Screening and analysis of differentially expressed genes from an alien addition line of wheat *Thinopyrum intermedium* induced by *barley yellow dwarf virus* infection

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Abstract: The alien addition line TAI-27 contains a pair of chromosomes of *Thinopyrum intermedium* that carry resistance against *barley yellow dwarf virus* (BYDV). A subtractive library was constructed using the leaves of TAI-27, which were infected by *Schizaphis graminum* carrying the GAV strain of BYDV, and the control at the three-leaf stage. Nine differentially expressed genes were identified from 100 randomly picked clones and sequenced. Two of the nine clones were highly homologous with known genes. Of the remaining seven cDNA clones, five clones matched with known expressed sequence tag (EST) sequences from wheat and (or) barley whereas the other two clones were unknown. Five of the nine differentially expressed sequences (WTJ9, WTJ11, WTJ15, WTJ19, and WTJ32) were highly homologous (identities >94%) with ESTs from wheat or barley challenged with pathogens. These five sequences and another one (WTJ18) were also highly homologous (identities >86%) with abiotic stress induced ESTs in wheat or barley. Reverse Northern hybridization showed that seven of the nine differentially expressed cDNA sequences hybridized with cDNA of *T. intermedium* infected by BYDV. Three of these also hybridized with cDNA of line 3B-2 (a parent of TAI-27) infected by BYDV. The alien chromosome in TAI-27 was microdissected. The second round linker adaptor mediated PCR products of the alien chromosomal DNA were labeled with digoxygenin and used as the probe to hybridize with the nine differentially expressed genes. The analysis showed that seven differentially expressed genes were homologous with the alien chromosome of TAI-27. These seven differentially expressed sequences could be used as ESTs of the alien chromosome of TAI-27. This research laid the foundation for screening and cloning of new specific functional genes conferring resistance to BYDV and probably other pathogens.

Key words: suppression subtractive hybridization (SSH), expressed sequence tag (EST), linker adaptor mediated polymerase chain reaction (LA-PCR), chromosome microdissection.

Résumé : La lignée d'addition TAI-27 contient une paire de chromosomes du *Thinopyrum intermedium,* laquelle porte un gène de résistance pour le virus de la jaunisse nanisante de l'orge (BYDV). Une banque soustractive a été produite à partir de feuilles de la lignée TAI-27, elles-mêmes infectées au stade trois feuilles par le *Schizaphis graminum* portant la souche GAV du BYDV ou un témoin. Neuf gènes exprimés différentiellement ont été identifiés parmi 100 clones choisis au hasard et séquencés. Deux des neuf gènes étaient très homologues à des gènes connus. Des sept autres clones d'ADNc, cinq montraient une homologie significative avec des séquences EST du blé ou de l'orge, tandis que les deux autres ne montraient aucune homologie. Cinq des neufs séquences exprimées différentiellement (WTJ9, WTJ11, WTJ15, WTJ19 et WTJ32) étaient très homologues (identité >94 %) avec des EST du blé ou de l'orge inoculés avec des pathogènes. Ces cinq séquences ainsi qu'une autre (WTJ18) étaient fortement homologues (identité >86 %) aussi à des EST induits par des stress abiotiques chez le blé ou l'orge. Des hybridations northern inversées ont montré que sept des neuf clones exprimés différentiellement hybridaient avec l'ADNc du *T. intermedium* infecté avec le BYDV. Trois de ceux-ci hybridaient également avec l'ADNc de la lignée 3B-2 (un des parents de la lignée TAI-27)

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infectée avec le BYDV. Le chromosome exotique présent chez TAI-27 a fait l'objet d'une microdissection. Les produits PCR issus d'une seconde ronde d'amplification avec un adaptateur ont été marqués à la dioxygénine et employés pour hybrider sur les neuf clones exprimés différentiellement. Cette analyse a montré que sept des gènes différentiellement exprimés étaient homologues à des séquences du chromosome exotique présent chez TAI-27. Ces séquences à expression différentielle pourraient servir d'EST pour le chromosome TAI-27. Cette recherche fournit des assises pour le criblage et le clonage de nouveaux gènes conférant la résistance au BYDV ou à d'autres pathogènes.

