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Resistance gene analogs associated with Fusarium head blight resistance in wheat

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Abstract Fusarium head blight (FHB) is one of the most destructive diseases in wheat. Identification of resistance gene analogs (RGAs) may provide candidate genes for cloning of FHB resistance genes and molecular markers for marker-assisted improvement of wheat FHB resistance. To identify potential RGAs associated with FHB resistance in wheat, 18 primer pairs of RGAs were screened between two parents (Ning7840 and Clark) and seven informative RGA primer combinations were analyzed in their recombinant inbred lines (RILs). Five PCR products amplified from three primer combinations showed significant association with FHB resistance, and their sequences are similar to the gene families of RGAs. Three of them (RGA14-310, RGA16-462, RGA18-356) were putatively assigned to chromosome 1AL and explained 12.73%, 5.57% and 5.9% of the phenotypic variation for FHB response in the F_7 population, and 10.37%, 3.37% and 4.53% in F_{10} population, respectively; suggesting that these RGAs may play a role in enhancing FHB resistance in wheat. Analysis of nucleotide sequence motifs demonstrated that all the RGA markers contain a heat shock factor that initiates the production of heat shock proteins. A sequence tagged site (STS) marker (FHBSTS1A-160) was successfully converted from RGA18-356, and validated in fourteen other cultivars. Significant interaction between the quantitative trait locus (QTL) on 1AL and the QTL on 3BS was detected. The marker FHBSTS1A-160 in combination with markers linked to the major QTL on 3BS could be used in marker-assisted selection (MAS) for enhanced FHB resistance in wheat.

Keywords *Triticum aestivum* · Resistance gene analog · Wheat scab · QTL tagging · Sequence tagged site

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Introduction

Epidemics of Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*, have recently become more severe and frequent in most wheat growing areas of the USA and many other countries (McMullen et al., 1997; Bai & Shaner, 2004). When

warm and wet weather coincides with wheat anthesis and early grain filling, severe fungal infection can dramatically reduce wheat grain yield and quality (Bai & Shaner, 1994). Molecular mapping provides a useful tool for characterization of the effect of quantitative trait loci (QTLs) for disease resistance, localization of the QTL on a chromosome region detected in a defined population, and understanding interactions between different disease resistance QTLs (Tanksley, 1993; Paterson, 1998). To date, several QTLs for FHB resistance have been reported (Bai & Shaner, 2004), and one QTL on chromosome 3BS from Sumai 3 and its derivative Ning7840 was consistently identified to have a major effect on FHB resistance in various genetic backgrounds (Bai et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2002; Guo et al., 2003). Several types of DNA markers closely linked to the QTL have been identified (Bai et al., 1999; Waldron et al., 1999; Buerstmayr et al., 2002; Guo et al., 2003; Xu & Ban, 2004), and some of them have been extensively used for marker-assisted selection (MAS) in breeding programs.

Recently, many resistance genes against various pathogens have been cloned from diverse plant species. Remarkably, most of these resistance genes share common domains in their encoded proteins, such as leucine-rich repeat (LRR), nucleotide-binding site (NBS) and serine/threonine kinase domains (PtoKin, Hammond-Kosack & Jones, 1997). These conserved domains existing in resistance genes across species offer an opportunity for the use of PCR-based strategies to isolate and clone new resistance gene family members or analogs in other plant species (Kanazin et al., 1996; Leister et al., 1996; Yu et al., 1996). Therefore, the RGA approach provides not only a powerful tool for the isolation of candidate resistance genes and for the analysis of the structure and evolution of resistance gene families, but also a useful marker source for MAS (Huang et al., 2004). Using this approach, putative RGAs were mapped and/or isolated from potato (Leister et al. 1996), soybean (Kanazin et al., 1996; Yu et al., 1996), rice (Mago et al., 1999; Zhou et al., 2004), and barley (Bulgarelli et al., 2004). In wheat, RGAs have been successfully used as molecular markers to tag resistance genes to various pests (Seah et al., 1998; Spielmeyer et al., 2000; Yan et al., 2003; Xie et al., 2004). However, RGA associated with FHB resistance QTL have not been reported. The objective of this study is to explore whether RGAs could be used as candidate genes for FHB resistance in wheat and as a new type of marker for tagging QTLs other than the one on 3BS for FHB resistance.

