Theor Appl Genet (2012) 124:1549–1560
DOI 10.1007/s00122-012-1809-7

ORIGINAL PAPER

High-density mapping and marker development for the powdery mildew resistance gene *PmAS846* derived from wild emmer wheat (*Triticum turgidum* var. *dicoccoides*)

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Received: 12 September 2011 / Accepted: 28 January 2012 / Published online: 18 February 2012

Abstract Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is an important foliar disease of wheat worldwide. The dominant powdery mildew resistance gene *PmAS846* was transferred to the hexaploid wheat lines N9134 and N9738 from wild emmer wheat (*Triticum dicoccoides*) in 1995, and it is still one of the most effective resistance genes in China. A high resolution genetic map for *PmAS846* locus was constructed using two F_2 populations and corresponding $F_{2:3}$ families developed from the crosses of N9134/Shaanyou 225 and N9738/Huixianhong. Synteny between wheat and *Brachypodium distachyon* and rice was used to develop closely linked molecular markers to reduce the genetic interval around *PmAS846*. Twenty-six expressed sequence tag-derived markers were mapped to the *PmAS846* locus. Five markers co-segregated with *PmAS846* in the F_2 population of N9134/Shaanyou 225. *PmAS846* was physically located to wheat chromosome 5BL bin 0.75–0.76 within a gene-rich region. The markers order is conserved between wheat and *Brachypodium distachyon*, but rearrangements are present in rice. Two markers, *BJ261635* and *CJ840011* flanked *PmAS846* and narrowed *PmAS846* to a region that is collinear with 197 and 112 kb genomic regions on *Brachypodium* chromosome 4 and rice chromosome 9, respectively. The genes located on the corresponding homologous regions in *Brachypodium*, rice and barley could be considered for further marker saturation and identification of potential candidate genes for *PmAS846*. The markers co-segregating with *PmAS846* provide a potential target site for positional cloning of *PmAS846*, and can be used for marker-assisted selection of this gene.

Communicated by X. Xia.

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Introduction

Powdery mildew is a foliar disease of wheat caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*). Due to potential high yield losses and decreased grain quality, powdery mildew is an economically important wheat disease worldwide in the cool and semi-humid wheat growing areas. Breeding for resistance is the most profitable and environmentally acceptable strategy to control powdery mildew. More than 60 powdery mildew resistance genes located at 41 loci (*Pm1-Pm45*, *Pm18* = *Pm1c*, *Pm22* = *Pm1e*, *Pm23* = *Pm4c*, *Pm31* = *Pm21*) (Ma et al. 2011; Hsam et al. 1998; Singrün et al. 2003; Hao et al. 2008; Xie et al. 2011) have been identified and designated in wheat and its wild relatives.

A significant problem in wheat breeding and production is the loss of resistance caused by virulent races. Therefore, it is urgent to search for new powdery mildew resistance genes. In order to improve resistance, wheat (*Triticum aestivum* L.) had been crossed with its related genera (Jiang et al. 1993), such as *Aegilops*, *Elytrigia*, *Secale*, *Haynaldia*, and related species of *Triticum*, for instance, *T. boeoticum*, *T. dicoccoides*, *T. carthlicum* and *T. timopheevii*, which represent a reservoir of genes for resistance to multiple diseases. Intergeneric and interspecific crosses have resulted in the transfer of desirable fungal resistance into wheat; for example, powdery mildew resistance genes, *Pm7*, *Pm8*,

Pm17, and *Pm20* originated from *Secale* (McIntosh et al. 2011); *Pm12*, *Pm13*, *Pm19*, *Pm29*, *Pm32*, *Pm34*, and *Pm35* originated from *Aegilops* (Miranda et al. 2007); *Pm21* originated from *Haynaldia* (Cao et al. 2011); *Pm40* and *Pm43* originated from *Elytrigia* (He et al. 2009); *Pm4b* and *Pm33* originated from *T. carthlicum* (Zhu et al. 2005); *Pm25* originated from *T. boeoticum* (Shi et al. 1998); and *Pm6*, *Pm27*, and *Pm37* originated from *T. timopheevii* (Perugini et al. 2008). All provide race-specific resistance to powdery mildew.

Wild emmer (*T. turgidum* var. *dicoccoides*, AABB, $2n = 28$) is a valuable source of resistance to pathogens, and genes transferred from this species included powdery mildew resistance genes *Pm16*, *Pm26*, *Pm30*, *Pm36*, *Pm41*, and *pm42*, which were transferred to wheat chromosomes 5BS, 2BS, 5BS, 5BL, 3BL and 2BS, respectively (Reader and Miller 1991; Chen et al. 2005; Rong et al. 2000; Liu et al. 2002; Blanco et al. 2008; Li et al. 2009; Hua et al. 2009). There is also potential, largely untapped of the rich genetic resource in wild emmer, for disease resistance, pest tolerance and various abiotic stresses (Xie and Nevo 2008).

Map-based cloning of genes in wheat is hampered by the large genome size (16,000 Mb) and complexity of polyploid genomes, with about 80% of repetitive DNA sequences. To date, only two powdery mildew resistance genes have been cloned, including *Pm3* allelic series (Yahiaoui et al. 2004; Srichumpa et al. 2005) and *Pm21* (Cao et al. 2011). However, remarkable achievements in the area of gene identification in wheat have been made recently. A total of 1,073,845 wheat expressed sequence tags (ESTs) (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html) are available in the public database (http://www.ncbi.nlm.nih.gov/), and more than 16,000 ESTs were located in specific chromosome deletion bins by the NSF wheat EST project (Qi et al. 2004). The development of many ESTs offers opportunities for a variety of studies including development of functional molecular markers (STS and SNP), particularly gene expression, and comparative genomics research.

The high level of conserved synteny to model grass genomes like *Brachypodium* (The International Brachypodium Initiative 2010), rice (Rota and Sorrells 2004) and barley (Drader and Kleinhofs 2010) provides useful efforts to the identification of candidate genes for traits of interest, predicting biological function of genes, fine mapping and genes cloning, such as *Vrn1* (Yan et al. 2003), *Vrn2* (Yan et al. 2004), and *Vrn3* (Yan et al. 2006).