Mots clés : hybridation soustractive suppressive (SSH), étiquette de séquence exprimée (EST), amplification PCR à l'aide d'un adaptateur, microdissection

[Traduit par la Rédaction]

Introduction

Barley yellow dwarf virus (BYDV) causes serious yield losses in all cereals worldwide. Annual yield reductions resulting from BYDV average 1–3%, although losses are 10-fold greater in some seasons (Burnett 1987). Resistance genes against BYDV are not found in common wheat but are available in barley, oat, and some wild Triticeae species. The *Yd2* gene from a number of Ethiopian barleys (*Hordeum vulgare*) has been the most effective means of providing resistance against BYDV in cultivated barley and it has been mapped to near the centromere on the long arm of barley chromosome 3 (Collins et al. 1996). To date, no resistance genes against BYDV have been cloned, but several polypeptide markers and PCR markers linked with BYDV resistance have been reported (Holloway and Heath 1992; Paltridge et al. 1998; Wang et al. 2002) and are being used routinely in wheat breeding (Ayala et al. 2001; Stoutjesdijk et al. 2001; Xin et al. 2001; W. Zhang et al. 2001).

Wheat *Thinopyrum intermedium* alien addition line TAI-27 is one of 14 alien addition lines carrying a pair of chromosomes from *T. intermedium* (*Agropyrom intermedium*) in common wheat (He et al. 1988). TAI-27 possesses resistance to BYDV located on the alien chromosomes (Zhang et al. 1991; Han et al. 1998; Tian et al. 2000). Tian et al. (2000) showed that TAI-27 possessed two pairs of St chromosomes, one being the disomic addition pair and the other pair substituting for a pair of wheat chromosomes. Liu et al. (2001) provided evidence that TAI-27 has one group 2 and one group 7 alien chromosome pair. Furthermore, Z.Y. Zhang et al. (2001) showed that a group 2 St chromosome derived from the same source (partial amphiploid Zhong 4 awnless), as TAI-27 is responsible for BYDV resistance. Therefore, it appears that the BYDV resistance bearing chromosome in TAI-27 is the same group 2 chromosome as addition line Z1 (Larkin et al. 1995; Han et al. 2003).

Suppression subtractive hybridization (SSH) was developed on the basis of representational difference analysis (Diatchnko et al. 1996) and has been used to study and clone differentially expressed genes, especially for human diseases (Yang et al. 1999; Yang et al. 2002; Boengler et al. 2003; Kiss et al. 2003). More recently, Luo et al. (2002) constructed an SSH cDNA library from wheat leaves inoculated by *Erysiphe graminis* DC to isolate genes that were involved in the powdery mildew resistance in wheat. Bahn et al. (2001) screened differentially expressed genes from low temperature induced winter barley. Some genes had been cloned using the SSH technique (Yoshimura et al. 1998; Kim et al. 1999*a*, 1999*b*; Bahn et al. 2001; Zhang et al. 2002). In this research, we screened for and characterized differentially expressed genes from wheat *Thinopyrum* alien addition line TAI-27 infected by BYDV using the SSH technique and characterized their genetic background. The information should be useful in understanding the mechanism of TAI-27 resistance against BYDV and for cloning resistance genes from the alien chromosome.

Materials and methods

Materials

Germplasm used included wheat *Thinopyrum* alien addition line TAI-27 (AABBDD plus a pair of St genome chromosomes derived from *T. intermedium*, 2*n* = 44) along with its two parents *T. intermedium* ($E_1E_1E_2E_2$StSt with the St genome originated from the St genome of *Pseudoroegneria*, 2*n* = 42) (Zhang et al. 2000) and 3B-2 (*Triticum aestivum*, AABBDD, 2*n* = 42, the maternal parent). All materials were kindly supplied by Professor Menyuan He of the Northeast Normal University and Professor Xiangqi Zhang of the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences.

