Saturation and comparative mapping of the genomic region harboring Hessian fly resistance gene \(^{H26}\) in wheat

Guo Tai Yu · Xiwen Cai · Marion O. Harris · Yong Qiang Gu · Ming-Cheng Luo · Steven S. Xu

Abstract  Resistance gene \(^{H26}\), derived from \textit{Aegilops tauschii} Coss., is one of the most effective \(R\) genes against the Hessian fly \([\textit{Mayetiola destructor} \text{(Say)}]\), an important pest of wheat \((\textit{Triticum aestivum} \text{L.})\). Using a limited number of PCR-based molecular markers a previous study mapped \(^{H26}\) to the wheat chromosomal deletion bin 3DL3-0.81-1.00. The objectives of this study were to saturate the chromosomal region harboring \(^{H26}\) with newly developed PCR-based markers and to investigate the collinearity of this wheat chromosomal region with rice \((\textit{Oryza sativa} \text{L.})\) and \textit{Brachypodium distachyon} genome. A population of 96 \(F_2\) individuals segregating at the \(^{H26}\) gene locus was used for saturation mapping. All wheat ESTs assigned to the deletion bin 3DL3-0.81-1.00 were used to design STS (sequence tagged site) primers. The wheat ESTs mapped near \(^{H26}\) were further used to BLAST rice and \textit{B. distachyon} genomic sequences for comparative mapping. To date, 26 newly developed STS markers have been mapped to the chromosomal region spanning the \(^{H26}\) locus. Two of them were mapped 1.0 cM away from the \(^{H26}\) locus. Comparative analysis identified genomic regions on rice chromosome 1 and \textit{Brachypodium} Super contig 13 which are collinear with the genomic region spanning the \(^{H26}\) locus within the distal region of 3DL. The newly developed STS markers closely linked to \(^{H26}\) will be useful for mapped-based cloning of \(^{H26}\) and marker-assisted selection of this gene in wheat breeding. The results will also enhance understanding of this chromosomal region which contains several other Hessian fly resistance genes.

Introduction

Hessian fly \([\textit{Mayetiola destructor} \text{(Say)}]\) is one of the most destructive insects in common wheat \((\textit{Triticum aestivum} \text{L.})\) and durum wheat \((\textit{Triticum turgidum} \text{L. var. durum})\) in the world \((\text{Berzonsky et al. 2003})\). Larval attack of the seedling causes plant death or stunting of growth while larval attack of the plant during stem elongation causes broken stems and shrunken seeds. Both result in significant economic losses. Chemical control is rarely used because timing of application is difficult and few effective insecticides are available. The most common cultural control practices are to delay fall seeding and to destroy the volunteer wheat. Biological control with parasites provides little protection for the current crop. The use of resistant cultivars is the
most effective and economical approach (Berzonsky et al. 2003).

To date, 32 Hessian fly resistance genes have been identified in common and durum wheat and their relatives, designated H1 through H32 (Williams et al. 2003; Liu et al. 2005; Sardesai et al. 2005; McIntosh et al. 2008). Deployment of some of the resistance genes has provided effective control of the insect in North America. However, wide use of resistant cultivars leads to the emergence of new virulent genotypes of Hessian fly (Gullon et al. 1961). Co-evolution of Hessian fly and its hosts, including wheat and its relatives, generates genetic variations of Hessian fly and then results in various biotypes (Kudagamage et al. 1990; Ratcliffe and Hatchett 1997). One resistance gene usually confers resistance to one or a few biotypes of Hessian fly.

Sixteen biotypes of Hessian fly have been identified according to their virulence to four differentials, H3 (in ‘Monon’), H5 (in ‘Magnum’), H6 (in ‘Caldwell’), and H7H8 (‘Seneca’) (Ratcliffe and Hatchett 1997). The 16 biotypes are designated Great Plains (GP), and A through O. The biotype L is virulent to all four differentials, while GP is avirulent to all of them. However, more biotypes, including vH9 and vH13, have been found (Formusoh et al. 1996; Zantoko and Shukle 1997), and more will be found in future. Some of them may have overcome the resistance from these four genes or other resistant genes. Although H9 and H13 confer resistance to the most virulent biotype L of the 16 biotypes, they do not confer resistance to the biotype vH9 and vH13, respectively. The complexity of the interaction between Hessian fly and resistance genes in wheat and its relatives, and variability of Hessian fly in the virulence necessitate the deployment of newly identified resistant genes.

The gene H26, derived from Ae. tauschii (Cox and Hatchett 1994), confers resistance to Hessian fly populations that are currently difficult or impossible to control with other available H genes (Cox and Hatchett 1994; Wang et al. 2006; Xu et al. 2006). H26 is one of only two genes that are highly effective against a Hessian fly population recently detected in Oklahoma (Ming-Shun Chen, personal communication). However, H26 has not been commercially deployed. H26 was previously assigned to chromosome 4D using monosomic analysis (Cox and Hatchett 1994). Recently, this gene was mapped to the deletion bin 3DL3-0.81-1.00 on chromosome 3D using molecular markers (Wang et al. 2006). Because fewer molecular markers have been assigned to this distal deletion bin on 3DL than the homologous regions on chromosomes 3A and 3B (Somers et al. 2004), H26 locus was only loosely mapped with a few SSR markers and closely linked PCR-based markers have not been identified.

