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ORIGINAL PAPER

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Development of novel PCR markers linked to the BYDV resistance gene *Bdv2* useful in wheat for marker-assisted selection

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Abstract The distal segment of the long arm of the *Thinopyrum intermedium* chromosome 7Ai1 carries the barley yellow dwarf virus (BYDV) resistance gene *Bdv2*. This segment was transferred to the distal region of the long arm of wheat chromosome 7D in the Yw series of translocation lines by using the *ph1b* mutant to induce homoeologous pairing. To transfer *Bdv2* to commercial varieties, we developed two resistance gene-analog polymorphism (RGAP) markers, Tgp-1_{350} and Tgp-2_{210}, and one randomly amplified polymorphic DNA (RAPD) marker, OPD04_{1300}. The diagnostic fragments of the RGAP marker Tgp-1_{350} and the RAPD marker OPD04_{1300} were cloned, sequenced and converted into sequence-characterized amplified region (SCAR) markers, named *SC-gp1* and *SC-D04*, respectively. *SC-gp1* and *SC-D04* were validated based on available translocation lines and segregating F_2 individuals. Our results indicated that the SCAR markers co-segregated with the BYDV resistance associated with *Bdv2*. Therefore, they can be used as a low-cost, high-throughput alternative to conventional phenotypic screening in wheat-breeding programs exploiting *Bdv2*. The marker-assisted selection for BYDV resistance was successfully performed in a wheat-breeding program.

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Introduction

Barley yellow dwarf virus (BYDV) is vectored by aphids and causes one of the most economically important virus diseases of wheat, barley, oat and other grain cereals (Conti et al. 1990). As no resistance to BYDV has been found in wheat despite widespread testing of germplasm collections, plant breeders have been forced to derive the resistance from the wild relatives of wheat (Brettell et al. 1988). *Thinopyrum intermedium* (Host) Barkworth and Dewey (also known as *Agropyron intermedium*, intermediate wheatgrass) carries BYDV resistance genes on both group 7 (7Ai1, Brettell et al. 1988; Xin et al. 1988; Sharma et al. 1995) and group 2 chromosomes (2Ai-2, Larkin et al. 1995). The BYDV resistance gene on 7Ai1(7X) was first identified in the disomic addition line L1 and was located on the long arm (Brettell et al. 1988; Xin et al. 1988; Friebe et al. 1992). This BYDV resistance gene was designated *Bdv2* (McIntosh et al. 2001).

Using L1 as the resistance source and cell culture technology, a series of wheat-*Th. intermedium* 7D-7Ai1 translocation lines carrying the *Bdv2* gene were developed: TC5, TC6, TC8 TC9, TC10 and TC14 (Banks et al. 1995). At the same time, a second series was developed—the Yw series, consisting of T7DS.7DL-7Ai1L translocation lines carrying *Bdv2* from L1—by inducing homoeologous pairing using the *ph1b* mutation (Xin et al. 1991, 2001). The results of genomic in situ hybridization (GISH) and restriction fragment length polymorphism (RFLP) analysis indicated that a small segment of 7Ai1L had been transferred to 7DL of wheat in the Yw translocation lines (Zhang et al. 1999, 2000). The Yw translocation lines show a better resistance to the GAV and GPV serotypes of BYDV that are prevalent in China and have been used as important resistant parents in wheat breeding.

Practical difficulties exist in the introgression of *Bdv2* carrying translocations into elite wheat varieties, particularly when resistance bioassays must be relied upon. In the field, natural infection is, in fact, rarely uniform. If

viruliferous aphids have to be used in the field, considerable skill and a time-consuming infrastructure are required to rear and release adequate populations. Field-based bioassays can be carried out only once a year. Laboratory-based bioassays can be conducted throughout the year, but they depend on maintaining viruliferous aphids and conducting large numbers of ELISA assays. Despite the availability of bioassay protocols and appropriate antibodies, plant breeders have proved reluctant to devote the resources needed for such bioassays. Marker-assisted selection (MAS) is one way to overcome these difficulties.

Although several RFLP markers and one randomly amplified polymorphic DNA (RAPD) marker for the TC series of translocations carrying *Bdv2* have been reported (Banks et al. 1995, 1996; Hohmann et al. 1996), none are suitable for large-scale selection. Ayala et al. (2001) identified the simple sequence repeat (SSR) marker gwm37, which is diagnostic for TC translocations. However, sequence-characterized amplified region (SCAR) markers are generally simpler and more amenable to high-throughput than SSR markers (Paran and Michelmore 1993). A SCAR marker (SC-A07$_{566}$) was converted from a RAPD marker OPA07 to detect the 7Ai1L segment in the TC series (Stoutjesdijk et al. 2001), but this marker was not capable of detecting the 7AilL segment in the Yw translocations in our Chinese laboratory. In addition, more diagnostic markers for the 7Ai1L segment containing *Bdv2* are necessary for fine mapping of the gene and for future genomic studies.

