences in flowering habit. All of the winter varieties showed the same haplotype in the first intron as Igri and H. vulgare subsp. spontaneum, which was different from the one observed in the varieties carrying the dominant Vrn-H3 allele (SI Fig. 9). These results suggest that regions in the first intron may play an important role in the regulation of HvFT by vernalization (SI Fig. 9). This possibility is also supported by previous reports indicating that FLC binds a region within FT first intron, which is critical for the regulation of this gene in Arabidopsis (14). We are developing several segregating populations to assess the roles of the HvFT promoter and first-intron polymorphisms in determining flowering time in different barley genetic backgrounds.
**FT Expression Profiles.** *HvFT* transcript levels were analyzed by quantitative PCR (SI Table 5) in 20 different F3 plants selected from the mapping population, 10 homozygous for the BGS213 *HvFT* allele and 10 homozygous for the *H. vulgare* subsp. *spontaneum* allele. Plants at the three-leaf stage carrying the BGS213 *HvFT* allele showed significantly higher *HvFT* transcript levels (*P* < 0.01) relative to those carrying the *H. vulgare* subsp. *spontaneum* allele [Fig. 2A and B, 0 weeks (w)], a difference that persisted for the next weeks in both vernalized and unvernalized plants (Fig. 2A and B).

A similar result was observed in wheat. RSLs carrying the Hope *TaFT* allele with the retrotransposon insertion showed significantly higher *TaFT* transcript levels (*P* < 0.01) than the RSLs carrying the CS allele (SI Fig. 10). Because *FT* induces flowering in other plant species, these results suggest that the earlier flowering of the lines carrying the dominant *Vrn3* alleles might be related to their higher *FT* transcript levels relative to lines carrying the *vrn3* allele.

Spring plants homozygous for the dominant *Vrn-H3* allele showed a rapid increase of *HvFT* transcripts with development (Fig. 2A and B). However, winter plants carrying the recessive *vrn-H3* exhibited low *HvFT* transcript levels in the absence of vernalization (Fig. 2A). After six weeks of vernalization, *HvFT* transcript levels in the vernalized winter plants were significantly higher than in the nonvernalized plants (Fig. 2B, 6w, vs. Fig. 2A, 6w; *P* = 0.002). Up-regulation of *FT* transcript levels by vernalization also was confirmed in *Triticum monococcum* L. winter accession G3116 (data not shown).

*HvFT* transcript levels were very low under SDs and were up-regulated when the plants were transferred to LDs (SI Fig. 11), suggesting a strong effect of photoperiod on *HvFT* transcript levels. A similar observation was reported before in barley (29).

*HvFT* transcription profiles were paralleled closely by those of *VRN-H1* (Fig. 2C and D), confirming the known interactions between these genes (9). In an opposite trend to that observed for *HvFT* and *VRN-H1*, the *VRN-H2* transcript levels in winter plants were reduced by vernalization (Fig. 2E). In the spring plants, *VRN-H2* levels were low even in the absence of vernalization (Fig. 2E), likely as a result of the higher transcript levels of *VRN1*. Previous studies suggest that *VRN1* directly or indirectly down-regulates *VRN2* (17, 23).

**Transformation of Winter Wheat Plants with the Hope *TaFT* Allele.** The complete genetic linkage between *FT* and *VRN3* in all mapping populations, together with the correspondence between polymorphisms in *FT* regulatory regions, *FT* transcript levels, and flowering time suggest that *FT* is in fact *VRN3*. To confirm the identity between *FT* and *VRN3*, we transformed winter wheat variety Jagger with the dominant *TaFT* allele from Hope, which carries the retrotransposon insertion (SI Appendix, section VI). A schematic representation of the Hope *TaFT* region cloned in the construct used in the transformation experiment is presented in Fig. 1F.