Wheat EST (expressed sequence tag) sequences can be used to develop user-friendly molecular markers such as STS (sequence tagged site) and SSR (simple sequence repeat) (Peng and Capitan 2005; Zhang et al. 2005; Perugini et al. 2008). There are 1,050,314 wheat ESTs available (http://www.ncbi.nlm.nih.gov/sites/entrez). A total of 16,000 of the ESTs were mapped to wheat deletion bins (Qi et al. 2004). These deletion-mapped ESTs are particularly useful for developing PCR-based DNA markers for saturation and fine mapping of a chromosomal interval harboring the genes of interest and gene cloning. So far, 120 wheat ESTs have been mapped to 3DL3-0.81-1.0 (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) and they are excellent resource for developing new PCR-based markers for saturation mapping of the genes within this chromosomal interval.

In addition to wheat EST, genomic sequence information from model species, such as rice (Oryza sativa L.) whose genome has been sequenced, has been used for molecular mapping and gene isolation through comparative analysis in wheat (Liu and Anderson 2003; Distelfeld et al. 2004; Francki et al. 2004). However, due to the many disruptions in collinearity between rice and the genomes of wheat and barley, Brachypodium distachyon has been proposed as another model species of cereals. The bacterial artificial chromosome (BAC) libraries (Foote et al. 2004; Hsu et al. 2006, 2008; Hasterok et al. 2006), ESTs (Vogel et al. 2006), and partial assembled genomic sequences have become available in B. distachyon. Limited data suggested that Brachypodium is likely more closely related to wheat than rice (Vogel et al. 2006; Bossolini et al. 2007). Thus, the genomic sequences and ESTs of Brachypodium could be another invaluable resource for molecular mapping and gene cloning in wheat.

The objectives of this study were to saturate the chromosomal region harboring the H26 locus using newly developed PCR-based STS (sequence tagged site) markers, which will facilitate not only deploying the H26 gene in wheat cultivars but also the genomic study of this chromosomal interval; and to determine the collinearity of this wheat genomic region with rice and Brachypodium genome.

Materials and methods

Plant materials and STS marker analysis

The mapping population of 96 F2 individuals derived from the cross between the resistant synthetic hexaploid wheat (SHW) line SW8 (Langdon/Ae. tauschii Cia 25) and the susceptible SHW line SW11 (Langdon/Ae. tauschii H-80-114-1) (Wang et al. 2006) was used for saturation mapping in the present study. DNA was extracted from the preserved (−80°C) young leaf tissues of the population as described...
by Dellaporta et al. (1983). One hundred and twenty wheat ESTs, assigned to the chromosomal bin 3DL3-0.81-1.0 where H26 resides (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi), were used to design primers with the computer program Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) (Rozen and Skaletsky 2000) to detect STS marker loci near the H26 locus. The primers of the STS markers mapped to this chromosomal region are listed in Table 1. Four SSR markers (Xcfd223, Xgwm3, Xcfd211, and Xbarc71) that linked to H26 locus (Wang et al. 2006) were used as the anchors in this study and their primer sequences were obtained from the GrainGenes Database (http://wheat.pw.usda.gov/GG2/index.shtml).

Bulked segregant analysis was performed to identify marker loci closely linked to H26. Two bulks of DNA were prepared by pooling equal amounts of DNA from eight homozygous resistant and eight homozygous susceptible F$_2$ individuals, respectively. The STSs were amplified at optimized PCR conditions. The annealing temperature for PCR was determined based on the melting temperature ($T_m$) of the primer pair. It was calculated by subtracting 5 from the $T_m$ of the primer with lower $T_m$ value (Innis and Gelfand 1990) and was adjusted based on the relative intensity of target band among the all amplified ones for a primer pair (Table 1). The SSRs were amplified as described by Röder et al. (1998). PCR products were separated on 6% non-denaturing polyacrylamide gels in 0.5 XTBE buffer at 120 W for 1 h. The gels were scanned with a Typhoon 9410 variable mode imager (Molecular Dynamics, Ithaca, NY, USA) after staining with GelRed (Sigma, St. Louis, MO, USA).

Linkage of molecular markers with H26 in the mapping population was analyzed using MAPMAKER 2.0 (Lander et al. 1987) for Macintosh at LOD 6.0 with the Kosambi mapping function (Kosambi 1944).

Comparative analysis

For comparative analysis with the rice or Brachypodium genome, tentative consensus (TC) or EST sequences were subjected to BLASTn searches of the rice genomic sequences in the Gramene database (Ware et al. 2002; http://www.gramene.org/Multi/blastview) or to search of Brachypodium super contigs (http://www.brachypodium.org). For BLASTn searches, the threshold limits for significant hits were at least 80% nucleotide identity for at least 60 bases. We set e-value <e$^{-7}$ in order to include maximum number of EST or TC hits with lowest e-value. When several significant hits were found, only the best hit was adopted.

To search wheat TCs corresponding to rice PAC AP003238, which is hit by the EST of the closest STS marker (Xrwgs12)–H26, the sequence of the rice PAC was subjected to BLASTn (Altschul et al. 1997) against wheat EST clusters (TIGR gene indices). A significant match for a TC was declared on the basis of e-value of <e$^{-31}$ which was used to reduce the similarity among the significant TCs. The TC sequences were obtained from the Annotator Search of DFCI (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/tblastx/tgi/tc_ann.pl?gudb=wheat). The TC sequences were then subjected to tBLASTx (Ware et al. 2002; http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Translations&PROGRAM=tblastx&BLAST_PROGRAMS=tblastx&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blashome) searches of the NCBI nucleotide collection (nr/nt) to identify the putative corresponding protein. A significant match for a protein was declared on the basis of a minimum 80% amino acid identity for at least 50 amino acid residues of the protein sequence and an e-value of <e$^{-7}$. The hit with the lowest e-value was considered the putative protein of a TC when several matches were found. The known genes within the rice genomic region AP003238 were obtained through TIGR v5 in Gramene (http://www.gramene.org/Oryza_sativa_japonica/index.html).