The presence of conserved domains in cloned resistance genes of plants—such as leucine-rich repeats (LRR), nucleotide-binding site (NBS) and serine/threonine kinase domains—offers the opportunity to isolate similar sequences—resistance gene analogs (RGAs)—in other plant species (Leister et al. 1996; Yan et al. 2003). The RGA approach has been used to isolate plant resistance gene candidates and to develop molecular markers using agarose-gel electrophoresis and RFLP analysis (Kanazin et al. 1996; Leister et al. 1996; Yu et al. 1996; Feuillet et al. 1997; Shen et al. 1998; Seah et al. 2000). However, the polymorphic markers obtained using RGAs as probes in the RFLP analysis are time-consuming to work with (Yan et al. 2003). Chen et al. (1998) improved the RGA approach by using high-resolution electrophoresis on polyacrylamide gel and a sensitive detection method to separate the PCR products. This technique was referred to as resistance gene-analog polymorphism (RGAP) (Chen et al. 1998). The RGAP technique has been used successfully to develop molecular markers for genes conferring resistance to stripe rust, leaf rust, scald, net blotch, barley yellow dwarf and scab of barley and to strip rust of wheat (Chen et al. 1998, 1999; Toojinda et al. 2000; Shi et al. 2001; Yan et al. 2003). However, to date no RGAP markers for *Bdv2* have been reported.

In this paper, two RGAP markers, Tgp-1$_{350}$ and Tgp-2$_{210}$, and one RAPD marker, OPD04$_{1300}$, all linked to *Bdv2* in the Yw series translocation lines, were developed. The markers of Tgp-1$_{350}$ and OPD04$_{1300}$ were cloned and converted into stable SCAR markers. The SCAR markers were analyzed in a segregating F_2 population and successfully used to introgress *Bdv2* into a current wheat-breeding program.

Materials and methods

Plant materials

The Yw translocation lines Yw642, Yw443, Yw243 and Yw1029 were developed using the *ph1b* mutant (Xin et al. 2001). LH64C was developed by CSIRO Plant Industry, Canberra, Australia, and the genotype is a BYDV-resistant Australian winter wheat (released as cv. Mackellar) carrying the cell culture-derived TC14 translocation. Both a wheat-*Thinopyrum intermedium* amphiploid TAF46 (2*n*=56) and a disomic addition line L1 (2*n*=44) derived from TAF46 were developed and kindly supplied by Dr. Y. Cauderon (Cauderon et al. 1973), INRA, Paris, France. A ditelosomic addition line of the 7Ai1 long arm (7Ai1L) from L1 was developed by one of the authors, Dr. P. Larkin (Larkin et al. 1995). The *Th. intermedium* accession was collected and conserved at the Institute of Crop Breeding and Cultivation, CAAS, Beijing, China. The addition line Z1 was derived from Zhong 4 Awnless, which has a different BYDV resistance gene on *Th. intermedium* chromosome 2Ai-2 (Larkin et al. 1995).

Wheat cvs. Zhong8601, Zhong7902, Chinese Spring (CS) and Vilmorin27 (Vi, a wheat parent of L1) were the wheat parents of the Yw translocation lines, and Zhongmai16 was the recurrent wheat parent used in the breeding program; all were collected or developed by the Institute of Crop Breeding and Cultivation, CAAS, Beijing, China. The susceptible line Yw641 was derived from Yw642. An F_2 population of 256 individuals was developed from the cross Yw642 × Zhong8601.

M53 is a synthetic wheat material with resistance to powdery mildew supplied by Dr. A. Mujeeb-Kazi, CIMMYT, Londres, Mexico, which was used as a resistance parent in the multi-resistant wheat breeding.

Py1 to Py6 are F_1 plants of Yw642/M53 × Zhongmai16. Bcy1 to Bcy630, backcross (BC) plants, were obtained by backcrossing Py1 to Py6, or their progenies possessing resistance to both BYDV and powdery mildew, to the elite variety Zhongmai16.

Field test for resistance to BYDV

Resistance to BYDV was tested in the field. At the three-leaf stage, F_1, F_2 and BC plants, as well as their parents, were infested with BYDV-infective aphids. Approximately ten aphids infected with the BYDV-GAV serotype were deposited at the base of each seedling. After 2 weeks, the aphids were eliminated by insecticide application. The infection type (IT), based on a 0–9 scale of leaf discoloration or plant dwarfness (http://wheat.pw.usda.gov/gg-pages/sxg/pheno.htm), was recorded 30 days after inoculation.

DNA extraction

DNA was extracted from fresh leaves following the protocol described by Sharp et al. (1988) and kept in 1× TE buffer.