The powdery mildew resistance gene *PmAS846* was transferred to the long arm of chromosome 5B to common wheat lines N9134 and N9738 from wild emmer wheat. The objective of this study was to construct a saturation synteny map between wheat, *Brachypodium* and rice, focusing on the genomic region harboring *PmAS846*. The markers tightly linked to *PmAS846* should be useful for marker-assisted selection (MAS) and cloning of this gene.

Materials and methods

Plant materials

Two segregating populations were developed for the mapping of *PmAS846*. The initial mapping population included 129 F_2 plants and the derived $F_{2:3}$ families from the N9738/Huixianhong cross, and the low level of polymorphism observed between the parental lines of this cross prompted the development of new mapping population. The second population included 362 F_2 plants and derived $F_{2:3}$ families from the cross of N9134/Shaanyou 225. N9134 is a resistant common wheat line carrying *PmAS846* introgressed from wild emmer accession As846 (*T. dicoccoides*). In a previous work, this gene was located on chromosome 5BL by the simple sequence repeat (SSR) marker *Xgwm67* with a genetic distance of 20.6 cM (Wang et al. 2007). It confers broad spectrum resistance to wheat powdery mildew. The common wheat line N9738, carrying *PmAS846*, was developed from the cross N9134/Zhong 4 (Wheat-*Thinopyrum intermedium* partial amphiploid, and highly susceptible to powdery mildew). Huixianhong and Shaanyou 225 are common wheat varieties that are highly susceptible to *Bgt* isolate E09. Chancellor was used as a susceptible control in the disease reaction tests. Chinese Spring (CS) and its chromosome 5B deletion lines (5BL-09, 5BL-11, 5BL-13, 5BL-14, 5BL-16 and 5BS-04, kindly provided by Drs Takashi Endo and Shuhei Nasuda, Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University, Japan) were used for chromosomal arm assignment and bin mapping of molecular markers.

Evaluation of powdery mildew reactions

Powdery mildew reactions of the F_2 mapping population and $F_{2:3}$ families (comprising 20 seedlings per family) were assessed via inoculation with *Bgt* isolate E09 (kindly provided by Drs Xiayu Duan and Yilin Zhou, State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China). E09 is a single-spore isolate with the following virulence response: *Pm1a*, *Pm3b*, *Pm3c*, *Pm3e*, *Pm5a*, *Pm6*, *Pm7*, *Pm8*, *Pm17* and *Pm19*, which was maintained on Chancellor until the leaf was fully expanded by conidia. Plants were inoculated by dusting conidia from sporulating seedlings of Chancellor at the two to three leaf stages. The susceptible parents Shaanyou 225, Huixianhong and Chancellor were used as the susceptible controls, and the resistant parents N9134 and

N9738 as the resistant controls in the disease reaction test. Phenotypes were recorded 12 days after inoculation when the susceptible controls were fully infected, and disease evaluations were performed according to Sheng (1988). Six infection type (IT) classes (0, 0;, 1, 2, 3, and 4) were scored, in which grade "0" represents immune reaction with no visible symptoms in plants; grade "0;" represents highly resistant reaction with hypersensitive necrotic flecks on leaves; grade "1" is highly resistant reaction with minute colonies on leaves, smaller than 1 mm in general with few conidia; grade "2" represents moderately resistant, at which leaves have moderately developed hyphae but colonies smaller than 1 mm, and some conidia; grades "3" and "4" are moderately susceptible (colonies with well-developed hyphae and abundant conidia, but colonies do not joint together) and highly susceptible (colonies with well-developed hyphae and abundant conidia, colonies are mostly joint together) types, respectively.

Genomic DNA isolation and SSR marker analysis

DNA was isolated from leaves of the entire F_2 population and of the parental lines by the CTAB method (Saghai-Maroof et al. 1984). Two resistant bulks and two susceptible bulks were made by pooling equal amounts of DNA from 10 resistant or 10 susceptible F_2 plants, respectively. Because *PmAS846* was previously assigned to wheat chromosome 5BL, 72 SSR markers specific for wheat chromosome 5BL were screened for polymorphisms on the parents and the bulks. Polymorphic markers indicative of linkage with *PmAS846* were further used to genotype the entire $F_{2:3}$ mapping population to determine genetic linkage between the gene and the markers. SSR markers were selected from the primer sets GPW (http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/), GWM (Röder et al. 1998), WMC (Somers et al. 2004), BARC (Song et al. 2005) and FCP (Zhang et al. 2009). The sequences of SSR primers were obtained from the GrainGenes Database (http://wheat.pw.usda.gov/GG2/index.shtml).

PCR amplifications were performed in a S1000 Thermal Cycler (Bio-Rad, California, USA) in 10 µl volume containing 1 µl of PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH 8.3), 1.5 mM of $MgCl_2$, 0.25 U of *Taq* DNA polymerase, 0.2 mM of dNTPs, 0.5 µM of each primer, and 50–100 ng of template DNA. PCR conditions for different markers included a 95°C denaturing step for 3 min, followed by 35 cycles of 95°C for 45 s, 50–65°C annealing (depending on annealing temperature of the primer pairs) for 45 s, and 72°C for 45 s to 1 min (depending on PCR product sizes), and a final extension at 72°C for 10 min. The amplification products from the markers were separated in 8% polyacrylamide gels or 2% agarose gels. PCR products were visualized with silver staining or ethidium bromide and photographed.

Chromosome arm assignment and physical bin mapping

The physical locations of markers linked to the resistance gene on chromosome 5BL were determined using CS chromosome 5B deletion lines characterized by Endo and Gill (1996).

Data analysis

Chi-squared (χ^2) tests were conducted to determine the goodness of fit of segregation ratios to theoretical Mendelian ratios. Linkages between markers and the resistance gene were established using JoinMap4.0 (http://www.kyazma.nl/index.php/mc.Join-Map/sc.General/), with a LOD threshold of 3.0.

Comparative mapping and marker development based on collinearity of *Brachypodium* and rice

Information regarding ESTs previously mapped to the deletion bin 5BL14-0.75–0.76 covering the *PmAS846* interval was obtained from GrainGenes wEST-SQL resources (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). These sequences were used for developing EST-STS markers using Primer 3 software (http://frodo.wi.mit.edu/primer3/). EST-STS primers (MAG set) assigned to chromosome 5BL (Xue et al. 2008), and EST-SSR marker *BJ261635* mapped to chromosome 5BL and closely linked with *Pm36* (Blanco et al. 2008) were also screened for polymorphism. The original sequence of marker *MAG2498* was kindly provided by Dr. Zhengqiang Ma, National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, China.