BYDV inoculating and material sampling

Wheat *Thinopyrum* alien addition line TAI-27, *T. intermedium*, and 3B-2 were planted and their leaves at the three-leaf stage were infected by *Schizaphis graminum* carrying the GAV strain of BYDV to induce resistance expression. Leaves were sampled once every 2 h, six times, on the day of inoculation and then once a day until 3B-2 turned yellow and ceased growing 3 weeks later. The samples collected were processed to isolate total RNA. Sampling of the control, that without BYDV inoculation, followed the same procedure as described above.

Subtractive hybridization and suppression PCR

Subtractive hybridization and suppression PCR were carried out according mainly to the method developed by Diatchenko et al. (1996).

Oligonucleotides

Adaptors

Adaptor I: 5′-GTAATACGACTCACTATAGGGCTCGAG-CGGCCGCCCGGGCAGGT-3′, 3′-CCCGTCCA-5′; adaptor II: 5′-TGTAGCGTGAAGACGACAGAAAGGGCGTGGTG-CGGAGGGCGGT-3, 3′-GCCTCCCGCCA-5′.

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PCR primers

P1: 5′-GTAATACGACTCACTATAGGGC-3′; P2: 5′-TGT-AGCGTGAAGACGACAGAA-3′; PN1: 5′-TCGAGCGGC-CGCCCGGGCAGGT-3′; PN2: 5′-AGGGCGTGGTGCGGA-GGGCGGT-3′.

Preparation of driver and tester, subtractive hybridization, and suppression PCR

The wheat *Thinopyrum* alien addition line TAI-27 inoculated with BYDV was used as tester and the control (noninoculated) as driver. Total RNA of tester and driver were isolated with a TRIzol kit (Gibco, BRL, Rockville, Md.), mRNA was isolated and purified with an mRNA isolation kit (Stratagene, La Jolla, Calif.), and cDNAs were synthesized with a ZAP-cDNA synthesis kit (Stratagene) using 5 μg of mRNA from tester and driver, respectively. After *Hae*III digestion, tester cDNA was divided into two groups and ligated to adaptor I and adaptor II, respectively. A 300× excess of driver was added to each of the testers, which were ligated to adaptor I and adaptor II, respectively, in two tubes and allowed to anneal for 10 h at 68 °C. After the first hybridization, the two samples were combined and a fresh portion of 60–80× excess driver was added and then hybridized for an additional 10 h at 68 °C. The final hybridization solution was diluted 200 times with dilution buffer. Two rounds of suppression PCR were performed according to the instructions of a Clontech PCR-Select^{TM} cDNA subtraction kit (Clonetech, Palo Alto, Calif).

Constructing the subtractive cDNA library

Products from the secondary suppression PCR were inserted into a PUCmT-Vector (Sagon, Shanghai, China). Recombinant plasmids (white colonies) were isolated by alkaline lysis. The Bio-Asia Company (Shanghai, China) carried out DNA sequencing. Nucleic acid homology searches were performed using the BLAST program at the GenBank–EMBL website.

Screening of differentially expressed genes from the subtractive library

Inserts of 100 randomly selected recombinant plasmids were released by PCR amplification using primers PN1 and PN2 and dotted onto nylon membranes (Hybond$^+$; Amersham, Piscataway, N.J.). Dot hybridization was carried out by using 1–2 μg of cDNA of tester and driver digested by *Hae*III and labeled by digoxygenin (DIG) as the probe. Two parallel membranes were prepared to hybridize with the tester and driver, respectively. Hybridization and detection were performed following the instructions of the DIG DNA labeling and detection kit (Roche, Indianapolis, Ind.).