RGAP analysis

The primers used in this study were designed based on conserved motifs of cloned resistance genes (Table 1). The RGAP protocol described by Chen et al. (1998) was used with a minor modification. For each PCR reaction, the 20-μl volume contained 50 ng of genomic DNA, 0.5 μM of each of two primers, 0.2 mM each of dNTPs (Dalian-Takara, Dalian, China), 1× PCR buffer (Dalian-

Table 1 Sequences of resistance gene analog primers used to develop markers specific for *Bdv2*

Primer	Sequence(5′–3′)	Gene	Domain[a]	References
Pto-Kin1IN	AAGTGGAACAAGGTTACG	*Pto*	Kinase	Shi et al. (2001)
Pto-Kin2IN	GATGCACCACCAGGGGG	*Pto*	Kinase	Shi et al. (2001)
Pto-Kin1	GCATTGGAACAAGGTGAA	*Pto*	Kinase	Chen et al. (1998)
Pto-Kin4	AGTGTCTTGTAGGGTATC	*Pto*	Kinase	Chen et al. (1998)
CLRR-INV1	GCAGCAACTTGTGC	*Cf9*	LRR	Shi et al. (2001)
NLRR Rev	TATAAAAAGTGCCGGACT	*N*	LRR	Chen et al. (1998)
RLRR For	CGCAACCACTAGAGTAAC	*Rps2*	LRR	Chen et al. (1998)
Xa1 LR-R	GAGATTGCCAAGCAATTGC	*Xa1*	LRR	Shi et al. (2001)
XLRR-INV1	TTGTCAGGCCAGATACCC	*Xa21*	LRR	Shi et al. (2001)
XLRR-INV2	GAGGAAGGACAGGTTGCC	*Xa21*	LRR	Shi et al. (2001)
Xa1NBS-F	GGCAATGGAGGGATAGG	*Xa1*	NBS	Shi et al. (2001)
Xa1NBS-R	CTCTGTATACGAGTTGTC	*Xa1*	NBS	Shi et al. 2001
S2	GGIGGIGTIGGIAAIACIAC	*N*, *Rps2*	NBS	Leister et al. (1996)
HD	A(A/G)AGCIA(G/A)IGGIA(G/A)ICC	*N*, *Rps2*	NBS	This study
S1	GGTGGGGTTGGGAAGACAACG	*N*, *Rps2*	NBS	Leister et al. (1996)
AS1	C(A/C)ACGCTAGTGGCAATCC	*N*, *Rps2*	NBS	Leister et al. (1996)
RLKF	GA(T/C)GTNAA(A/G)CCIGA(A/G)AA	*Lr10 k*	Kinase	Feuillet et al. (1997)
RLKR	TC(T/C)GG(T/C)GC(A/G)AT(A/G)TANCCNGGITGICC	*Lr10 k*	Kinase	Feuillet et al. (1997)
Cf9F	CGAGACGACAGGACAAGTGA	*Cf9*	315–334	Wang et al. (1998)
Cf9R	CAACCGTAACCCACGAGAAC	*Cf9*	2516–2497	Wang et al. (1998)
PtoF	GTTTACAAGGGTGTTTTGCG	*Pto*	163–182	Wang et al. (1998)
PtoR	TATTATGCGACTCCACTGCC	*Pto*	784–765	Wang et al. (1998)

[a] LLR, Leucine-rich repeat; NBS, nucleotide-binding site

Takara), 2.0 m*M* $MgCl_2$ and 1 U *Taq* DNA polymerase (Dalian-Takara). Amplification was performed in DNA thermocyclers (Perkin Elmer and Ericomp, San Diego, Calif.) programmed for 5 min at 94°C, followed by 45 cycles of 45 s at 94°C,45 s at 45°C, 1 min at 72°C and a final 7-min extension at 72°C. A 2.5-min ramp time was used between the 94°C denaturing and 45°C annealing steps during cycles. Following amplification, 6 μl of formamide loading buffer was added to the PCR product, and 7 μl of denatured PCR samples was loaded onto a 5% denaturing polyacrylamide gel that was pre-run for 40 min at 1,600 V. The gel loading samples was run at 1,350 V for 2.5–3.5 h. The gel was silver-stained according to the method of Promega silver-staining (http://www.promega.com).

RAPD analysis

RAPD analysis was according to the method of Williams et al. (1990) with minor modifications (Zhang et al. 2000). The amplified products were fractionated on a 1.2% agarose gel. A total of 320 ten-mer RAPD primers ordered from Operon (Alameda, Calif.) were screened, including OPA01-20, OPB01-20, OPC01-20, OPD01-20, OPG01-20, OPK01-20, OPM01-20, OPN01-20, OPO01-20, OPQ01-20, OPS01-20, OPU01-20, OPV01-20, OPW01-20, OPX01-20, and OPY01-20.

Cloning and sequencing of RGAP and RAPD fragments

The RGAP diagnostic band was excised from the dried polyacrylamide gel, soaked in 10 μl H_2O and heated for 10 min at 94°C. Following centrifugation, 2 μl of the solution was used as template for re-amplification with the original RGA primers. The re-amplification product with a single band of the same size as the original band was selected for cloning into a pGEM-Teasy vector (Promega, Madison, Wis.). After *Eco*RI restriction, plasmids from ten single colonies derived from each cloning reaction were tested to determine the size of the inserted fragments. Two clones with the expected insert size were sequenced using the ABI 377 sequencer (Applied Biosystems, Foster City, Calif.).

The RAPD diagnostic band was excised and cloned into a pGEM-Teasy vector. White colonies were selected for testing for inserts of the appropriate size by means of PCR screening and *Eco*RI restriction analysis. The initial PCR products of resistant and susceptible DNAs were transferred onto Hybond N^+ nylon membrane (Amersham Pharmacia Biotech, Freiburg, Germany) and hybridized with the candidate-inserted fragments of the putative recombination clone. Two candidate clones were sequenced.