Mapped EST sequences were blasted against the *Brachypodium* (http://www.brachypodium.org/) and rice genomes (http://rice.plantbiology.msu.edu/) using BLASTn. *Brachypodium* and rice genes with the best hit (*e* values >1E−10 and >80% nucleotide identity) and/or within the syntenic regions were then used as queries in BLASTn searches of *Triticum* sequences (http://wheat.pw.usda.gov/GG2/blast.shtml). The *Triticum* sequences were aligned with *Brachypodium* and rice CDS and genomic sequences. A total of 156 conserved primer pairs were designed using Primer 3 and Conserved Primers 2.0 softwares (You et al. 2009).

PCR amplification was performed in a similar procedure to the SSR marker survey described above. For EST markers that were monomorphic between the parents on

PAGE gels, single-stranded conformational polymorphism (SSCP) analyses were employed (Sunnucks et al. 2000). The PCR products were mixed with equal volume of formamide loading dye (98% formamide, 10 mM EDTA, 0.25% bromophenol blue, and 0.25% xylene cyanol), denatured at 95°C for 10 min, then placed on ice, and 5.0 µl of the mixture was loaded onto 8% non-denaturing polyacrylamide gels (37.5:1 acrylamide: bis-acrylamide). Gels were run at 4°C and 4 W for 10 h and the DNA fragments were visualized by 0.2% silver staining.

Results

Evaluation of powdery mildew reactions

When inoculated with the *Bgt* isolate E09, N9134 and N9738 were highly resistant (IT = 0 to 0;), "0;" is a IT, whereas Shaanyou 225 and Huixianhong were highly susceptible (IT = 4). The N9738/Huixianhong population with 129 F_2 plants segregated in 94 resistant and 35 susceptible when inoculated with E09 (Table 1), indicative of a single dominant resistance gene conferring powdery mildew resistance ($\chi^2_{3:1} = 0.313$, $df = 1$). A total of 362 F_2 plants derived from the cross N9134/Shaanyou 225 were also inoculated with E09 and showed a segregation as 266 resistant and 96 susceptible, which fit the expected 3:1 ratio for a single dominant gene ($\chi^2_{3:1} = 0.446$, $df = 1$). The segregation in both $F_{2:3}$ families fits 1:2:1 ratios ($\chi^2_{1:2:1} = 2.379$ and 1.889, respectively, $df = 2$), confirming that the powdery mildew resistance in N9134 and N9738 is conferred by a single dominant gene.

Identification and bin mapping of SSR markers

Initially, 72 wheat SSR markers on chromosome 5BL were screened for polymorphisms between N9738 and Huixianhong and between resistant and susceptible bulks. Twelve polymorphic markers, *Xbarc88*, *Xbarc74*, *Xwmc75*, *Xwmc415*, *Xwmc537*, *Xgwm408*, *Xgwm67*, *Xgpw3035*, *Xgpw358*, *Xgpw3191*, *Xgpw7346* and *XFCP1*, were selected

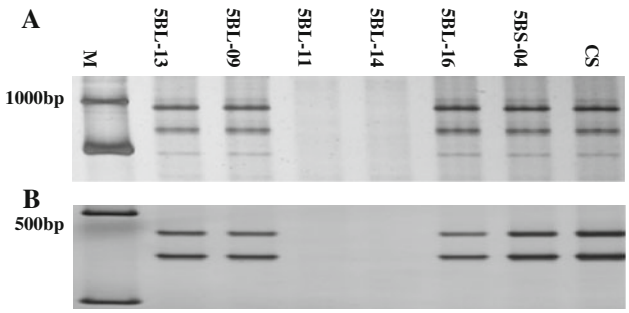

Fig. 1 Amplification pattern of markers *Xgpw7346* (**a**) and *XFCP1* (**b**) in Chinese Spring and its 5B chromosome deletion lines. *M* D2000 ladder, *CS* Chinese Spring, *CS* deletion line: 5BL-09 (FL = 0.76), 5BL-11 (FL = 0.59), 5BL-13 (FL = 0.82), 5BL-14 (FL = 0.75), 5BL-16 (FL = 0.79), 5BS-04 (FL = 0.43), *FL* fraction length

to genotype 129 F_2 plants of N9738/Huixianhong for the construction of linkage map. Two flanking SSR markers, *Xgpw7346* and *XFCP1*, were closely linked to the resistance locus at 1.7 and 1.3 cM, respectively. SSR markers previously mapped between two specified loci *Xbarc74* and *Xwmc75* (http://wheat.pw.usda.gov) were also tested for polymorphism between N9134 and Shaanyou 225. Seven markers (*Xgpw7309*, *Xgpw3191*, *Xgpw7346*, *Xgpw7425*, *XFCP1*, *XFCP620* and *XFCP394*) revealed polymorphisms between two bulks and were subsequently used to genotype 362 F_2 plants in N9134/Shaanyou 225 population. *PmAS846* was flanked by SSR loci *Xgpw7346* and *XFCP1* with genetic distances of 0.8 cM proximal and 0.9 cM distal, respectively.

In order to determine on which chromosome bin the target gene resides, CS and its deletion lines of chromosome 5B were used to physically map flanking markers. Both *Xgpw7346* and *XFCP1* were located to the distal end of chromosome 5BL (bin 5BL14), indicating that the *PmAS846* gene was located in the physical bin 5BL14 (0.75–0.76) (Fig. 1).