Results

More than 500 STS primer pairs were designed based on the sequences of the 120 wheat ESTs assigned to the deletion bin 3DL3-0.81-1.00. These primer pairs were then tested for polymorphisms between the two parents of the mapping population. Fifty-one pairs of primers were found to amplify polymorphic bands between two parents (Fig. 1). Bulked segregant analysis identified 24 STS co-dominant markers linked to H26 and they were mapped to the deletion bin 3DL3-0.81-1.00 (Table 1; Fig. 1). Thus, 20% of the ESTs within this chromosomal interval were converted to STS markers.

In order to exploit the genomic sequences of the model species, rice and Brachypodium, the collinearities of the wheat genomic region harboring the H26 locus with rice and Brachypodium genomes were studied to develop additional markers for wheat. We designed 48 and 46 pairs of STS primers from the sequences of the rice and Brachypodium genomic regions which are collinear with the wheat genomic region harboring H26, respectively. One STS marker (Xrwgs17) developed from rice and one (Xrwgs10) from Brachypodium genomic sequences were mapped to the H26 region (Table 2). A total of 26 STS markers were mapped to the distal region of 3DL that resides within the deletion bin 3DL3-0.81-1.00 (Fig. 2).

The genetic map of partial 3DL, which spans a genetic distance of 42.5 cM with 30 molecular markers, was constructed in the population. This linkage map represents an average density of one marker per 1.4 cM. All markers were mapped at LOD $\geq 6.0$. Two co-segregating STS
Table 1  STS markers developed from wheat ESTs, rice genomic sequences, and *Brachypodium* genomic sequences

| STS marker | PCR primers | Annealing temperature (°C) | EST accession/genomic group |
|------------|-------------|----------------------------|-----------------------------|
| Xrwgs1     | GCTGTCGCAACAAACATATAA 55 | BE404125                  |
| Xrwgs2     | TTTTGAAACAACATTGAATCT 48 | BE590549                  |
| Xrwgs3     | TGACTTATCCCCAGTGACAGCAG 55 | BF485004                  |
| Xrwgs4     | ATGGCTACCCCAGTGGACAAG 55 | BE443397                  |
| Xrwgs5     | GTTCTCGGCAATCAATCCTCT 55 | BG262734                  |
| Xrwgs6     | AAGGACGACGTCACAGGCTCAT 55 | BE591925                  |
| Xrwgs7     | CCAGGAGCTCGGAGAAAAAC 57 | BE444335                  |
| Xrwgs8     | TGCTCCCAAAAGCTTCTACCT 55 | BE498661                  |
| Xrwgs9     | CCATTTGGCACAATGACTTG 55 | BE405038                  |
| Xrwgs10    | CCTAACTGAGGTCCCCACCAA 55 | Brachypodium Super contig 13 |
| Xrwgs11    | GGAGAGTCGCAAGATCCA 55 | BE403428                  |
| Xrwgs12    | CTCTCTGCCAGTCCCAACTTT 55 | BE426418                  |
| Xrwgs13    | ACAACCAGGGACTGATCGAC 55 | BM138635                  |
| Xrwgs14    | CATGACCGGAGAGATGCAA 55 | PSR1205                   |
| Xrwgs15    | GAGGCCATCAAGTCCAAGTT 55 | BE426763                  |
| Xrwgs16    | ATGCATGCTAATTAGCCTGAT 47 | BG608151                  |
| Xrwgs17    | TCTCTGAGGGGAAGCAAGAA 55 | Rice chromosome 1L distal end |
| Xrwgs18    | TGAAGCAATCAGCAATGAGGA 55 | BE490274                  |
| Xrwgs19    | CTGTTTACGCCAATGAGGATT 55 | BE444864                  |
| Xrwgs20    | ACCGACATCATCACCATGCTT 55 | BE605103                  |
| Xrwgs21    | GGAAGACGATCACAAGGACA 55 | BE446756                  |
| Xrwgs22    | ACAATTGCTAGCTATGAGATGT 55 | BE444579                  |
| Xrwgs23    | CTCAAGGAGCTCGGAGAGAC 55 | BE489841                  |
markers, Xrwgs11 and Xrwgs12, have distances of 1 cM from the H26 locus. Another marker, Xrwgs10, is 3.2 cM proximal to H26. Several STS markers, such as Xrwgs4, Xrwgs5, and Xrwgs6, were co-segregating in the mapping population (Fig. 2). However, they were developed from different ESTs or TCs.

To evaluate the local collinearity of the deletion bin 3DL3-0.81-1.00 with rice genome we blasted the rice genome using 24 ESTs or corresponding TCs from which the mapped STS markers were developed. Fifteen of the ESTs or TCs hit the distal region of rice chromosome 1, two hit chromosome 3, one hit chromosomes 8 and 10, whereas five of the ESTs or TCs did not hit any rice genomic region under the significance threshold (Table 2). In general, there is good collinearity between the distal region of wheat 3DL and rice chromosome 1. However, wheat EST BE426418 detected a locus close to the one that EST BE444864 corresponded to (Fig. 3).

Since the STS marker Xrgws12 derived from the EST BE426418 is 1 cM from H26 locus we blasted rice genomic sequences using BE426418 as a query (Table 3). The rice genomic sequence collinear with BE426418 is derived from the PAC AP003238. Within this rice genomic region 14 known genes were found (Table 3). Among them are genes for a membrane attach component, lipase, DNA binding, Leucine rich repeat (LRR), and fungal lignin peroxidase (Table 3).