Analysis of genetic background of the differentially expressed genes by reverse Northern hybridization

Total RNA of *T. intermedium* and 3B-2 infected by BYDV (sampled by same procedures as those for tester and driver) were isolated with a TRIzol kit (Gibco) and the first strand of cDNAs were synthesized with 10 μg of total RNA using a cDNA synthesis kit (TaKaRa, Japan). The hybrid strands of cDNA and mRNA were adsorbed by magnetic beads (mRNA Isolation System III; Promega, Madison, Wis.) for purification. The second strand of cDNA was synthesized directly on the magnetic beads (cDNA synthesis kit, TaKaRa) and washed away from the magnetic beads with *Sau*3A buffer three times after synthesis. The cDNA was digested with 0.2 U of *Sau*3A for 2 h at a 50-μL volume and then purified by phenol–chloroform extraction and finally resuspended in 20 μL of distilled water. Five nanograms of prepared *Sau*3A linker adaptors and 1 U of T4 ligase (Roche) were added to 4 μL of digested cDNA. The ligation between the adaptor and digested cDNA was performed at 16 °C for 16 h. The *Sau*3A linker adaptors, with the sequences 5′-GATCCTGAGCTCGAATTCGACCC-3′ and 5′-GGGTCGAATTCGAGCTCAG-3′, were prepared as described by Albani et al. (1993). Two rounds of PCR were performed to amplify cDNA with the primer 5′-GGGTCG-AATTCGAGCTCAG-3′, which corresponds to the *Sau*3A linker adaptor. The first-round PCR was performed in a 20-μL volume with 35 cycles of 30 s at 94 °C, 30 s at 60 °C, and 1 min at 72 °C, followed by a final extension at 72 °C for 7 min. The second-round PCR was carried out using 1 μL of the first-round products as template. The PCR program was the same as described above, except 20 cycles of amplification were used.

The differentially expressed gene fragments were released by PCR, separated by electrophoresis, and transferred onto nylon membranes (Hybond$^+$, Amersham). One to two micrograms of products of the second-round PCR of the cDNA of *T. intermedium* and 3B-2 was labeled by digoxigenin-11-dUTP as the probe. Reverse Northern hybridization and detection were performed following the instructions of the DIG DNA labeling and detection kit (Roche).

Characterization of chromosome location of the differentially expressed genes

The alien chromosome in TAI-27 was microdissected and its DNA was amplified according to the procedures described by Wan et al. (2000). In brief, the alien chromosome in TAI-27 was microdissected by using the fixed glass needle on the arm of a LeitZ microoperation instrument on an inverted microscope. The microdissected chromosome was digested with 20 μL of proteinase K solution (19 ng/μL in 1× T4 ligase buffer) and then 0.02 unit of *Sau*3A (Promega) in an Eppendorf tube. Subsequently, the chromosomal DNA was amplified using *Sau*3A linker adaptor mediated PCR (LA-PCR). *Sau*3A linker adaptors were prepared with the 23mer DNA sequence 5′-GATCCTGAGCTCGAATTCGAC-CC-3′ and the 19mer DNA sequence 5′-GGGTCGAATTCG-AATTCGAGCTCAG-3′. The digested chromosomal DNA was linked with the *Sau*3A adaptor (2 μL, 5 ng/μL) using T4 DNA 1igase (0.5 μL, 3U/μL) (Promega) in a total volume of 24.5 μL. Two rounds of PCR were performed. The first round was carried out in the same tube by adding 10 μL of 10× *Taq* buffer, 6 μL of 25 mmol/L $MgCl_2$, 2 μL of 10 mmol/L dNTPs, 1 μL of 19mer primer (50 ng/μL), 2 U of *Taq* DNA polymerase (Promega), and double distilled water in a 100-μL total volume. After denaturing at 94 °C for 5 min, amplification was performed with 35 cycles of 1 min at 94 °C, 1.5 min at 50 °C, and 3 min at 72 °C, followed by a final 15-min extension at 72 °C. The second round was done under the same conditions described above except that only a 2-μL product from the first round was used as the template. The second-round PCR products were labeled with

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Fig. 1. Electrophoresis pattern of the second-round suppressing PCR with the subtracted cDNA as template. Lane 1, amplification with double primers (PN1 and PN2); lane 2, molecular mass marker DL2000; lanes 3 and 4, amplification with a single primer, PN1 or PN2, respectively.