Bin-mapped EST marker analysis

To further delineate the genetic location of *PmAS846*, 21 EST-STS markers and one EST-SSR marker *BJ261635*

Table 1 Segregation ratios of *PmAS846* in the two F_2 populations

Cross	Generation	Number of the F_2 plants or $F_{2:3}$ families	Observed ratio			Expected ratio	χ^2 value
			HR	Seg	HS		
N9738/Huixianhong	F_2	129	94		35	3:1	0.313
N9738/Huixianhong	$F_{2:3}$	129	38	56	35	1:2:1	2.379
N9134/Shaanyou 225	F_2	362	266		96	3:1	0.446
N9134/Shaanyou 225	$F_{2:3}$	362	98	168	96	1:2:1	1.889

$\chi^2_{0.05} = 3.841$, $df = 1$; $\chi^2_{0.05} = 5.991$, $df = 2$

HR homozygous resistant, *Seg* segregating (heterozygous resistant), and *HS* homozygous susceptible

were screened. Five markers (*BF482522*, *BF202652*, *BF484437*, *MAG2498* and *BJ261635*) were polymorphic between N9738 and Huixianhong (Table 2). These were subsequently mapped in the N9738/Huixianhong F_2 population. The EST-SSR marker *BJ261635* displayed a nearly consistency between genotype and phenotype (Fig. 2).

High-density genetic mapping of *PmAS846* based on collinearity between wheat, *Brachypodium* and rice

The high level of collinearity exists between wheat chromosome 5BL, *Brachypodium* chromosome 4 and rice chromosome 9 (The International Brachypodium Initiative 2010; Linkiewicz et al. 2004; Rota and Sorrells 2004), which may provide useful information for fine mapping of this gene. To develop additional markers, we evaluated the levels of collinearity between the *PmAS846* region, *Brachypodium* and rice using sequences of five mapped ESTs and one SSR marker *XFCP620*. SSR marker *XFCP620* is at the locus of gene *WK35* (Faris et al. 2010), and its orthologous gene in *Brachypodium* and rice were *Bradi4g38010* and *Os09g38910*, respectively. BLAST searches revealed that all the five mapped wheat sequences had significant similarity to sequences on *Brachypodium* chromosome 4 except *MAG2498*, and three of them had similarity to sequences on rice chromosome 9 based on searches of rice sequences except *BF484437* and *MAG2498* (Table 2). The region around the *PmAS846* locus on wheat chromosome 5BL was identified to be syntenic to part of *Brachypodium* chromosome 4 and rice chromosome 9.

Table 2 Summary of *Brachypodium* and rice collinearity-based marker development for the *PmAS846* region

Wheat marker	*Brachypodium*			Rice			Marker type
	Gene	*E* value BLASTn	Position (bp)	Gene	*E* value BLASTn	Position (bp)	
CJ778922[a]	*Bradi1g49997*	2E−53	na	*Os07g14350*	8.60E−45	na	Conserved
BF482522	*Bradi4g36800*	E−109	41949410	*Os09g37100*	1.60E−64	21391293	EST-STS
BF202652	*Bradi4g36800*	1E−69	41949410	*Os09g37100*	1.80E−51	21391293	EST-STS
CJ927263	*Bradi4g36880*	5E−68	42043874	*Os09g37230*	3.30E−48	21487652	Conserved
CJ679871	*Bradi4g36910*	E−120	42074105	*Os09g37270*	3.90E−113	21525469	Conserved
BF620979	*Bradi4g36976*	E−176	42181605	*Os05g40160*	2.30E−96	na	Conserved
BF484437	*Bradi4g36976*	E−137	42181605	*Os05g40160*	1.40E−75	na	EST-STS
RG-36976	*Bradi4g36976*	0.0	42181605	*Os05g40160*	6.10E−281	na	Conserved
CV765558	*Bradi4g37002*	2E−63	42117455	*Os09g37495*	2.60E−32	21606038	Conserved
CK207184	*Bradi4g37002*	2E−51	42195697	*Os09g37495*	8.20E−31	21606038	Conserved
CK211420	*Bradi4g37002*	3E−41	42195697	*Os09g37495*	1.30E−33	21606038	Conserved
BJ212117	*Bradi4g37030*	6E−41	42209527	*Os09g37510*	2.90E−31	21609609	Conserved
CK210589	*Bradi4g37030*	3E−59	42209527	*Os09g37510*	9.40E−108	21609609	Conserved
CD871658	*Bradi4g37230*	3E−53	42411610	*Os02g51750*	7.90E−07	na	Conserved
MAG2498	na	ns	na	na	ns	na	EST-STS
BF200076	*Bradi4g37267*	1E−78	42460155	*Os09g37949*	8.10E−78	21880614	Conserved
BG904722	*Bradi4g37560*	1E−35	42627756	*Os02g57060*	9.20E−45	na	Conserved
BJ261635	*Bradi4g37680*	2E−84	42777206	*Os09g38520*	1.40E−53	22160396	EST-SSR
AL819406	*Bradi4g37770*	5E−46	42830702	*Os09g38620*	5.30E−48	22211909	Conserved
CJ694617	*Bradi4g37770*	3E−64	42830702	*Os09g38620*	8.50E−100	22211909	Conserved
CJ540214	*Bradi4g37800*	3E−15	42843356	*Os03g31010*	2.50E−12	na	Conserved
RG-37900	*Bradi4g37900*	5E−55	42918857	*Os09g38700*	4.40E−50	22243412	Conserved
CJ840011	*Bradi4g37960*	8E−56	42970820	*Os09g38755*	5.60E−62	22268621	Conserved
XFCP620[b]	*Bradi4g38010*	na	42996968	*Os09g38910*	na	22348258	SSR
BI955376	*Bradi4g38090*	2E−53	43084761	*Os09g38980*	4.60E−59	22382443	Conserved
RG-38170	*Bradi4g38170*	1E−46	43165360	*Os06g15750*	2.80E−125	na	Conserved
CA744029	*Bradi4g38210*	1E−60	43204228	*Os03g46740*	1.40E−77	na	Conserved

na not applicable, *ns* not significant

[a] *CJ778922* sequence with the best hit genes on *Brachypodium* were *Bradi1g49997* and *Bradi4g37046* (*E* value = 1E−48, 42247705–42253327)

[b] Faris et al. (2010)

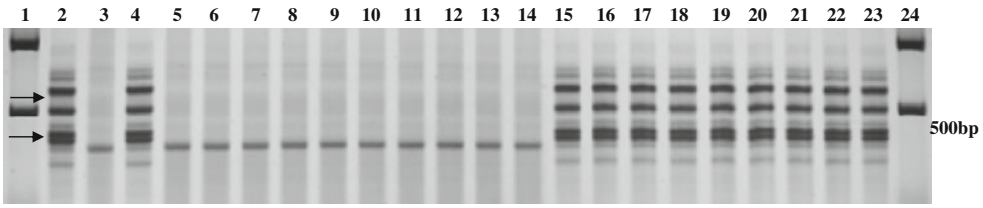

Fig. 2 Polyacrylamide gel electrophoresis of PCR products amplified with marker *BJ261635* in the F_2 population of N9134/Shaanyou 225. *Lanes 2* N9134; *3* Shaanyou 225, *4* resistant bulk, *5* susceptible bulk; *6–14* nine susceptible F_2 plants; *15–23* nine resistant F_2 plants. *Arrow* indicates the polymorphic amplification products. A D2000 ladder is shown in *lanes 1* and *24*