To search for putative genes corresponding to the wheat TCs that were hit by the rice PAC AP003238 we blasted the wheat EST clusters with AP003238 (Table 4). Twelve significant TCs were identified. Using tBLASTx, we blasted the NCBI nucleotide collection (nr/nt) with these TC sequences and obtained eleven predicted proteins, including one integral membrane protein-2B and one similar to ETS domain (DNA-binding domain). However, functions for rest of the predicted proteins are unknown (Table 4).

**Discussion**

Hessian fly resistance gene H26 was previously mapped to the 3DL distal region 3DL3-0.81-1 (Wang et al. 2006). Because this chromosomal interval has fewer PCR-based
markers than other regions according to the consensus genetic map (Somers et al. 2004) the H26 locus was roughly mapped with only eight SSR markers and one TRAP marker in the previous mapping endeavor (Wang et al. 2006). The molecular markers tightly linked to H26 have not been identified previously. Through saturation mapping in the present study we successfully developed 26 new STS markers and mapped them onto this region. Two of the STS markers (Xrwgs11 and Xrwgs12) are 1 cM away from H26 locus. Another marker, Xrwgs10, is 3.2 cM proximal to H26. Since H26 conditions resistance to multiple biotypes of Hessian fly, including vH13 (Cox and Hatchett 1994; Wang et al. 2006; Xu et al. 2006), these three STS markers will be useful for marker-assisted selection in wheat breeding and germplasm development.

In addition to H26, several other economically and genetically important genes reside within the 3DL distal region 3DL3-0.81-1, including H24 (Ma et al. 1993) and H32 (Sardesai et al. 2005) for resistance to Hessian fly, Lr24 for resistance to leaf rust (Puccinia triticina Erikss) (Boyko et al. 1999), R1 for red kernel color (Nelson et al. 1995), Chl2 for hybrid chlorosis (Koba and Tsunewaki 1978; Erayman et al. 2004), and the genes for Esterase-5 (Devos and Gale 1993) and /afii9826-(1-3)-Glucanase (Li et al. 2001). Thus, the 26 newly developed STS markers in our study will facilitate the genetic study of a number of important traits or genes in this region.

The homoeologous group 3 of wheat is considered to be collinear to chromosome 1 of rice (Ahn et al. 1993; Kurata et al. 1994; Munkvold et al. 2004; Dilbirligi et al. 2006), but detailed studies on the microcollinearity particularly

Table 2  STS markers, their corresponding ESTs and TCs, and similarity to the *Brachypodium* and rice genomic sequences

| Marker | EST accession/genicomic region | TC accession | *Brachypodium* | Rice |
|--------|---------------------------------|--------------|----------------|------|
|        |                                 |              | Contig         |      |
|        |                                 |              | e-Value        |      |
|        |                                 |              | Chromosome     | e-Value |

| Xrwgs1 | BE404125 | TC236858 | Super 13 | 2.0e−77 | 1 | 5.9e−123 |
| Xrwgs2 | BE590549 | TC265775 | Super 3  | 8.0e−10 | NSb |
| Xrwgs3 | BF485004 | TC263479 | Super 13 | 1.0e−130| 1 | 2.4e−291 |
| Xrwgs4 | BE443397 | TC252434 | Super 3  | 4.0e−96 | 10 | 1.7e−168 |
| Xrwgs5 | BG262734 | NA        | Super 13 | 7.0e−37 | 1 | 8.8e−42 |
| Xrwgs6 | BE591925 | TC272750 | Super 13 | 4.0e−27 | 1 | 4.9e−34 |
| Xrwgs7 | BE444335 | TC241376 | Super 13 | 2.0e−16 | 1 | 1.5e−08 |
| Xrwgs8 | BE498661 | TC253823 | NS        | 1      | NS |
| Xrwgs9 | BE405038 | NA        | NS        | 1      | NS |
| Xrwgs10| Brachypodium Super contig 13 | NA |
| Xrwgs11| BE403428 | TC255189 | NS        | 3      | 5.9e−29 |
| Xrwgs12| BE426418 | NA        | Super 13 | 9.0e−14 | 1 | 2.0e−13 |
| Xrwgs13| BM138635 | TC247552 | Super 13 | 9.0e−91 | 1 | 1.7e−133 |
| Xrwgs14| PSR1205  | NA        | NS        | 1      | NS |
| Xrwgs15| BE426763 | TC270760 | Super 2  | 1.0e−33 | 1 | 3.1e−82 |
| Xrwgs16| BG608151 | TC257542 | NS        | 8      | 1.5e−10 |
| Xrwgs17| Rice chromosome IL | NA |
| Xrwgs18| BE490274 | TC238164 | Super 13 | 2.0e−38 | 1 | 1.3e−32 |
| Xrwgs19| BE444864 | NA        | Super 13 | 2.0e−40 | 1 | 8.0e−90 |
| Xrwgs20| BE605103 | TC251323 | NS        | 3      | 2.4e−227 |
| Xrwgs21| BE446756 | TC244950 | Super 13 | 2.0e−28 | 1 | 2.0e−59 |
| Xrwgs22| BE444579 | TC268986 | Super 13 | 2.0e−32 | 1 | 5.7e−43 |
| Xrwgs23| BE489841 | TC233450 | Super 13 | 5.0e−58 | 1 | 4.2e−223 |
| Xrwgs24| BE637789 | TC233150 | Super 13 | 8.0e−76 | 1 | 5.0e−84 |
| Xrwgs25| BM137927 | NA        | Super 13 | 4.0e−60 | 1 | 1.7e−50 |
| Xrwgs26| BE591864 | NA        | Super 13 | 1.0e−20 | NS |