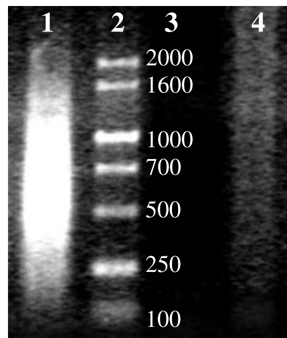
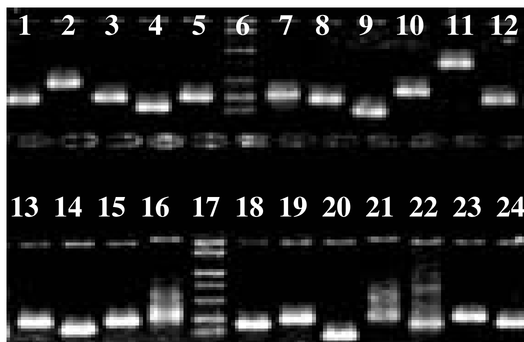


Fig. 2. Analysis of some clones in the suppressed subtractive library of wheat *Thinopyrum* alien addition line TAI-27. Lanes 6 and 17 are molecular mass marker DL2000.



DIG-11-dUTP as the probe. The released differentially expressed gene fragments were separated by electrophoresis and transferred onto nylon membranes (Hybond$^+$, Amersham) and hybridized with the probe described above.

Results

Performance of TAI-27, *T. intermedium*, and 3B-2 infected by BYDV

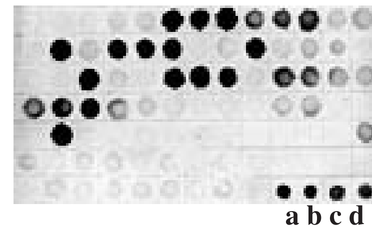
3B-2 started to show symptoms 15 days postinfection with BYDV and leaf tips began to turn yellow. The symptoms became more severe 3 weeks later and the plants eventually died without seedset. TAI-27 showed few BYDV symptoms 17 days postinfection and its moderate resistance enabled the plant to set seeds. *Thinopyrum intermedium* expressed high resistance against BYDV through its life cycle.

Construction of the subtractive library

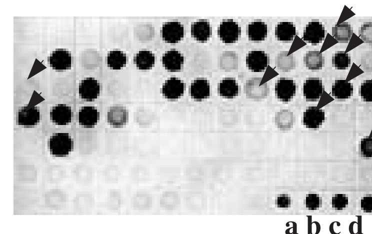
After the second hybridization of cDNA, two rounds of suppressed PCR were performed using a single primer, PN1 or PN2, and double primers PN1 and PN2. There were no evident PCR products when PN1 was used as the primer and there was a weak smear of high molecular mass DNA when PN2 was used alone (Fig. 1). When double primers PN1 and PN2 were used, many PCR products ranging from 100 to 2000 bp were obtained (Fig. 1), indicating that hybridization and suppression were successful in the experiment.

The fragments ranging from 150 to 1000 bp produced by the second-round PCR with PN1 and PN2 as the primers were recovered and used to construct a library by using a PUCmT-Vector. Nearly 400 recombinant clones were obtained. The molecular mass of most clone inserts was around 200 bp (Fig. 2).