Materials and methods

Plant materials and FHB evaluation

A population with 133 recombinant inbred lines (RILs) was developed from a cross between an FHB-resistant cultivar Ning7840 and a susceptible cultivar Clark by single-seed descent (SSD) (Bai et al., 1999). The mapping population was evaluated for FHB resistance as described by Bai et al. (1999). In brief, a hypodermic syringe was used to inject 1,000 conidiospores into a central floret of a wheat spike. Inoculated plants were returned to their original positions on greenhouse benches after a 3 days misting period at 23–25 °C with 100% relative humidity. Disease symptoms were recorded by counting the number of symptomatic spikelets on each inoculated spike 21 days after inoculation. Disease severity was calculated as the proportion of symptomatic spikelets (PSS) per inoculated spike at 21st day after inoculation and used for QTL analysis. To validate the STS marker, additional 14 cultivars were evaluated for disease severities by the same method (Bai et al., 2001).

RGA analysis

DNA was isolated from seedlings of the two parents and the F_{10} RILs with the cetylmethylammonium bromide (CTAB) procedure (Saghai-Maroof et al., 1984). The RGA primers (Table 1) were designed from the conserved sequences of LRR, NBS, and PtoKin motifs from cloned resistance genes (Bent et al., 1994; Kanazin et al., 1996; Leister et al., 1996; Yu et al., 1996; Chen et al., 1998; Joyeux et al., 1999; Seah et al., 1998; Rajesh et al., 2002).

To visualize the RGA PCR products, each forward primer was labeled with ^{33}P-ATP. PCR products were separated on 5% denaturing polyacrylamide gels and visualized on an autoradiograph. Two bulks contrasting in FHB responses were constructed by pooling five RILs in each bulk for bulked segregant analysis (BSA) (Bai et al., 1999). The bulks and two parents were initially screened with all 18 RGA primers,

Table 1 Name, sequences, and sources of primers of resistance gene analogs

Name	Forward	Sequence 5′-3′[a]	Reverse	Sequence 5′-3′[a]	Reference
RGA1	RGA1-F	AGTTTATAATTYSATTGCT	RGA1-R	ACTACGATTCAAGACGTCCT	Kanazin et al. (1996)
RGA2	RGA2-F	AGTTTATAATTYSATTGCT	RGA2-R	CACACGGTTTAAAATTCTCA	Kanazin et al. (1996)
RGA3	RGA3-F	AGTTTATAATTYSATTGCT	RGA3-R	CTCTCGATTCAAAATATCAT	Kanazin et al. (1996)
RGA4	Kin2	TGATACTGGATGATGTCTGG	EGF	GTGCTTCTTATGAACCCTTC	Seah et al. (1998)
RGA5	PLRR1-F	TGGGGTGGGAAGACAAC	PLRR1-R	CTCGTGGGATCGTCTGAAG	Bent et al. (1994), Joyeux et al. (1999)
RGA6	PLRR2-F	TGGGGTGGGAAGACAAC	PLRR2-R	GTCAGTTTCGGGCATATGAG	Bent et al. (1994), Joyeux et al. (1999)
RGA7	RGA7-F	AGTTTATAATTYSATTGCT	RGA7-R	CCGAAGCATAAGTTGCTG	Kanazin et al. (1996)
RGA8	XLRR-F	CCGTTGGACAGGAAGGAG	XLRR-R	CCCATAGACCGGACTGTT	Chen et al. (1998)
RGA9	NLRR-F	TAGGGCCTCTTGCATCGT	NLRR-R	TATAAAAAGTGCCGGACT	Chen et al. (1998)
RGA10	RLRR-F	CGCAACCACTAGAGTAAC	RLRR- R	ACACTGGTCCATGAGGTT	Chen et al. (1998)
RGA11	Pto kin1	GCATTGGAACAAGGTGAA	Pto kin2	AGGGGGACCACCACGTAG	Chen et al. (1998)
RGA12	CLRR-F	TTTTCGTGTTCAACGACG	CLRR-R	TAACGTCTATCGACTTCT	Chen et al. (1998)
RGA13	NPLOOP-F	TCAATTAATGTTTGAGTTATTGTA	NKIN2-R	GTAACTAAGGATAGA	Chen et al. (1998)
RGA14	Ptokin3-f	TAGTTCGGACGTTTACAT	Ptokin4-r	AGTGTCTTGTAGGGTATC	Chen et al. (1998)
RGA15	NBS-F	GGAATGGGNGGNGTNGGNAARAC	NBS-R	YCTAGTTGTRAYDATDAYYYTRC	Yu et al. (1996)
RGA16	S1	GGTGGGGTTGGGAAGACAACG	AS1	CAACGCTAGTGGCAATCC	Leister et al. (1996)
RGA17	PtoFen-S	ATGGGAAGCAAGTATTCAAGGC	PtoFen-AS	TTGGCACAAAATTCTCATCAAGC	Leister et al. (1996)
RGA18	NLRR-inv1	TGCTACGTTCTCCGGG	NLRR-inv2	TCAGGCCGTGAAAAATAT	Rajesh et al. (2002)