a These sequence data were produced by the US Department of Energy Joint Genome Institute [http://www.jgi.doe.gov/](http://www.jgi.doe.gov/) (with consent of the *Brachypodium* Genome Sequencing Project co-directors Drs. John Vogel, Michael Bevan, and David Garvin)

b Not significant based on the criteria (at least 60 bases, and an e-value of <e−7)

c A TC was not available
between 3DL and rice chromosome 1 have not been reported. The results from our study showed that 15 of the 24 ESTs mapped to the deletion bin 3DL3-0.81-1 (63%) had homology to the sequences on rice chromosome 1, suggesting collinearity between the distal region of 3DL and the distal region on the long arm of rice chromosome 1. This result also agrees with the study by Munkvold et al. (2004) in which the distal half of group 3 chromosomes (3L3-0.81-1) has better homology with rice chromosome 1 than the proximal half.

Although the result from this study showed good conservation in the deletion bin 3DL3-0.81-1.00 in terms of the homology with rice chromosome 1 disruptions in the collinearity between 3DL3-0.81-1.00 and rice chromosome 1 were observed. For example, the local inversion and distant translocation were observed for wheat in this study (Fig. 3). These rearrangements were also reported by Munkvold et al. (2004). Yet, the difference between 3DL3-0.81-1.00 and corresponding region on rice chromosome 1 is still too large to efficiently develop STS markers for wheat chromosome 3D using rice genomic sequence based on the collinearity. We designed 48 pairs of STS primers, but only one was mapped to the collinear region on 3DL (Table 2; Fig. 2). Therefore, a cautious approach should be taken when utilizing the rice genomic sequence for fine mapping in wheat.
Due to the limited degree of collinearity observed in wheat–rice comparative studies, *Brachypodium* has been proposed as a new model grass to study genomics of large-genome cereals (Draper et al. 2001). However, little is known about the collinearity between wheat and *Brachypodium* at the chromosome level. In the comparative mapping of *Lr34* orthologous regions (Bossolini et al. 2007), six of 11 wheat markers detected the collinearity between *Brachypodium* and wheat chromosome 7A, and six of 11 markers revealed the collinearity between *Brachypodium* and wheat chromosome 7D. The results from our study revealed that 15 of 24 (63%) wheat ESTs evaluated had similar sequences as rice chromosome 1. Fourteen of the 15 ESTs and one marker are collinear between the distal region of wheat 3DL and *Brachypodium Super contig 13* (Table 2). This points to the utility of *B. distachyon* as a model for the understanding of the large and complex genomes of wheat.

On the other hand, the collinearity between wheat 3DL-0.81-1.00 and *Brachypodium Super contig 13* is not perfect. The discrepancies between wheat 3DL-0.81-1.00 and *Brachypodium* Super contig 13 limit the development of STS markers for wheat chromosome 3D from the genomic sequences of the *Brachypodium* Super contig 13 based on the collinearity. We designed 46 pairs of STS primers with *Brachypodium* Super contig 13 sequences based on the collinearity, but only one was mapped to wheat chromosome 3D (Table 2; Fig. 2). Therefore, as seen with rice, cautions need to be taken when employing the *Brachypodium* genomic sequence for molecular mapping and gene cloning in wheat.

*Brachypodium* was reported to be more closely related to wheat than to rice (Vogel et al. 2006; Bossolini et al. 2007). The genomic regions of *Brachypodium* and rice corresponding to the wheat 3DL-0.81-1.00 were found to be similar instead in this study. Both *Brachypodium* and rice had 63% of evaluated ESTs collinear with wheat (Table 2). The wheat EST BE426418 detected a locus close to the ones that EST BE444864 corresponded to in both rice and *Brachypodium* (Fig. 3). These results indicate complexity of the collinearity of wheat genomes with *Brachypodium* genome. Therefore, local comparative mapping is suggested before using *Brachypodium* genomic sequences for fine mapping or gene cloning in wheat.

The majority of the cloned resistance genes, including those for disease and insect resistance, encode proteins with a nucleotide-binding site (NBS) domain and a LRR domain (Hammond-Kosack and Jones 1997). NBS domains have been shown to bind and hydrolyze ATP in plants and animals, and ATP binding appears to be essential for signal transduction. The LRR domain is a key determinant of protein–protein interactions (Ellis et al. 2000). For examples, the *Xanthomonas* resistance gene, *Xa1*, in rice (Yoshimura et al. 1998) and the nematode resistance gene, *Cre3*, in wheat (Lagudah et al. 1997) have been shown to be NBS-LRR-like genes. NBS-LRR-like genes can be viable candidates for genes conditioning resistance to pest. In the present study, three genes with DNA binding, or ATP binding, or LRR were found within the rice PAC AP003238. Since the

![Fig. 3](image-url)  
**Fig. 3** Collinearity of the H26 region of wheat (left) with corresponding genomic region of rice chromosome 1 (right). Physical locations corresponding to the EST markers on the genetic map of 3DL are indicated as base pairs on the genomic region of rice. Genetic distances between the markers were indicated as cM to the left of the genetic map. The STS marker *Xrwgs17* was developed directly from the rice genomic sequence.