Fig. 3. Partial differentially expressed cDNA sequence screening. Hybridization between partial differentially expressed cDNA sequences with two probes: (*i*) non-virus-induced TAI-27 cDNA and (*ii*) virus-induced TAI-27 cDNA. a, tester; b, driver; c, genomic DNA of wheat *Thinopyrum* alien addition TAI-27; d, PCR products of the alien addition chromosome. Arrows indicate some differentially expressed cDNA sequences between Figs. 3*i* and 3*ii*.



i



ii

Screening of differential expressed genes from the suppression subtractive library

Hybridization between released inserts from 100 randomly selected recombinant clones in the suppression subtractive library and tester and driver cDNA was carried out. Differences in the intensity of hybridization signals were observed for many clones (Fig. 3). Twenty clones that showed positive or stronger signals when hybridizing with the tester cDNA but possessing no or weaker signals with the driver cDNA were selected. Further screening with electrophoresis was undertaken to eliminate duplicates in the differentially expressed genes. Nine clones were selected, sequenced, and analyzed with a Blast search in the GenBank/EMBL database. Results were listed in Table 1. Of the nine differentially expressed cDNA sequences, two clones (WTJ9 and WTJ11) were highly homologous (identities >90%) with known genes. Of the remaining seven cDNA clones, five (WTJ3, WTJ15, WTJ18, WTJ19, and WTJ32) matched known expressed sequence tag (EST) sequences from wheat and (or) barley, but two clones (WTJ26 and WTJ34) had no hits in the database. Five of the nine differentially expressed sequences (WTJ9, WTJ11, WTJ15, WTJ19, and WTJ32) were highly homologous with ESTs

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Table 1. Nine differentially expressed genes.

Name	Length (bp)	GenBank accession No.	Homological comparison with the data in the GenBank–EMBL database: identities	Sequence property
WTJ3	198	BQ788525	EST of barley and wheat: 141/142–87/92	poly(A) tail
WTJ9	168	BQ788527	*Hordeum vulgare* (pMaW25) mRNA for β-ketoacyl-ACP synthase: 152/163	
			EST from cold-acclimated and salt-stressed wheat: 164/164	
			EST from heat stress in wheat (*Triticum aestivum*): 164/164	
			EST from wheat after treatment with 6-iodo-3-propyl-2-propyloxy-4(3H)-quinazolinone: 164/164	
			EST from wheat after inoculation with *Erysiphe graminis* and 6-iodo-3-propyl-2-propyloxy-4(3H)-quinazolinone: 164/164	
			EST from wheat after inoculation with *Erysiphe graminis*: 164/164	
			EST from wheat yellow rust infested tissue JIC: 164/164	
			EST from wheat after inoculation with leaf rust pathogen *Puccinia triticina* race BBB carrying the avirulence gene *Avr1*: 164/164	
			Wheat *Fusarium graminearum* infected spike cDNA library: 164/164	
WTJ11	161	BQ788528	*Triticum turgidum* mRNA for ADP/ATP carrier: 161/161	
			SSH cDNA library from genotype CI14106 (wheat line) cold hardened: 161/161	
			Chinese spring wheat drought stressed leaf cDNA library: 161/161	
			EST from cold-acclimated and salt-stressed wheat: 160/161	
			KSU wheat *Fusarium graminearum* infected spike cDNA library: 159/161	
WTJ15	180	BQ788529	Wheat, Polk cultivar (resistant), infected with *Septoria tritici* strain A: 149/150	
			EST from heat-shocked seedlings *Sorghum bicolor*: 136/156	
			An EST database from *Sorghum*, floral-induced meristems: 135/156	
WTJ18	275	BQ788530	Functional genomics of abiotic stress (cold acclimation) in wheat: 163/184	
WTJ19	384	BQ788526	EST from wheat inoculation with leaf rust pathogen *Puccinia triticina* race BBB carrying the avirulence gene *Avr1*: 324/328	poly(A) tail
			SSH cDNA library from CI14106 (wheat line) genotype cold hardened: 316/326	
			EST from wheat heat-stressed seedling cDNA library: 286/291	
			EST from wheat after inoculation with *Erysiphe graminis* f.sp tritici: 300/310	
			Drought-stressed Dicktoo barley epidermis cDNA library: 297/316	
			Wheat *Fusarium graminearum* infected spike cDNA library: 168/173	
			Riband (susceptible) wheat leaves infected with *Septoria tritici* strain A: 86/90	
WTJ26	141	BQ788531	Unknown	
WTJ32	353	BQ788532	EST from heat-stressed flag leaf cDNA library of *Triticum aestivum* cDNA clone: 234/236	
			Barley ESTs from pathogen (*Blumeria graminis*) attacked leaf epidermis: 332/350	
WTJ34	210	BQ788533	Unknown	