[a]Code for mixed bases: Y = C/T, N = A/T/G/C, R = A/G, S = C/G and D = A/G/T.

but only primers showing unambiguous polymorphic bands between bulks were used for further segregation analysis. PCR amplification was conducted in a 10 μl volume containing 40 ng of template DNA, 1 X PCR buffer, 3.3 mM $MgCl_2$, 230 μM each of dATP, dTTP, dGTP and dCTP, 0.5 μM of each primer, and 0.6 U *Taq* DNA polymerase. The PTC-100 DNA thermal cycler (MJ Research Inc., Waltham, Mass, USA) was programmed for 3 min at 94 °C followed by 6 cycles of 1 min at 94 °C, 1 min at 58 °C (−2 °C/cycle) and 1 min at 72 °C, then 39 cycles of 30s at 94 °C, 30s at 45 °C and 1 min at 72 °C. A step of 7 min at 72 °C was used for a final PCR extension.

The RGA data were combined with AFLP and SSR marker data (Bai et al., 1999; Guo et al., 2003) for linkage map construction using the JoinMap 3.0 program (Van Ooijen & Voorrips, 2001). Recombination fractions were converted to centiMorgans (cM) according to the Kosambi mapping function (Kosambi, 1944). A logarithm of odds (LOD) threshold of 3 was used to claim genetic linkage among molecular markers. For QTL analysis, simple regression and interval mapping analysis was performed using the QGene program (Nelson, 1997). To identify an appropriate threshold LOD score for declaring a significant QTL, 1,000 times permutation test was conducted using the same program resulting in a LOD threshold of 2.21 to claim presence of a QTL.

Converting a RGA into a STS marker

The procedure from Guo et al. (2003) was used to convert a RGA into a STS marker with slight modification. In brief, a RGA band co-segregating with an FHB-resistance QTL was excised with a needle from a dried polyacrylamide gel, and re-hydrated for 3 min in 1 ml deionized H_2O. Excess water was removed and the gel was crushed using a pipette tip. A total of 200 μl deionized H_2O was added to the crushed gel. The crushed gel in a sealed microfuge tube was immersed into boiling water for 3 min. The supernatant was transferred into a new tube for further PCR amplification after being centrifuged at 14,000 rpm for 10 min. To verify the size of the target band, the isolated DNA fragment was re-amplified with the original RGA primers, and PCR products were analyzed on a 5% denaturing polyacylamide gel with the original RGA reaction products as a control. If other bands were amplified in addition to the target band, the target band was cut out again and the DNA extraction process and gel analysis was repeated. If re-amplified PCR generated only one band of the correct size, the PCR product was further used for RGA cloning.