Genes located in the collinear regions of *Brachypodium* chromosome 4 and rice chromosome 9 were selected to develop conserved markers for saturating the *PmAS846* region. Based on the identified *Triticeae* sequences, 156 conserved primers were designed using the Conserved Primers 2.0 and Primer 3 online. Twenty-one markers were closely linked to *PmAS846* in the two mapping population. Most of these markers are co-dominant, such as *RG-36976* and *RG-37900* (Fig. 3). The marker *RG-36976* with a NBS-LRR analog was designed to allow unequivocal distinction of homozygous and heterozygous genotypes by both agarose gel and polyacrylamide gel electrophoresis (Fig. 3). Seven closely linked markers were mapped within a genetic interval of 0.8 cM (0.5 and 0.3 cM on either side of the gene) region carrying *PmAS846*, and five of them, *BJ261635*, *AL819406*, *CJ694617*, *CJ540214*, and *RG-37900*, co-segregated with *PmAS846* in the N9134/Shaanyou 225 population (Fig. 4).

In summary, the genetic linkage map of the *PmAS846* region contained 42 molecular markers including 16 SSR markers and 26 EST-derived markers. Comparative sequence analysis revealed good levels of collinearity in the *PmAS846* region, *Brachypodium* chromosome 4 and rice chromosome 9, but with a couple of exceptions between *PmAS846* region and *Brachypodium* chromosome 4, and six exceptions between *Brachypodium* chromosome 4 and rice chromosome 9. The collinear region covered ~1.25 Mb (41.95–43.20 Mb) and ~0.99 Mb (21.39–22.38 Mb) on *Brachypodium* chromosome 4 and rice chromosome 9, respectively (Table 2).

Comparative mapping in the two populations revealed that *BJ261635* was the proximal marker which was 1.1 cM from *PmAS846* in the N9738/Huixianhong population, but co-segregated with *PmAS846* in the N9134/Shaanyou 225 population. Therefore, the two flanking EST markers *BJ261635* and *CJ840011* are the most closely linked

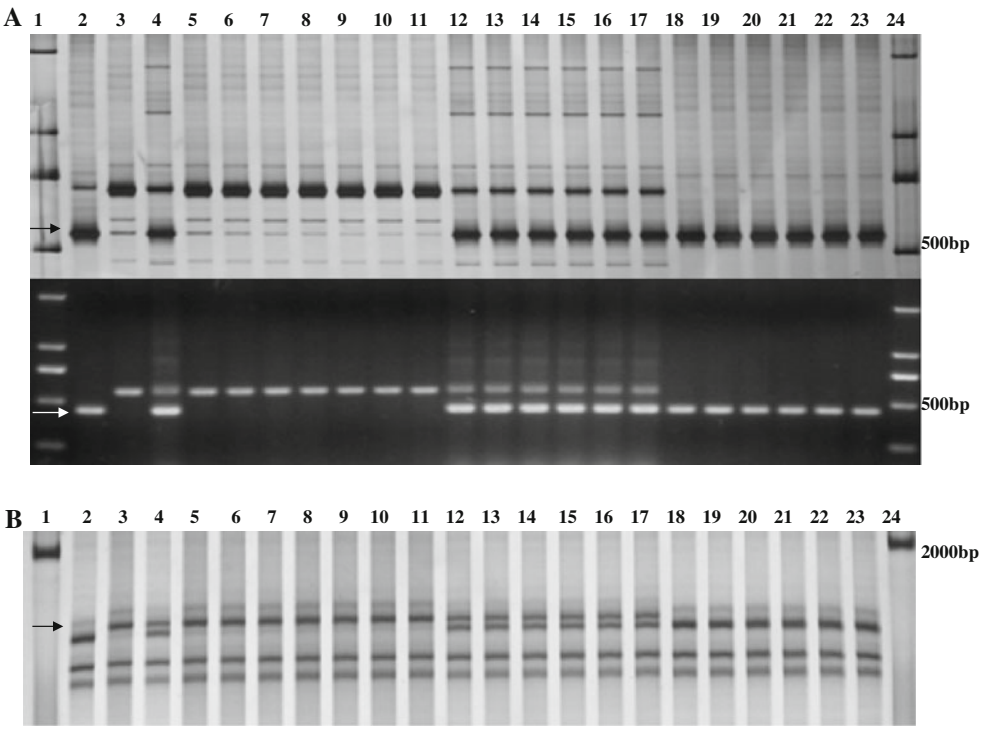

Fig. 3 PCR products of markers *RG-36976* (**a** polyacrylamide and agarose gel electrophoresis, respectively) and *RG-37900* (**b** polyacrylamide gel electrophoresis) amplified in the F_2 population of N9134/Shaanyou 225. *Lanes 2* N9134, *3* Shaanyou 225, *4* resistant bulk, *5* susceptible bulk, *6–11* six homozygous susceptible individual genotypes, *12–17* six heterozygous resistant individual genotypes, *18–23* six homozygous resistant individual. *Arrow* indicates the polymorphic amplification products. A D2000 ladder is shown in *lanes 1* and *24*