from wheat or barley after inoculation with pathogen (identities >94%). The five sequences mentioned above and another one (WTJ18) were highly homologous with abiotic stress induced ESTs from wheat or barley (identities >86%). Two of the nine differentially expressed cDNA sequences (WTJ3 and WTJ19) possessed a polyA tail. These nine differentially expressed cDNA sequences cloned from wheat *Thinopyrum* alien addition line TAI-27 have been registered in GenBank (Table 1).

Analyzing the genetic origin of the differentially expressed genes by reverse Northern hybridization

The nine differentially expressed cDNA sequences were released by PCR, separated by electrophoresis, and transferred onto nylon membranes (two parallel membranes) and hybridized with DIG-11-dUTP-labeled cDNA of infected *T. intermedium* and 3B-2, respectively. The hybridization results showed that seven of the nine differentially expressed cDNA sequences could be hybridized with *T. intermedium*. Three of these could also be hybridized with 3B-2, although with weaker hybridization signals than those from hybridization with *T. intermedium*. The other four cannot be hybridized with 3B-2. Two of the nine clones (WTJ15 and WTJ26) could not be hybridized with *T. intermedium* and 3B-2 (Figs. 4*b* and 4*c*). However, one of these two clones (WTJ26) was detected by Southern hybridization using PCR products from the alien chromosome in TAI-27 as the probe (Fig. 4*d*).

Chromosome location of the nine differentially expressed cDNA sequences

The alien chromosome in TAI-27 was isolated by a glass needle and its DNA was amplified by Sau3A linker adaptor mediated PCR. The nine differentially expressed cDNA se-

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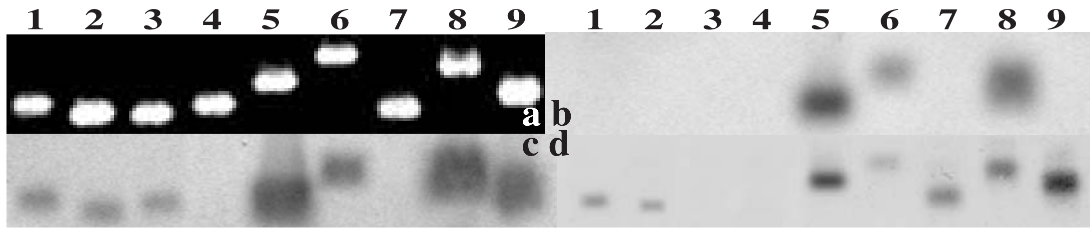
Fig. 4. Analysis of genetic background of the nine differentially expressed cDNA sequences. (*a*) Electrophoresis of nine differentially expressed cDNA fragments released by PCR with PN1 and PN2. (*b* and *c*) Reverse Northern hybridization of nine differentially expressed cDNA fragments by using cDNA of 3B-2 and *T. intermedium* induced with BYDV inoculation as the probe, respectively. (*d*) Southern hybridization of nine differentially expressed cDNA fragments by using the PCR products of the alien chromosome of TAI-27 as the probe. 1, WTJ3; 2, WTJ9; 3, WTJ11; 4, WTJ15; 5, WTJ18; 6, WTJ19; 7, WTJ26; 8, WTJ32; 9, WTJ34.



quences were tested by hybridizing with PCR products of the microdissected chromosome. Seven of the nine cDNA sequences hybridized with the isolated alien chromosome, but only six hybridized with *T. intermedium*. Although WTJ11 hybridized with *T. intermedium*, it could not be hybridized with the PCR products of the isolated alien chromosome. It is possible that the PCR products of the alien chromosome did not cover the whole alien chromosome (Jung et al. 1992) or that WTJ11 was lost because of partial deletion of the alien chromosome (Wan et al. 2000). On the other hand, WTJ26 that did not hybridize with cDNA of 3B-2 and *T. intermedium* hybridized with the alien chromosome in TAI-27 (Fig. 4*d*).