---

**Fig. 2.** Transcript levels of *HvFT*, *VRN-H1*, and *VRN-H2* in F3 plants homozygous for the *HvFT* *H. vulgare* subsp. *spontaneum* allele (red, late flowering) or BGS213 allele (blue, early flowering). Plants at the three-leaf stage grown at room temperature (RT = 20–24°C) under LD (16 h of light) were either maintained at the same nonvernalizing condition (A, C, and E) or transferred to a cold room at 4°C under LD (B, D, and F; Ver., vernalization). Time is indicated in weeks (w) after the transfer to the separate chambers (0 w RT). Unvernalized plants carrying the BGS213 allele were flowering at the time of the 6-w RT sampling. Values are the means of 10 independent F2:3 plants, and bars are SE of the means. The $2^{−ΔΔC_{ct}}$ method (38) was used to normalize and calibrate transcript values relative to the *ACTIN* endogenous control. Scales can be compared within the same genes but not across genes. Asterisks indicate significant differences (*P* < 0.05).
We analyzed two independent transgenic events (Fig. 3), including six or seven T1 transgenic plants and eight or nine nontransgenic controls per transformation event. Leaf RNA samples were extracted from five-leaf-old unvernalized plants grown under LD. Jagger plants transformed with the Hope allele showed TaFT transcript levels >80-fold higher than the nontransgenic controls (Fig. 3A). Average flowering time for the transgenic lines was typical of spring lines (40 ± 1 and 51 ± 2 days after sowing), whereas the nontransgenic plants remained in vegetative stage until the experiment was terminated 110 days after sowing (Fig. 3B and C). The conversion of a winter wheat variety into a spring one supports the identity between FT and VRN-3.

**Interactions Between FT and Vernalization.** In both wheat and barley, factorial ANOVAs for flowering time by using vernalization treatment and FT allelic classes as factors yielded highly significant interactions (P < 0.0001) (SI Table 6). Vernalized wheat and barley plants showed smaller differences in flowering time between the two VRN3 allelic classes than unvernalized plants (SI Fig. 12). Based on these results and the observed up-regulation of FT transcript levels by vernalization (Fig. 2A and B), we conclude that, in the temperate cereals, FT interacts with the vernalization pathway.

In Arabidopsis, no association between natural variation in vernalization requirement and FT has been described so far. However, overexpression of FT (or TSF) strongly suppresses the FLC-mediated late-flowering phenotype of winter annual Arabidopsis accessions without affecting FLC mRNA levels (34). This suggests that activation of FT and/or TSF can bypass the block to flowering created by FLC, confirming that FT acts downstream of FLC (34). A similar result is reported here for wheat, where the increased expression of TaFT in transgenic winter variety Jagger bypassed the VRN2 repression, resulting in a spring growth habit.

To test whether the interaction between vernalization and FT in wheat was related to the presence of the vernalization gene VRN2, which is unique to temperate cereals, we studied the transcript levels of FT in isogenic lines of T. monococcum differing in their VRN2 alleles (SI Fig. 13). Spring accession Dv92 has a recessive vrn2 allele generated by a point mutation in the CCT domain, whereas the winter accession G3116 has a functional Vrn-2 allele (11). No significant differences in VRN2 transcript levels (P > 0.05) were detected at the five-leaf stage between the isogenic lines. In contrast, at the same developmental stage, FT transcript levels were 170-fold higher (P < 0.05) in the isogenic lines carrying the mutant vrn2 allele from Dv92 than in those carrying the dominant Vrn2 allele at the same developmental stage (SI Fig. 13).

This result suggests that VRN2 modulates the quantitative levels of FT (directly or indirectly), providing a link between the vernalization pathway and FT in the temperate cereals. This genetic interaction is not completely unexpected, because the CCT domain in VRN2 is related to the one present in CO, which was shown to be involved in the regulation of FT transcript levels (35). It is tempting to speculate that the allelic differences in the FT regulatory regions described in this study may be responsible for the disruption of the interactions between FT and VRN2 (or a VRN2-regulated gene) and for their differential responses to the vernalization treatment. A tentative model summarizing the interactions between VRN1, VRN2, and VRN3 is presented in Fig. 4.