---

Theor Appl Genet (2009) 118:1589–1599

© Springer
### Table 3  Genes within the rice PAC AP003238

| Gene      | Description                                                      | InterPro                                      |
|-----------|------------------------------------------------------------------|-----------------------------------------------|
| NP_001045455.1 | MAC/Perforin domain containing protein, expressed                | Membrane attack complex component/perforin/complement C9 |
| NP_001045456.1 | Expressed protein                                                | Lipase, active site                           |
| NP_001045457.1 | MUTL protein homolog 1, putative, expressed                      | DNA mismatch repair protein/ATP-binding region, ATPase-like |
| NP_001045458.1 | Serine/arginine repetitive matrix protein 1, putative, expressed | Pistil-specific extensin-like protein/RNA recognition motif, RNP-1/HMG-I and HMG-Y, DNA-binding |
| NP_001045459.1 | Abscisic stress ripening protein 1, putative, expressed         | ABA/WDS-induced protein                       |
| NP_001045460.1 | Abscisic stress ripening protein 2, putative, expressed         | ABA/WDS-induced protein                       |
| Q5JN43_ORYSJ | Pentatricopeptide repeat protein PPR1106-17, putative, expressed | Pentatricopeptide repeat                     |
| NP_001045461.1 | Phosphatidylserine decarboxylase, putative, expressed            | Phosphatidylserine decarboxylase-related/C2 calcium/lipid-binding region, CaLB/CaLB-binding EF-hand/Phosphatidylserine decarboxylase |
| NP_001045462.1 | Expressed protein                                                | Histone H5/Pollen allergen Poa pIX/Ph1 pVI, C-terminal/Antifreeze protein, type I |
| NP_001045463.1 | Expressed protein                                                | Protein of unknown function/Glycyl-tRNA synthetase, alpha2 dimer |
| NP_001045464.1 | Expressed protein                                                | Protein of unknown function/Glycyl-tRNA synthetase, alpha2 dimer |
| NP_001045465.1 | tRNA uridine 5-carboxymethylaminomethyl modification enzyme gidA, putative, expressed | FAD-dependent pyridine nucleotide-disulfide oxidoreductase/Glucose-inhibited division protein/Fumarate reductase/succinate dehydrogenase flavoprotein,N-terminal/Pyrindine nucleotide-disulfide oxidoreductase, class I |
| NP_001045466.1 | ATP binding protein, putative, expressed                         | Serine/threonine protein kinase/Leucine rich repeat, N-terminal/Tyrosine protein kinase/Protein kinase, core/Leucine-rich repeat, typical subtype |
| NP_001045467.1 | Copine-4, putative, expressed                                    | Zinc finger, RING-type/Fungal lignin peroxidase/Copine/von Willebrand factor, type A |

### Table 4  Wheat TCs and their predicted proteins

| Wheat TC | Protein                                            | e-Value | Length (No. of amino acid residues) | Identity (%) |
|----------|----------------------------------------------------|---------|------------------------------------|--------------|
| TC236864 | Unknown (Zea mays)                                 | 9.0e−108| 166                                | 95           |
| TC236865 | Unknown (Zea mays)                                 | 2.0e−88 | 111                                | 91           |
| TC236866 | Unknown (Hordeum vulgare)                          | 2.0e−61 | 67                                 | 86           |
| TC236867 | Integral membrane protein -2B (Taeniopygia guttata)| 0.0e+00 | 224                                | 97           |
| TC237661 | Unknown (Hordeum vulgare)                          | 2.0e−69 | 87                                 | 98           |
| TC237663 | Similar to ETS domain-containing protein EIK-4 (Gallus gallus) | 4.0e−122| 166                                | 98           |
| TC238164 | Unknown (Oryza sativa japonica)                    | 1.0e−97 | 199                                | 80           |
| TC238349 | Unknown (Oryza sativa japonica)                    | 9.0e−49 | 50                                 | 92           |
| TC238351 | Unknown (Zea mays)                                 | 1.0e−63 | 116                                | 87           |
| TC239719 | Protein coding (Danio rerio)                        | 0.0e+00 | 522                                | 99           |
| TC244941 | Unknown (Oryza sativa japonica)                    | 5.0e−125| 207                                | 91           |
| TC244950 | Unknown (Oryza sativa japonica)                    | 3.0e−14 | 54                                 | NS           |
| TC255809 | Unknown (Oryza sativa japonica)                    | 0.0e+00 | 303                                | 82           |
H26 is 1 cM from the marker Xrwws12 derived from the wheat EST BE426418, and the AP003238 is the corresponding PAC of the wheat EST BE426418 in rice, these three genes could be candidate genes for resistance to Hessian fly. On the wheat side, one gene corresponding to TC237663 that was hit by the rice PAC AP003238, was found to have DNA-binding site (ETS domain) (Table 4). However, fine mapping as well as efforts toward cloning are needed to confirm these results.

Acknowledgments We thank Drs. Chao-Chien Jan and Bin Ye for critically reviewing the manuscript. This material is based upon work supported by the USDA-ARS CRIS Project No. 5442-22000-033-00D.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

Ahn S, Anderson JA, Sorrells ME, Tankesly SD (1993) Homoeologous relationships of rice, wheat and maize chromosomes. Mol Genet 241:483–490

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) A new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Berzonsky WA, Ding H, Haley SD, Harris MO, Lamb RJ, Mckenzie RIH, Ohm HW, Patterson FL, Pears F, Porter DR, Ratcliffe RH, Shanower TG (2003) Breeding wheat for resistance to insects. Plant Breed Rev 22:221–296

Bossolini E, Wicker T, Knobel PA, Keller B (2007) Comparison of orthologous loci from small grass genomes Brachypodium and rice: implications for wheat genomics and grass genome annotation. Plant J 49:704–717