SCAR primers and SCAR-PCR amplifications

Based on the sequences of the clones derived from the RGAP and RAPD diagnostic bands, we designed SCAR primers using the software OLIGO6.0 (Molecular Biology Insights, Cascade, Colo.) and these were synthesized by AuGCT Biotech, Beijing, China.

For each PCR reaction, the 25-μl volume contained 20–40 ng genomic DNA, 0.2 μ*M* of each primer, 200 μ*M* of each dNTP, 1× PCR buffer (Sino-America, Beijing, China), 2.0 m*M* $MgCl_2$ and 1 U *Taq* polymerase (Sino-America). The PCR reaction was performed at 72°C for 5 min, 96°C for 1 min, followed by 35 cycles of 94°C for 1 min, 64°C for 1 min, 72°C for 2 min, with a final extension at 72°C for 10 min. The PCR products were fractionated on 1% agarose gel.

Results

Genetic and phenotypic analyses

In the resistance test, the resistant donor *Th. intermedium*, the Yw translocation lines Yw642, Yw443, Yw243, and Yw1029, the addition lines L1 and 7Ai1L as well as amphiploid TAF46 showed good resistance to the GAV serotype of BYDV with IT 0 scale (no visible symposium). The susceptible line Yw641, all wheat parents and varieties were susceptible showing serious symptoms (IT

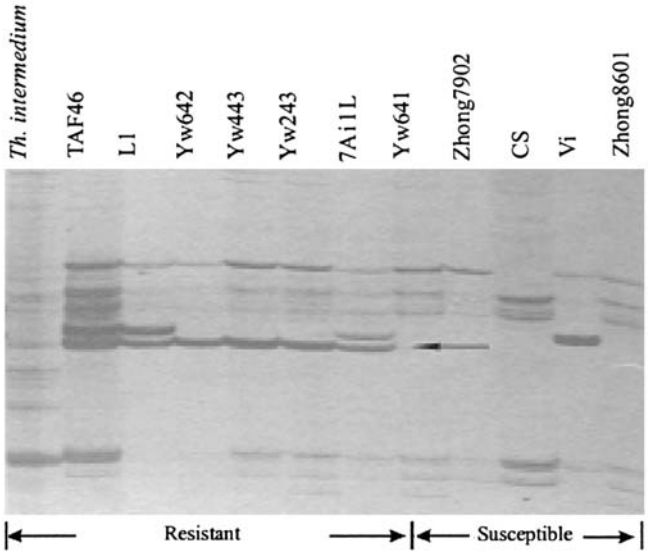

Fig. 1 Silver-stained denaturing polyacrylamide gel showing the resistance gene-analog polymorphism (RGAP) marker Tgp-1_{350} (*arrowhead* 350-bp band) amplified with primers Cf9F and PtoKin2IN in BYDV-resistant and -susceptible lines

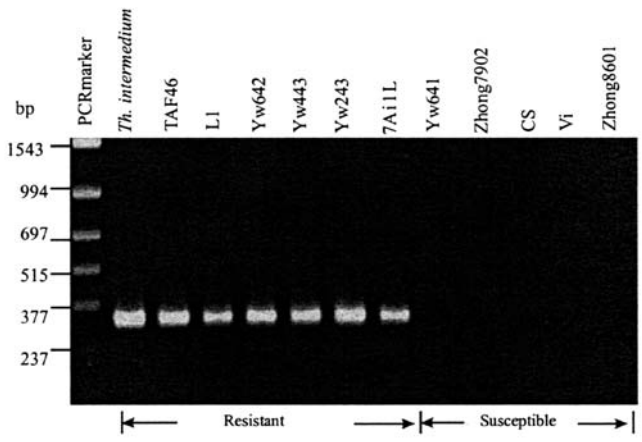

Fig. 2 PCR products of barley yellow dwarf virus (BYDV)-resistant and -susceptible lines amplified with the *SC-gp1* primers

8–9 scale) 30 days after inoculation. F_1 plants of the cross Yw642 × Zhong8601 were resistant. Of 256 F_2 individuals tested, 183 were resistant, and 73 were susceptible, which fits a 3:1 ratio (p=0.22). These results confirmed that *Bdv2* is genetically dominant and that the alien translocation block in Yw642 carrying *Bdv2* was inherited as a single dominant genetic unit without significant distortion from Mendelian segregation.

RGAP markers linked to *Bdv2*

One hundred and eighty-seven pairs of RGA primer combinations were used to identify diagnostic markers for *Bdv2*. Two RGAP markers, Tgp-1_{350} and Tgp-2_{210}, were developed with the primer combinations of Cf9F/PtoKin2IN and PtoF/XLRR-INV2. These primer combinations amplified respectively diagnostic bands of about 350 bp and 210 bp, which were present in all resistance materials possessing *Bdv2*, including Yw642, Yw443, Yw243, and Yw1029, L1, 7Ai1L, TAF46 and *Th. intermedium*, but were absent in all susceptible materials (Fig. 1). The presence of the diagnostic bands Tgp-1_{350} and Tgp-2_{210} was further tested using 256 individuals of a segregating F_2 population obtained from the cross Yw642 × Zhong8601. The results showed that both RGAP markers co-segregated with the resistance and should now be used as RGAP markers diagnostic for *Bdv2*.