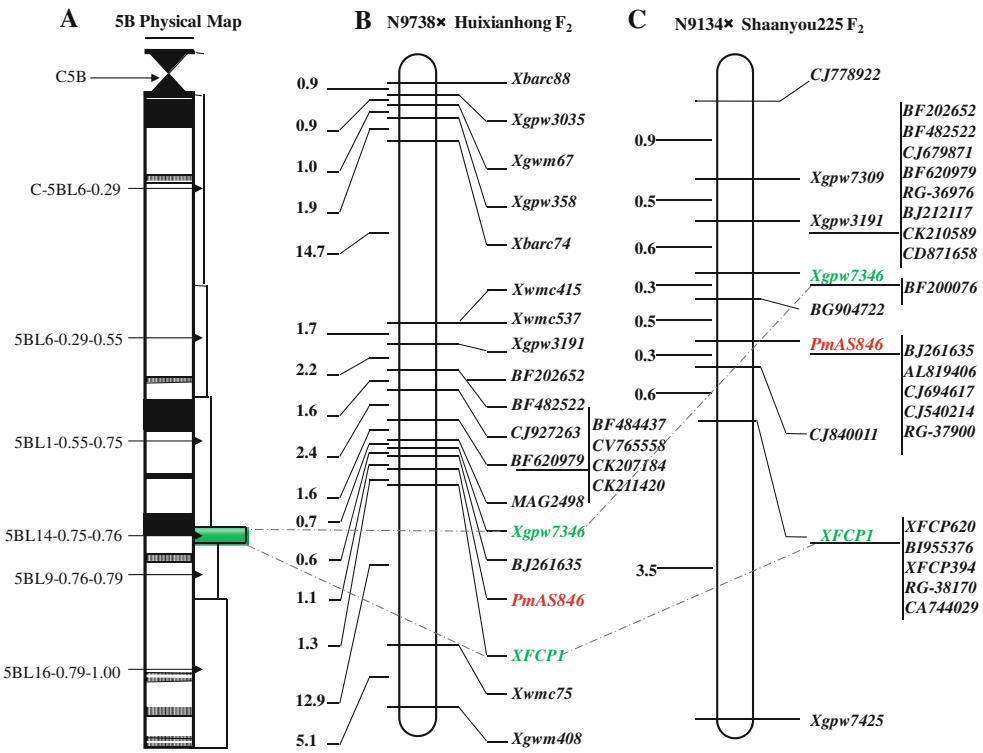

Fig. 4 Molecular mapping of the *PmAS846* locus in the F_2 populations derived from N9738/Huixianhong (**b**) and N9134/Shaanyou 225 (**c**). The genetic linkage map of the *PmAS846* region corresponds to the 5BL deletion bin 5BL14-0.75–0.76 (**a**). Markers are indicated to the *right side* of the genetic map. Flanking SSR markers for *PmAS846* are shown in green and connected with *dashed lines*

markers, and narrowed *PmAS846* locus to a region that is collinear with 197 and 112 kb genomic regions on *Brachypodium* chromosome 4 (*Bradi4g37680* to *Bradi4g37960*) and rice chromosome 9 (*Os09g38520–Os09g38755*), respectively.

A synthetic and orthologous gene comparison map is presented in Fig. 5; only *Bradi4g36976* (chromosome 4: 42.18 Mb) and *Bradi4g38170* (chromosome 4: 43.17 Mb) in the *Brachypodium* collinear region spanning *PmAS846* are annotated as disease resistance gene located at the distal end of chromosome 4L (http://www.gramene.org/Brachypodium_distachyon/Info/Index). Two markers, *RG-36976* and *RG-38170*, in the N9134/Shaanyou 225 population are orthologous to *Bradi4g36976* and *Bradi4g38170* in the collinear region of *Brachypodium*, and they linked with *PmAS846* at 1.4 and 0.9 cM, respectively.

There are 28 gene sequences (CDS, TIGR accession numbers from *Bradi4g37680* to *Bradi4g37960*) among the corresponding homologous region in *Brachypodium* that could be considered for further marker saturation. Twenty-three of these genes on *Brachypodium* chromosome 4 with their annotations are shown in Table 3. No significant homologies with disease resistance protein sequences were found in the collinear regions of *Brachypodium* (*Bradi4g37680* to *Bradi4g37960*) and rice (*Os09g38520* to *Os09g38755*) (http://www.gramene.org/). However, *Bradi4g37710* was annotated as homing "serine/threonine phosphatase activity" and *Bradi4g37900* as "leucine-rich repeat protein kinase". *Bradi4g37800* was homologous with the *Arabidopsis* gene *AT2G03070*, located on *Arabidopsis* chromosome 2 that is known to play a role in defense responses to fungi (http://www.arabidopsis.org/index.jsp). Ontology analysis of these genes on collinear region of *Brachypodium* indicated that *Bradi4g37720* and *Bradi4g37740*, like *Bradi4g37710* and *Bradi4g37900*, are responsive to external or biotic stimuli. These results indicated that all the genes and their homologs might play important roles in defense response processes in wheat and could be considered as potential candidate genes for *PmAS846*.

Discussion

The powdery mildew resistance gene *PmAS846* was introgressed into common wheat line N9134 from the wild emmer accession As846 and subsequently transferred to common wheat line N9738. *PmAS846* provides a potent resistance that is effective against 21 Chinese *Bgt* isolates with different virulence patterns (our unpublished date), and it should be a valuable resource in wheat breeding programs. In the present study, a genetic linkage map for *PmAS846* was developed using 42 SSR and EST-based markers and spanned a genetic distance of 50.6 and 7.2 cM in the two mapping populations, respectively. SSR markers *Xgpw7346* and *XFCP1* flanking *PmAS846* were located in bin 5BL14-0.75–0.76, within a gene-rich region identified previously.