Boyko EV, Gill KS, Mickelson-Young L, Nasuda S, Raupp WJ (1999) A high-density genetic linkage map of Aegilops tauschii, the D-genome progenitor of bread wheat. Theor Appl Genet 99:16–26

Cox TS, Hatchett JH (1994) Hessian fly resistance gene H26 transferred from Tritium tauschii to common wheat. Crop Sci 34:958–960

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation Version II. Plant Mol Biol Rep 1:19–21

Devos KM, Gale MD (1993) Extended genetic maps of the homoeologous group 3 chromosomes of wheat, rye, and barley. Theor Appl Genet 85:649–652

Dilbirligi M, Erayman M, Campbell BT, Randhawa HS, Baenziger PS, Dweikat I, Gill KS (2006) High-density mapping and comparative analysis of agronomically important traits on wheat chromosome 3A. Genomics 84:78–87

Distelfeld A, Uauy C, Olmos S, Schlatter A, Dubcovsky J, Fahima T (2004) Microcolinearity between a 2-cM region encompassing the grain protein content locus Gpc-6B1 on wheat chromosome 6B and a 350-kb region on rice chromosome 2. Funct Integr Genom 4:59–66

Draper J, Mur LAJ, Jenkins G, Ghosh-Biswas GC, Bahlak P, Hasterok R, Routledge APM (2001) Brachypodium distachyon: a new model system for functional genomics in grasses. Plant Phys 127:1539–1555

Ellis J, Dodds P, Pryor T (2000) Structure, function and evolution of plant resistance genes. Curr Opin Plant Biol 3:278–284

Erayman M, Sandhu D, Sidhu D, Dilbirligi M, Baenziger PS, Gill KS (2004) Demarcating the gene-rich regions of the wheat genome. Nucl Acids Res 32:3546–3565

Foote T, Griffiths S, Allouis S, Moore G (2004) Construction and analysis of a BAC library in the grass Brachypodium sylvaticum and its use as a tool to bridge the gap between rice and wheat in elucidating gene content. Funct Integr Genomics 4:26–33

Formusoh ES, Hatchett JH, WC Black IV, Stuart JJ (1996) Sex-linked inheritance of virulence against wheat resistance gene H9 in the Hessian fly (Diptera: Cecidomyiidae). Ann Entomol Soc Am 89:428–434

Francki M, Carter M, Ryan K, Hunter A, Bellgard M, Appels R (2004) Comparative organization of wheat homoeologous group 3S and 7L using wheat–rice synteny and identification of potential markers for genes controlling xanthophyll content in wheat. Funct Integr Genomics 4:118–130

Gallun RL, Deay HO, Cartwright WB (1961) Four races of Hessian fly selected and developed from an Indiana population. Purdue University Agriculture Experiment Station Research Bulletin 732, West Lafayette, Indiana

Hammond-Kosack KE, Jones JDG (1997) Plant disease resistance genes. Annu Rev Plant Physiol Plant Mol Biol 48:575–607

Hasterok R, Marasek A, Donnison IS, Armstead I, Thomas A, King IP, Wolny E, Idziak D, Draper J, Jenkins G (2006) Alignment of the genomes of Brachypodium distachyon and temperate cereals and grasses using bacterial artificial chromosome landing with fluorescence in situ hybridization. Genetics 173:349–362

Huo N, Lazo G, Vogel J, Coleman-Derr D, Luo MC, Thilmony R, Garvin DF, Anderson OD, Gu Y-Q (2006) Construction and characterization of two BAC libraries from Brachypodium distachyon, a new model for grass genomics. Genome 49:1099–1108

Huo N, Lazo G, Vogel J, You FM, Ma Y, Hayden DM, Coleman-Derr D, Hill TA, Dvorak J, Anderson OD, Luo MC, Gu Y-Q (2008) The nuclear genome of Brachypodium distachyon: analysis of BAC end sequences. Funct Integr Genomics 8:135–147

Innis MA, Gelfand DH (1990) Optimization of PCR’s. In: Innis MA, Gelfand DH, Sninsky JI, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 3–12

Koba T, Tsunewaki K (1978) Mapping of the s and Ch2 genes on chromosome 3D of common wheat. Wheat Inform Serv 45:46–18

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Kudagamage CJ, Foster JE, Taylor PL, Chen BH (1990) Biotypes of the Hessian fly (Diptera: Cecidomyiidae) identified in the southeastern United States. J Entomol Soc Sci 25:575–580

Kurata N, Moore G, Nagamura Y, Yano M, Yan Y, Minobe Y, Gale M (1994) Conservation of genomic structure between rice and wheat. Bio Technol 12:276–278

Lagudah ES, Moulet O, Appels R (1997) Map-based cloning of a gene sequence encoding a nucleotide-binding domain and a leucine-rich region in the Cre3 nematode resistance locus of wheat. Genome 40:659–665

Lander E, Abrahamson J, Barlow A, Daly M, Lincoln S, Newburg L, Green P (1987) Mapmaker: a computer package for constructing genetic-linkage maps. Cytogenet Cell Genet 46:642

Li WL, Faris JD, Muthukrishnan S, Liu DJ, Chen PD, Gill BS (2001) Isolation and characterization of novel cDNA clones of acidic chitinase and ß-1, 3-glucanase from wheat spikes infected by Fusarium graminearum. Theor Appl Genet 102:353–362

Liu SX, Anderson JA (2003) Targeted molecular mapping of a major wheat QTL for Fusarium head blight resistance using wheat ESTs. Genome 46:817–823