Conversion of the RGAP marker Tgp-1_{350} into a SCAR marker

Ten putative clones derived from the Tgp-1_{350} fragment amplified in the resistant translocation line Yw642 were analyzed with the restriction enzyme *Eco*RI. Two clones with the expected insertion size were sequenced. The sequence of Tgp-1_{350} showed an actual size of 350 bp, which is bordered by the original primers (see electronic supplementary material). The sequence of Tgp-1_{350} had no significant homology with any of the sequences deposited in the public databases of Genbank + EMBL + DDBL + PDS on the basis of BLAST analysis through website http://www.ncbi.nlm.nih.gov/BLAST/; there was a very low alignment [score = 40.1 bits(20), expect = 2.7, identities = 20/20] with *Cf-9* and *Cf-4A*.

Similarly, the diagnostic fragment of Tgp-2_{210} was sequenced. The sequence of Tgp-2_{210} showed an actual size of 208 bp bordered by the original primers (see electronic supplementary material). The sequence of Tgp-2_{210} had no significant homology with any of the RGA sequences deposited in the public databases of Genbank + EMBL + DDBL + PDS on the basis of BLAST analysis through website http://www.ncbi.nlm.nih.gov/BLAST/; there was a very low alignment [score = 40.1 bits(20), expect = 2.7, identities = 20/20] with *Pto*.

Based on the sequence of Tgp-1_{350}, the primer pair of SC-gp1U (5′- CAGGACAAGTGAAAGCACCTAAGC-3′) and SC-gp1L (5′-GTCCACAAGTCATATGGGGA-GAC-3′) were designed, synthesized and used to amplify templates from resistant and susceptible materials. The *SC-gp1* primers amplified a robust band of about 330 bp from resistance lines possessing *Bdv2*, but not from any of the susceptible lines (Fig. 2). The SCAR marker *SC-gp1* was dominant for the 7Ai1L segment carrying *Bdv2*.

The F_2 population of 256 plants was used to investigate linkage between *SC-gp1* and *Bdv2*. The 183 resistant plants showed an amplified *SC-gp1* marker, while none of the 73 susceptible plants generated this band. These results confirmed that the SCAR marker co-segregated with *BYDV* resistance.

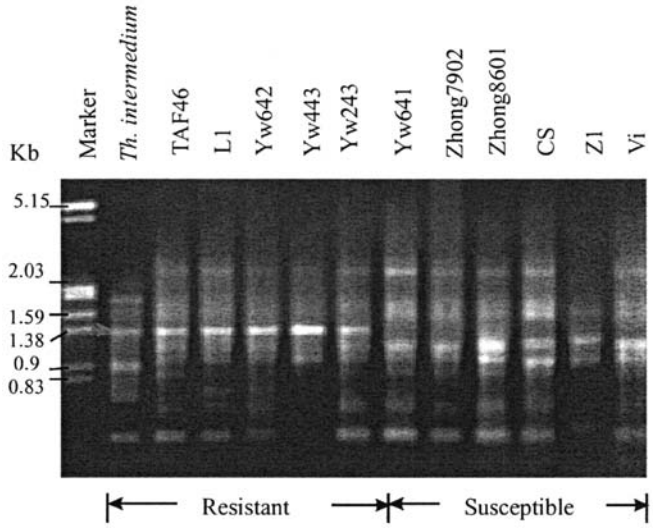

Fig. 3 PCR products of BYDV-resistant and -susceptible lines amplified with the RAPD primer OPD04. The *arrowhead* indicates the position of the marker specific to *Bdv2*

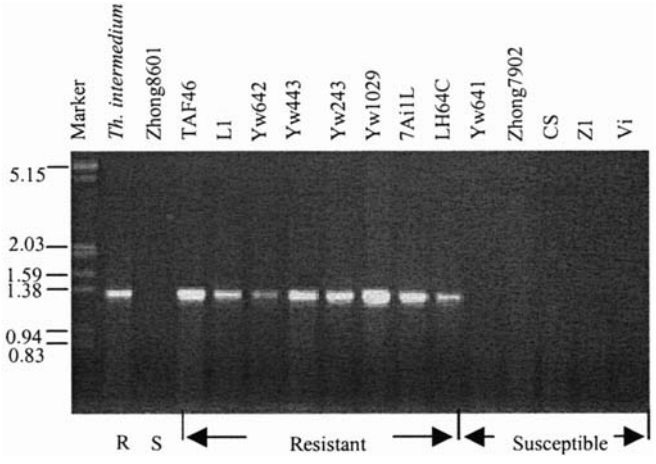

Fig. 4 PCR products of BYDV-resistant and -susceptible lines amplified with the *SC-D04* primers

The RAPD marker is linked to *Bdv2*

Of the 320 arbitrary 10-mer RAPD primers screened, only primer OPD04 (5′-TCTGGTGAGG-3′) reproducibly amplified a diagnostic band of about 1,300 bp. The diagnostic band of $OPD04_{1300}$ was present in the resistant germplasm possessing *Bdv2*, including the Yw translocation lines, L1 and 7Ai1L as well as *Th. intermedium* but was absent in the susceptible materials (Fig. 3). It was also absent from the *Th. intermedium* group 2 addition line Z1, which has a different resistance to *Bdv2*. Only F_2 individuals showing resistance had the $OPD04_{1300}$ diagnostic band. These results showed that the diagnostic band $OPD04_{1300}$ can be used as a marker for the 7Ai1L segment carrying the *Bdv2* gene.