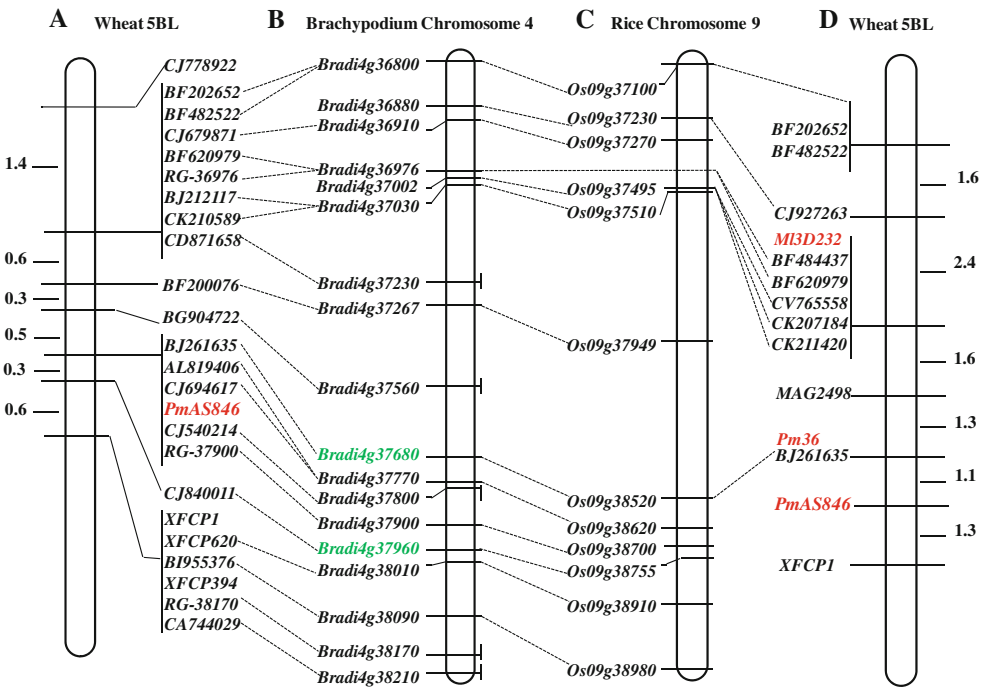

Fig. 5 Comparison of the *PmAS846* region on wheat chromosome 5BL (**a**, **d**) with *Brachypodium distachyon* chromosome 4 (**b**) and rice chromosome 9 (**c**). Markers of wheat and genes of *Brachypodium* or rice with collinearity are connected with *dashed lines*. The collinear genes are indicated to the left of *Brachypodium* chromosome 4 and rice chromosome 9 based on chromosome Mb positions. Collinear region comprising the genes *Bradi4g37680* and *Bradi4g37960*, which span *PmAS846*, are indicated in *green*. Map position of *Pm36* is according to Blanco et al. (2008); map position of *Ml3D232* is according to Zhang et al. (2010) (color figure online)

Table 3 Candidate genes in the *Brachypodium* genome bd21 8X release sequence located in the collinear region comprising the markers *BJ261635* and *CJ840011*, which span *PmAS846* in wheat N9134 and N9738

Brachypodium gene	*Brachypodium* gene annotation	Chromosome location	Wheat marker (cM)	*E* value (tBLASTx)
Bradi4g37680	Dopamine beta-monooxygenase activity	42777206–42780259	*BJ261635* 0.0/1.1	5.0E−69
Bradi4g37690	Putative protein	42788799–42792647		
Bradi4g37700	Copper ion binding	42793335–42794023		
Bradi4g37710	Protein serine/threonine phosphatase activity	42793702–42799764		
Bradi4g37720	Putative protein	42805672–42807281		
Bradi4g37730	THX transcription factor	42810385–42814102		
Bradi4g37740	Intracellular cyclic nucleotide activated cation channel activity, voltage-gated potassium channel activity	42814832–42819740		
Bradi4g37750	BTB	42819916–42821259		
Bradi4g37760	Zinc ion binding	42829632–42830090		
Bradi4g37770	NADPH-hemoprotein reductase activity, iron ion binding, FAD binding, nitric-oxide synthase activity	42830702–42835920	*AL819406* 0.0 *CJ694617* 0.0	4.0E−48 1.0E−97
Bradi4g37780	Zinc ion binding	42839307–42840809		
Bradi4g37800	Transcription coactivator activity	42843356–42858402	*CJ540214* 0.0	2.0E−15
Bradi4g37820	4-Nitrophenylphosphatase activity	42864395–42866457		
Bradi4g37840	4-Nitrophenylphosphatase activity	42872859–42875754		
Bradi4g37860	Putative protein	42884981–42899992		
Bradi4g37870	4-Nitrophenylphosphatase activity	42905754–42908139		
Bradi4g37890	Organic anion transmembrane transporter activity, organic cation transmembrane transporter activity	42914371–42918432		
Bradi4g37900	Leucine-rich repeat protein kinase, putative, subfamily LRR-V	42918857–42922981	*RG-37900* 0.0	1.0E−95
Bradi4g37910	Putative protein	42923216–42927858		
Bradi4g37917	Putative protein	42931923–42935991		
Bradi4g37930	Putative protein	42955371–42957953		
Bradi4g37940	ATPase activity	42958773–42960113		
Bradi4g37960	Phosphoinositide 5-phosphatase activity	42970820–42974182	*CJ840011* 0.3	7.0E−59

Many important disease resistance genes were located to wheat chromosome 5BL, including *Pm36* (Blanco et al. 2008), *Tsn1* (Faris et al. 2010), *Ml3D232* (Zhang et al. 2010) and many others. *Pm36* was transferred into durum wheat line 5BIL-29 and 5BIL-42 from wild emmer accession MG29896. *Ml3D232* was transferred into the hexaploid wheat line 3D232 from wild emmer accession I222. EST marker *CJ683537* (a putative NBS-LRR sequence) co-segregated with *Ml3D232* is orthologous to *Bradi4g36976* (*Bradi4g36980*, previously designated) in the collinear region of *Brachypodium* (Zhang et al. 2010). The EST sequences *CJ683537* and *BF484437* belong to a member of UniGene *Ta.25929*. To characterize the relationship between *Ml3D232* and *PmAS846*, two markers *BF484437* and *RG-36976* were designed based on the sequences of wheat EST *BF484437* and UniGene *Ta.25929*. Both *BF484437* and *RG-36976* were located on the proximal side of *PmAS846* with 1.4–4.0 cM in the two genetic linkage maps of the *PmAS846*. EST sequences *CJ832481* and *BJ261635* belong to the same UniGene *Ta.50830*, which was homologous with the *Brachypodium* gene *Bradi4g37680*. Marker *CJ832481* was located on the distal side of *Ml3D232* at a genetic distance of 2.7 cM (Zhang et al. 2010). *BJ261635* was the proximal marker with a 1.1 cM from *PmAS846* in the N9738/Huixianhong population and co-segregated with *PmAS846* in the N9134/Shaanyou 225 mapping population (Figs. 4, 5). Comparative genetic map analysis confirmed that *PmAS846* was located in a more distal interval (on the distal side of *Ml3D232*) defined by markers *CJ679871*, *RG-36976/BF484437/CJ683537*, *CK210589*, *CD871658* and *BJ261635/CJ832481* (Zhang et al. 2010) (Fig. 5).