Liu XM, Brown-Guedira GL, Hatchett JH, Owuoche JO, Chen MS (2005) Genetic characterization and molecular mapping of a Hessian fly-resistance gene transferred from T. turdium ssp. diococcus to common wheat. Theor Appl Genet 111:1308–1315
Ma Q, Gill BS, Sorrels ME, Tanksley SD (1993) RFLP markers linked to Hessian fly-resistance genes in wheat (*Triticum aestivum* L.) from *Triticum tauschii* (Coss.) Schmal. Theor Appl Genet 85:750–754

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers J, Appels R (2008) Catalogue of gene symbols for wheat. MacGene 2008. http://www.shigen.nig.ac.jp/wheat/komugi/genes/download.jsp. Accessed on 02 Jan 2009

Munkvold JD, Greene RA, Bermudez-Kandianis CE, La Rota CM, Edwards H, Sorrells SF, Dake T, Benschler K, Kantety R, Linkiewicz AM, Dubcovsky J, Akhunov ED, Dvorak J, Miftahudin Gustafson JP, Pathan MS, Nguyen HT, Matthew DE, Chao S, Lazo GR, Hummel DD, Anderson OD, Anderson JA, Gonzalez-Hernandez JL, Peng JH, Lapitan N, Qi LL, Echalier B, Gill BS, Hassain KG, Kalavacharla V, Kianian SF, Sandhu D, Erayman M, Gill KS, McGuire PE, Quaiser CO, Sorrells ME (2004) Group 3 chromosome bin maps of wheat and their relationship to rice chromosome 1. Genetics 168:639–650

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Negre S, Bernard M, Leroy P (1995) Molecular mapping of wheat homeologous group 3. Genome 38:525–533

Peng JH, Capitan NLV (2005) Characterization of EST-derived microsatellites in the wheat genome and development of eSSR markers. Funct Integr Genom 5:80–96

Perugini LD, Murphy JD, Marshall D, Brown-Guedira G (2008) *Pm37*, a new broadly effective powdery mildew resistance gene from *Triticum timopheevii*. Theor Appl Genet 116:417–425

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD, Akhunov ED, Dvorak J, Linkiewicz AM, Ratnasiri A, Dubcovsky J, Bermudez-Kandianis CE, Greene RA, Kantety R, La Rota CM, Munkvold JD, Sorrells SF, Sorrells ME, Dilbirligi M, Sidhu D, Erayman M, Randhawa HS, Sandhu D, Bondareva SN, Gill KS, Mahmoud AA, Ma XF, Miftahudin Gustafson JP, Conley EJ, Nduati V, Gonzalez-Hernandez JL, Anderson JA, Peng JH, Lapitan NL, Hassain KG, Kalavacharla V, Kianian SF, Pathan MS, Zhang DS, Nguyen HT, Choi DW, Fenton RD, Close TJ, McGuire PE, Quaiser CO, Gill BS (2004) A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics 168:701–712

Ratcliffe RH, Hatchett JJ (1997) Biology and genetics of the Hessian fly and resistance in wheat. In: Bondari K (ed) New developments in entomology. Research Signpost, Scientific Information Guild, Trivandrum, India, pp 47–56

Ratcliffe RH, Safranski GG, Patterson FL, Ohm HW, Taylor PL (1994) Biotype status of Hessian fly (Diptera: Cecidomyiidae) populations from the eastern United States and their response to 14 Hessian fly resistance genes. J Econ Entomol 87:1113–1121

Röder MS, Korzun V, Wendehake K, Plasschke J, Tixier M, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rozen S, Skaltsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Akrawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana, Totawa, NJ, pp 365–368

Sardesai N, Nemacheck JA, Subramaniam S, Williams CE (2005) Identification and mapping of *H32*, a new wheat gene conferring resistance to Hessian fly. Theor Appl Genet 111:1167–1173

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi LL, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25

Vogel JP, Gu Y-Q, Twigg P, Lazo GR, Laudencia-Chingcuanco D, Hayden DM, Donze TJ, Vivian LA, Stumova B, Coleman-Deer D (2006) EST sequencing and phylogenetic analysis of the model grass *Brachypodium distachyon*. Theor Appl Genet 113:186–195

Wang T, Xu SS, Harris MO, Hu J, Liu L, Cai X (2006) Genetic characterization and molecular mapping of Hessian fly resistance genes derived from *Aegilops tauschii* in synthetic wheat. Theor Appl Genet 113:611–618

Ware DH, Jaiswal P, Ni J, Yap IV, Pan X, Clark KY, Teytelman L, Schmidt SC, Zhao W, Chang K, Cartinhour S, Stein LD, McCouch SR (2002) Gramene, a tool for grass genomics. Plant Physiol 130:1606–1613

Williams CE, Collier CC, Sardesai N, Ohm HW, Cambron SE (2003) Phenotypic assessment and mapped markers for *H31*, a new wheat gene conferring resistance to Hessian fly (Diptera: Cecidomyiidae). Theor Appl Genet 107:1516–1523

Xu SS, Cai X, Wang T, Harris MO, Friesen TL (2006) Registration of two synthetic hexaploid wheat germplasms resistant to Hessian fly. Crop Sci 46:1401–1402

Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang Z-X (1998) Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc Natl Acad Sci USA 95:1663–1668

Zantoko L, Shulke RH (1997) Genetics of virulence in the Hessian fly to resistance gene *H3* in wheat. J Hered 88:120–123

Zhang LY, Bernard M, Leroy P, Feuillet C, Sourdille P (2005) High transferability of bread wheat EST-derived SSRs to other cereals. Theor Appl Genet 111:667–687