Conversion of the RAPD marker into a SCAR marker

Of the 20 clones derived from the $OPD04_{1300}$ fragment of *Th. intermedium* and Yw642 analyzed, 11 had the expected fragment. Three clones were used as probes to Southern hybridize the PCR products amplified by the primer OPD04. The clones Tidz4 and Tidz8 were able to hybridize with the $OPD04_{1300}$-specific band derived from resistant materials but could not hybridize with PCR products derived from susceptible ones, indicating that the cloned fragments were highly homologous to the $OPD04_{1300}$ diagnostic band. The sequencing of the inserted fragments of Tidz4 and Tidz8 proved that they were identical and bordered by the original ten bases of the OPD04 primer. The actual size of $OPD04_{1300}$ was 1,347 bp (see electronic supplementary material). The sequence had no significant homology with any of the sequences deposited in the public databases on the basis of BLAST analysis through the website http://www.ncbi.nlm.nih.gov/BLAST/.

The primer pair of SCD04U (5′-TCTGGTGAGGC-AAACCTTCTGG-3′) and SCD04L (5′-TCTGGTGAGG-GAGGTGTGATGACG-3′) were designed and amplified a single robust band of about 1,300 bp in the resistant germplasm possessing *Bdv2* but not in susceptible ones (Fig. 4). The SCAR marker *SC-D04* was dominant for the 7Ai1L segment carrying *Bdv2*. The F_2 population of 256 plants was used to investigate the linkage between *SC-D04* and *Bdv2*. Our results showed that the 183 resistant plants had the *SC-D04* marker and that this marker was absent in the 73 susceptible plants, thereby confirming that the SCAR marker co-segregated with BYDV resistance and indicating the stability of the alien chromatin block in the Yw642 translocation.

Wheat breeding with assisted selection by SCAR markers

The SCAR marker *SC-D04* was used to select BYDV-resistant genotypes during five generations in the context of an active breeding program. Of the 636 plants obtained that showed resistance to powdery mildew—from the BC_1 to BC_5 generations, including Py1 to Py6 and Bcy1 to Bcy630—300 individuals with BYDV resistance, and resistance parents *Th. intermedium*, L1 and Yw642, were positive for *SC-D04*, while 336 individuals susceptible to BYDV, and susceptible wheat parents Zhong 8601, Zhongmai 16 and M53 (the resistance donor of powdery mildew), were negative for *SC-D04*. The SCAR assay results were in total agreement with field-based viral bioassays for resistance.

Discussion

Using the disomic addition line L1 as the resistance source and tissue culture techniques, we developed a series of 7D-7Ail translocations (TC5, TC6, TC7, TC8,

TC9, TC10, and TC14) (Banks et al. 1995). Hohmann et al. (1996) reported that TC14 had the smallest *Th. intermedium* translocation, located in the distal 44% of the long arm of chromosome 7D in TC14. Using L1 as the resistance source and the *ph1b* mutant to induce homoeologous pairing, Xin and his group developed the Yw series of translocation lines, including Yw642, Yw443, Yw243 and Yw1029 (Xin et al. 1991, 2001). The translocated segment of *Th. intermedium* chromosome 7AilL in the Yw lines replaced about 10% of the long arm of 7D, which should then be smaller than that in TC14 (Xin et al. 2001). In China, the Yw series of translocation lines show better resistance to the GPV and GAV serotypes of BYDV and have been used as resistant parents in wheat breeding programs.

To facilitate the transfer of the *Bdv2* gene into elite varieties, we developed two RGAP markers and one RAPD marker diagnostic for resistance in the Yw series of translocation lines induced by the *ph 1b* mutant. To enable high-throughput MAS, we converted the RGAP marker Tgp-1_{350} and the RAPD marker OPD04_{1300} into SCAR markers *SC-gp1* and *SC-D04*, respectively. These markers co-segregated with the BYDV resistance gene *Bdv2* located on the 7AiL segment. Our results indicated that these markers were inherited with the resistance as a block. Due to the large phylogenetic distance between the X (St) genome of *Th. intermedium* and the D genome of *Triticum aestivum*, an incomplete chromosome pairing in F_1 plants occurs, and crossovers between 7Ai1 and 7D chromosomes are rare (Friebe et al. 1992; Wang and Zhang 1996; Ayala et al. 2001). The entire alien segment and its markers are inherited as a block, as has been demonstrated for other alien blocks by Schachermayr et al. (1995) and Seah et al. (2000) and demonstrated for TC14 by Ayala et al. (2001). Therefore, the markers located on the alien segment should detect genes from *Th. intermedium*. The markers for BYDV resistance in the Yw series could also detect the *Th. intermedium* segment carrying *Bdv2* in the Australian resistant line LH64C derived from TC14.