The EST-SSR marker *BJ261635* closely linked to *Pm36* was located on chromosome 5BL bin 5BL6-0.29-0.76 and on the distal side of *Pm36* at 0.3–0.4 cM (Blanco et al. 2008). A combination of genetic maps (Fig. 5) indicated that *Pm36* and *PmAS846* are likely to either allelic or tightly linked, and an allelism test would be necessary to understand the relationships between *Pm36*, *Ml3D232* and *PmAS846*.

In addition, SSR markers *XFCP394* and *XFCP620* flanking *Tsn1* (Faris et al. 2010) were located on the distal side of *PmAS846* at a 0.9 cM in the N9134/Shaanyou 225 mapping population (Figs. 4, 5). *Pm36*, *Ml3D232*, *PmAS846* and *Tsn1* may belong to the same gene clusters. Clusters of genes conferring resistance to wheat diseases on wheat chromosomes are not randomly distributed (Dilbirligi et al. 2004). Genes within a cluster can be allelic or closely linked, for example, the powdery mildew resistance genes at the *Pm1* (Singrün et al. 2003) and *Pm3* loci (Srichumpa et al. 2005).

Chromosome 5B is nearly 870 Mb (5BL, 580 Mb and 5BS, 290 Mb) and is known to contain a number of resistance genes such as *Pm36* (Blanco et al. 2008), *Tsn1* (Faris et al. 2010), *Ml3D232* (Zhang et al. 2010) and *PmAS846*, *Ph1* involved in homoeologous pairing (Sidhu et al. 2008), and *SKr* and *Kr1* related to intergeneric crossability of wheat (Alfares et al. 2009). Chromosome 5B seems to have important biological functions in wheat breeding. Sequencing chromosome 5B will provide a platform for map-based cloning of interesting genes located on this chromosome (http://www.wheatgenome.org).

Wheat has an extremely large genome with more than 80% repetitive DNA sequences which make cloning of agronomically important genes very difficult (Gupta et al. 2008). However, gene-rich regions contain less repetitive DNA sequences and recombinations occur much more frequently in the gene-rich regions than gene-poor regions. The bp/cM estimates vary from 118 kb for gene-rich regions to 22 Mb for gene-poor regions (Gill et al. 1996; Gupta et al. 2008). The *PmAS846* locus was delineated to a 0.8 cM interval flanked by the EST marker *BG904722* on the proximal side and the EST marker *CJ840011* on the distal side, close to the end of chromosome 5BL in a region known to be a recombination "hot spot", and much recombination within the targeted interval 5BL 0.75–0.79 occurred toward the distal end (physical to genetic distance ratio ~400 kb/cM) (Faris et al. 2000). Several recombinations that occurred will give sufficient genetic resolution of the mapping populations utilized and thus an attempt at map-based cloning might be successful.

In the present study, the synteny between wheat, *Brachypodium* and rice was used to develop closely linked markers and to increase the density of markers around *PmAS846*. Within the corresponding *Brachypodium* genomic region (*Bd4g37680–Bd4g37960*), no significant homologies to a known NBS-LRR resistance gene analogy were found. In some cases, multiple rearrangements in gene order and content (non-syntenic genes) occurred, such as fungal disease resistance genes *Lr10* (Feuillet et al. 2003), *Lr21* (Huang et al. 2003) and *Pm3* (Yahiaoui et al. 2004), the rice genome contains genes homologous to wheat genes but at non-orthologous positions. Similar situations were also found between wheat and barley and even between wheat sub genomes (Wicker et al. 2011). It seems to be consensus that macro-collinearity (collinearity on the genetic map level) is better preserved than micro-collinearity (collinearity at the sequence level).

However, even if a gene is not present on its orthologous position in *Brachypodium* or rice, the flanking genes are often sufficiently conserved to provide a collection of markers that can be used to saturate the target region in the other cereal genomes. For example, comparative analysis of the *Tsn1* genomic region of wheat chromosome 5B with the homologous regions of rice and *Brachypodium* indicated a conserved level of collinearity with rice chromosome 9 and

Brachypodium chromosome 4, *Bradi4g38050* and *Bradi4g38060* in the *Brachypodium* collinear region, which spans *Tsn1* within a genetic interval of ~100 kb on wheat chromosome 5BL (Faris et al. 2010). Good collinearity has been shown between wheat chromosome 5BL, *Brachypodium* chromosome 4 and rice chromosome 9 (Faris et al. 2010; Sidhu et al. 2008; Zhang et al. 2010). In our research, the genetic linkage map of *PmAS846* and *Brachypodium* chromosome 4 exhibited highly conserved synteny with only one exception, which seems to be better preserved than the collinearity between the wheat EST markers mapped to wheat 5BL and their putative orthologs on rice chromosome 9. The *PmAS846* genomic region of wheat chromosome 5BL (*BJ261635–CJ840011*) also corresponds to the distal region of barley chromosome 5H (123.08-125.81 cM). As barley is more closely related to wheat than *Brachypodium* and rice, the recent sequencing of the barley genome (Mayer et al. 2011) should provide a new comparative genomics approach for fine mapping and cloning of genes in wheat. Markers co-segregated with *PmAS846* and highly conserved synteny of this region between wheat, *Brachypodium* and rice, may allow map-based cloning of *PmAS846*. Initial attempts at chromosome walking in wheat will be performed with these orthologous gene probes that delimited physical distances of 197 and 112 kb in *Brachypodium* and rice, respectively.

It may be easier to use comparative maps to isolate *PmAS846* from a gene-rich region on wheat chromosome 5BL using related plants with small genomes such as *Brachypodium*. Saturation mapping of the *PmAS846* gene region with DNA markers, especially newly developed EST markers is underway. Cloning of the gene(s) will contribute to better understanding the allelic relationships, gene structure and function in this gene-rich region. In addition, most of the tightly linked or co-segregating markers for *PmAS846* locus characterized in this study were inherited as co-dominant markers. They are particularly useful for MAS of *PmAS846* in wheat breeding programs to quickly introgress this gene into commercial varieties or pyramid different resistance genes in a single genotype for more durable resistance.

Acknowledgments This research was supported by National Natural Science Foundation (31071409), National 863 Program (2011AA100103) and National 973 Program (2009CB118301) of China.

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