Discussion

Previous studies showed that TAI-27 expressed resistance against BYDV in the field (He et al. 1988; Zhang et al. 1991; Tian et al. 2000). Our results further confirmed the previous work. According to our results, the resistance of TAI-27 is stronger than that of common wheat but weaker than that of *T. intermedium*, suggesting that only some of the resistance genes in *T. intermedium* were transferred into common wheat with the addition of a pair of chromosomes from *T. intermedium* in the TAI-27. Other resistance genes must be located on other chromosomes of *T. intermedium*.

The normalization step of SSH procedures equalizes the wide differences in abundance of different mRNA species. Consequently, differentially expressed genes of low abundance and high abundance can be isolated. In a model system, the SSH technique enriched rare sequences over 1000-fold in one round of subtractive hybridization (Diatchenko et al. 1996; Gurskaya et al. 1996). In our research, WTJ26 may be a low abundantly expressed gene from the alien chromosomes of TAI-27, although it is present in moderately high copy number in the dissected chromosome of *T. intermedium* in TAI-27. The normalization step in the subtractive hybridization procedure equalizes the abundance of cDNA within the target population and enriched the rare sequence WTJ26 in virus-induced TAI-27.

Defense response genes belong to a broad class of genes involved in plant defense, including hypersensitive response genes, pathogenesis-related genes, genes for the flavonoid metabolic pathway, genes encoding proline/glycine-rich proteins, ion channel regulators, lipoxygenase, lectin, and others (Li et al. 1999). WTJ3, WTJ26, and WTJ34 cannot be grouped into the classes mentioned above for lack of information. Six out of the nine differentially expressed sequences (WTJ9, WTJ11, WTJ15, WTJ18, WTJ19, and WTJ32) are homologous with ESTs from wheat or barley induced by abiotic or biotic stress. It suggests that TAI-27 infected by BYDV is induced in gene expression, and most of which are commonly expressed genes induced by other abiotic and (or) biotic stresses. WTJ26 and WTJ34 may be specifically expressed genes induced by BYDV and need to be further investigated.

WTJ18, WTJ19, and WTJ32 are expressed in 3B-2 and *T. intermedium*, and TAI-27, WTJ3, WTJ9, and WTJ34 are expressed in *T. intermedium* and TAI-27 but not in 3B-2. These specially expressed genes in TAI-27 were from *T. intermedium* and were further confirmed by their hybridization patterns with the alien chromosome DNA of TAI-27.

Chromosome microdissection and microcloning are efficient tools to do chromosome-specific or chromosome-region-specific molecular biology (Scalenghe et al. 1981; Jung et al. 1992; Chen and Armstrong 1995). In this experiment, we initially used chromosome microdissection and microcloning techniques to approximately characterized the location of differentially expressed genes in TAI-27 induced by BYDV. Seven differentially expressed genes, WTJ3, WTJ9, WTJ18, WTJ19, WTJ26, WTJ32, and WTJ34, were located on the alien chromosome of TAI-27. The seven differentially expressed genes can be taken as ESTs of the alien chromosome of TAI-27 induced by BYDV.

In conclusion, we firstly constructed the subtractive cDNA library of wheat *Thinopyrum* alien addition line TAI-27 induced by BYDV and then selected nine differentially expressed genes from the library and further analyzed them by reverse Northern and chromosome microdissection and microcloning techniques. Seven differentially expressed genes were located in the alien chromosome of TAI-27. Two unique ESTs are reported here that require further investigation to determine if they play a specific role in the virus resistance of TAI-27.

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