Stoutjesdijk et al. (2001) converted the RAPD marker OPA07 into a SCAR marker for the TC series of recombinants carrying the *Bdv2* gene. However, the RAPD and SCAR markers couldn't amplify the band diagnostic for the segment of *Th. intermedium* 7Ail in the Yw lines in our Chinese laboratory under the conditions reported by Stoutjesdijk et al. (2001). One explanation may be that the reagents, including the *Taq* polymerase and the PCR buffer with different pHs, and the amplification programs used by the two groups were different.

RGAP technology uses high-resolution electrophoresis and a sensitive detection of DNA fragments amplified by primers designed on the conserved domains of plant resistance genes (Chen et al. 1998; Yan et al. 2003). The RGAP approach has been used efficiently to identify tightly linked markers for disease resistance genes, such as *Yr9* and *Yr5* of wheat, and for genes conferring resistance to stripe rust, leaf rust, scald, net blotch, barley yellow dwarf and scab of barley (Chen et al. 1998, 1999; Toojinda et al. 2000; Shi et al. 2001; Yan et al. 2003). In the present study, the RGAP technology was used to develop markers tightly linked to *Bdv2* in the Yw series of translocation lines. Although 187 primer combinations were used to develop the diagnostic markers for *Bdv2*, only two RGAP markers—Tgp-1_{350} and Tgp-2_{210}—were identified. The number of RGAP markers identified for *Bdv2* was lower than that for *Yr9* and *Yr5* and, contrary to our expectation, the sequences of the RGAP markers had no significant homology with the cloned plant resistance genes in public databases. We therefore assume that the amplified sequences were not resistance genes. Possible explanations for our results may include the following. (1) In this study we substituted the original components of the silver-staining kit of Promega with reagents from a Beijing chemical company. The latter were most likely not sufficiently sensitive to detect weak bands, with the result that some marker bands may have been missed. (2) The segment of *Th. intermedium* 7Ai1L carrying *Bdv2* may have different domain sequences from the domains that were used to design these primers in this study. (3) The RGA primers used in this study were not sufficient to identify additional direct markers for *Bdv2*. If more suitable primers were to be used and PCR products detected using more sensitive methods, markers directly diagnostic for *Bdv2* may be identified in the future.

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References

Ayala L, Henry M, González-de-León D, Ginkel M van, Mujeeb-Kazi A, Keller B, Khairallah M (2001) A diagnostic molecular marker allowing the study of *Th. intermedium*-derived resistance to BYDV in bread wheat segregating populations. Theor Appl Genet 102:942–949

Banks PM, Larkin PJ, Bariana HS, Lagudah ES, Appels R, Waterhouse PM, Brettell RIS, Chen X, Xu HJ, Xin ZY, Qian YT, Zhou M, Cheng ZM, Zhou GH (1995) The use of cell culture for subchromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. Genome 38:395–405

Banks PM, Larkin PJ, Kammholz SJ (1996) Barley yellow dwarf virus resistant wheat breeding germplasm. In: 5th Int Wheat Conf. Ankara, pp 114–115

Brettell RIS, Banks PM, Cauderon Y, Chen X, Cheng ZM, Larkin PJ, Waterhouse PM (1988) A single wheatgrass chromosome reduces the concentration of barley yellow dwarf virus in wheat. Ann Appl Biol 113:599–603

Cauderon Y, Saigne B, Dauge M (1973) The resistance to wheat rusts of *Agropyron intermedium* and its use in wheat improvement. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp Wheat Improv. University of Missouri, Columbia, Mo., pp 401–407

Chen XM, Line RF, Leung H (1998) Genome scanning for resistance-gene analogs in rice, barley and wheat by high resolution electophoresis. Theor Appl Genet 97:345–355

Chen XM, Line RF, Hayes PM, Toojinda T, Vivar H, Kleinhofs A, Kudrna D (1999) Mapping barley genes for resistance to stripe rust, leaf rust and scab using resistance gene analog polymorphism and restriction fragment length polymorphism. Phytopathology 89:S15

Conti M, D'Arcy CJ, Jedlinski H (1990) The "yellow plague" of cereals, barley yellow dwarf virus. In: Burnett PA (ed) World perspectives on barley yellow dwarf. CIMMYT, Mexico, pp 1–6

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like-kinase gene encoded at the *Lr10* disease resistance locus of wheat. Plant J 11:45–52

Friebe B, Mukai Y, Gill BS, Cauderon Y (1992) C-banding and *in situ* hybridization analysis of *Agropyron intermedium*, a partial wheat × *Agropyron intermedium* amphiploid, and six derived chromosome addition lines. Theor Appl Genet 84:899–905

Hohmann U, Badaeva K, Busch W, Friebe B, Gill BS (1996) Molecular cytogegetic analysis of *Agropyron* chromatin specifying resistance to barley yellow dwarf virus in wheat. Genome 39:336–347