According to this model, VRN2 is a repressor of flowering down-regulated by vernalization and SD (11, 16, 17), which negatively regulates VRN3 and VRN1 (directly or indirectly). VRN3 is a promoter of flowering up-regulated by LD, which positively regulates VRN1, the meristem identity gene. A secondary effect of the increase in VRN1 transcripts is the down-regulation of VRN2, as part of a feedback regulatory loop described in refs. 16 and 17. Unvernalized winter plants grown under LD exhibit high levels of VRN2 transcripts and low levels of VRN1 and VRN3 (Fig. 2A, C, and E). Vernalization under LD results in the down-regulation of VRN2 and the up-regulation of VRN3 and VRN1. Under SD, all three genes show low transcript levels, but a rapid up-regulation of VRN1 and VRN3 is observed when plants are transferred from SD to LD (SI Fig. 11). This model also attempts to explain the strong epistatic interactions observed among these three genes. The recessive vrn2 allele eliminates the effects of VRN1 and VRN3 allelic differences on flowering time (18, 19, 36). We suggest that in the absence of a functional VRN2 repressor, the different mutations in the VRN1 (20, 21) or VRN3 (Fig. 1F) regulatory regions have no effect on flowering. It is also known that the dominant Vrn1 and Vrn3 alleles reduce or eliminate the effect of VRN2 allelic differences on flowering time. We propose that a mutation in a regulatory region of VRN1 or VRN3 is sufficient to preclude its recognition by the VRN2-mediated repression and to initiate the flowering cascade.
In summary, this study provides strong evidence supporting the identity between FT and VRN3 in wheat and barley. It also shows that allelic variation in FT is associated with large differences in flowering time and that there are significant interactions between FT allelic variation and vernalization requirements in these species. This allelic variation provides an additional source of adaptive diversity to these economically important crops.

Materials and Methods

Genetic and Physical Maps. SI Appendix, section I, describes the accessions and markers used in the wheat- (SI Table 1) and barley- (SI Table 3) mapping populations. The complete list of the barley BACs used to construct the physical contigs and the sequencing coverage for each sequenced BAC is available in SI Appendix, section II. The phylogenetic analysis is described in SI Appendix, section III.

Allelic Variation. The description of the materials used for the characterization of the FT allelic differences is included in SI Appendix, section IV. This includes a list of the wheat accessions tested for the presence of the retrotransposon insertion on the TaFT promoter (SI Table 4). The map comparisons used to determine the location of the QTLs for flowering time discovered in the cross Fredrickson × Stander (37) on barley chromosome arm 7HS also is included in this section.

Transcription Profiles and Transgenic Plants. The materials and methods used in the expression experiments are presented in SI Appendix, section V. This information includes the environmental conditions and the primers used in the quantitative PCR experiments (SI Table 5). The constructs and procedures used in the transgenic experiments are detailed in SI Appendix, section VI, whereas the statistical analyses for the interactions between FT and vernalization are presented in SI Appendix, section VII.

We thank the Okayama University (Kurashiki, Japan), the John Innes Centre (Norwich, U.K.), and the National Small Grain Collection (Aberdeen, ID) for the germplasm used in this study; L. Valarikova, Xiaoting Zhang, Lydia Li, and Qunen Lam for excellent technical assistance; and Dr. P. San Miguel (Purdue University, West Lafayette, IN) for the BAC sequencing. This work was supported by U.S. Department of Agriculture National Research Initiative Grant 2005-02356. G.T. thanks the Argentinian Fulbright Commission for an Exchange Program grant.

17. Takahashi R (1983) Catalogue of the Barley Germplasm Preserved at the Okayama University (Institute of Agricultural and Biological Sciences, Okayama University, Kurashiki, Japan).