Kanazin V, Marek F, Shoemaker RC (1996) Resistance gene analogs are conserved and clustered in soybean. Proc Natl Acad Sci USA 93:11746–11750

Larkin PJ, Baeva K, Banks PM, Lagudah ES, Appels R, Chen X, Xin ZY, Ohm HW, McIntosh RA (1995) Disomic *Thinopyrum intermedium* addition lines in wheat with barley yellow dwarf virus resistance and with rust resistances. Genome 38:385–394

Leister D, Ballvora A, Salamini F, Gebhardt C (1996) A PCR-base approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nat Genet 14:421–429

McIntosh RA, Devos KM, Dubcovsky J, Rogers WJ (2001) Catalog of gene symbols for wheat: Supplement pp 14. http://wheat.pw.usda.gov/ggpages/wgc/2001 upd.html

Paran I, Michelmore RW (1993) Development of reliable PCR-markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Schachermayr GM, Messmer MM, Feuillet C, Winzeler H, Winzeler M, Keller B (1995) Identification of molecular markers linked to the *Agropyron elongatum*-derived leaf rust resistance gene *Lr24* in wheat. Theor Appl Genet 90:982–990

Seah S, Spielmeyer W, Jahier J, Sivasithamparam K, Lagudah ES (2000) Resistance gene analogs within an introgressed chromosomal segment derived from *Triticum ventricosum* that confers resistance to nematode and rust pathogens in wheat. Mol Plant Microbe Interact 13:334–341

Sharma H, Ohm H, Goulart L, Lister R, Appels R, Benlhabib O (1995) Introgression and characterization of barley yellow dwarf virus resistance from *Thinopyrum intermedium* into wheat. Genome 38:406–413

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Location of beta-amylase sequences in wheat and its relatives. Theor Appl Genet 75:286–290

Shen KA, Meyers BC, Islam-Faridi MN, Chin DB, Stelly DM, Michelmore RW (1998) Resistance gene candidates identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. Mol Plant Microbe Interact 11:815–823

Shi ZX, Chen XM, Line RF, Wellings CR (2001) Development of resistance gene analog polymorphism markers for the *Yr9* gene resistance to wheat stripe rust. Genome 44:509–516

Stoutjesdijk P, Kammhozl SJ, Kleven S, Matsay S, Banks PM, Larkin PJ (2001) PCR-based molecular marker for the *Bdv2 Thinopyrum intermedium* source of barley yellow dwarf virus resistance in wheat. Aust J Agric Res 52:1383–1388

Toojinda T, Broers LH, Chen XM, Hayes PM, Kleinhofs A, Korte J, Kudrna D, Leung H, Line RF, Powell W, Ramsay L, Livar H, Waugh R (2000) Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). Theor Appl Genet 101:580–589

Wang RRC, Zhang XY (1996) Characterization of the translocated chromosome using fluorescence in situ hybridization and random amplified polymorphic DNA on two *Triticum aestivum-Thinopyrum intermedium* translocation lines resistant to wheat streak mosaic or barely yellow dwarf virus. Chromos Res 4:583–587

Wang SP, Liu KD, Wang J, Zhang Q (1998) Identifying candidate disease resistance genes in rice by sequence homology and chromosomal locations (in Chinese). Acta Bot Sin 40:42–50

Williams JGK, Kubelik AR, Livak KJ, Rafalski JV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Xin ZY, Brettell RIS, Cheng ZM, Waterhouse PM, Appels R, Banks PM, Zhou GH, Chen X, Larkin PJ (1988) Characterization of a potential source of barley yellow dwarf virus resistance for wheat. Genome 30:250–257

Xin ZY, Xu HJ, Chen X, Lin ZS, Zhou GH, Qian YT, Chen ZM, Larkin P J, Banks PM, Apples R, Clarke B, Brettell RIS (1991) Development of common wheat germplasm resistant to barley yellow dwarf virus by biotechnology. Sci China Ser B 34:1055–1062

Xin ZY, Zhang ZY, Chen X, Lin ZS, Ma Y, Xu HJ, Banks PM, Larkin PJ (2001) Development and characterization of common wheat-*Thinopyrum intermedium* translocation lines with resistance to barley yellow dwarf virus. Euphytica 119:161–165

Yan GP, Chen XM, Line RF, Wellings CR (2003) Resistance gene-analog polymorphism markers co-segregating with the Yr5 gene for resistance to wheat stripe rust. Theor Appl Genet 106:636–643

Yu YG, Buss GR, Maroof MA (1996) Isolation of a superfamily of candidate disease resistance genes in soybean based on conserved nucleotide-binding site. Proc Natl Acad Sci USA 93:11751–11756

Zhang ZY, Xin ZY, Ma YZ, Chen X, Xu QF, Lin ZS (1999) Mapping of a BYDV resistance gene from *Thinopyrum intermedium* in wheat background by molecular markers. Sci China Ser C 42:663–668

Zhang ZY, Xin ZY, Chen X, Qian YT, Lin ZS, Ma YZ (2000) Molecular cytogenetic characterization of a new wheat line Yw243 with resistance to barley yellow dwarf virus (in Chinese). Acta Genet Sin 27:614–620