Target PCR products were ligated into the pGEM T-Easy vector (Promega, Madison, WI, USA), and transformed into DH5α (*Escherichia. coli*) competent cells. After transformation, 40 white colonies from each transformation were selected and separately cultured overnight in test tubes containing 5 ml Luria Borth (LB) media with 50 μl/ml of Ampicillin. A 2 μl aliquot of each cultural solution was used as template for PCR in a 50 μl volume containing 1 X PCR buffer, 1.5 mM $MgCl_2$, 240 μM each dNTP, 0.26 μM each of M13 forward and reverse primers, and 0.2 U *Taq* DNA polymerase. Thermocycling conditions consisted of an initial step of 95 °C for 1 min, followed by 45 cycles of 45 s at 94 °C, 45 s at 53 °C and 1 min at 72 °C, and by a step of 10 min at 72 °C for final extension. PCR products were visualized on a 2% agarose gel stained with ethidium bromide. Sixteen clones with similar size of PCR inserts were sequenced for each RGA.

Plasmid DNA from selected clones was isolated using QIAprep spin Miniprep Kit (Qiagen, Valencia, CA, USA) and sequenced with an ABI 3700 DNA Analyzer in the core facilities of Oklahoma State University. Sequences with at least six identical copies for each RGA were selected to design primers for further analysis. The STS primers were initially utilized to amplify the genomic DNA from the two parents (Ning7840 and Clark) in a 30 μl volume containing 1 $\times$ PCR buffer, 2.5 mM $MgCl_2$, 230 μM each dNTP, 0.3 μM each forward and reverse primer, 1U *Taq* DNA polymease. PCR amplification started at 94 °C for 3 min, followed by 38 cycles of 45 s at 94 °C for denaturing, 45 s at 56–58 °C for annealing, varied with Tm of different primer sets, and 1 min at 72 °C for extension. A final extension step was 72 °C for 7 min. The primers that amplified at least one polymorphic band between the two parents were further analyzed for segregation in the RILs. If a primer pair amplified a PCR product showing the same banding pattern as the original RGA marker, it was considered to be a candidate for a STS marker. A RGA marker was designated as a combination of RGA primer name and target fragment size. To assess the potential application of a STS marker for MAS, 14 diverse cultivars and breeding lines were analyzed with the STS marker.

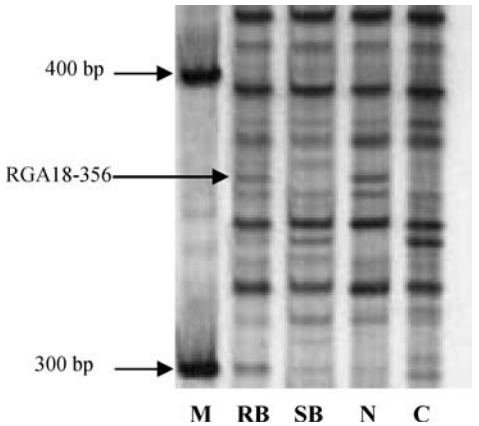

Fig. 1 Bulk segregant analysis in a 5% denaturing polyacrylamide gel showing the RGA polymorphism between the resistant bulk (RB) and susceptible bulk (SB), and between the resistant (Ning7840 (N)) and susceptible (Clark (C)) parents. The PCR product was amplified with the RGA18 primer combination. "M" represents molecular size marker (100-bp DNA ladder from Life Technologies, Inc, USA)

Results

Resistance gene analogs

Among 18 RGA primer pairs screened between the parents and bulks, seven primer combinations produced 22 unambiguous polymorphic bands that differentiated Ning7840 and the resistant bulk from Clark and the susceptible bulk (Fig. 1). These primer pairs were further analyzed in the population (Fig. 2). Five RGA markers (RGA14-310, RGA14-339, RGA16-462, RGA18-356 and RGA18-754) showed significant association with FHB response. The determination coefficients between these markers and FHB severities were 0.13, 0.02, 0.056, 0.059, 0.036 in F_7 generation, and 0.104, 0.028, 0.034, 0.045, 0.049 in F_{10} generation, respectively.

Linkage analysis with AFLP and SSR markers previously developed (Bai et al., 1999; Guo et al., 2003) showed that three RGAs (RGA14-310, RGA16-462, and RGA18-356) associated with FHB resistance were tightly linked to the SSR marker BARC017 which was associated with FHB resistance by 0.034 and 0.023 of phenotypic variation for FHB resistance in F_7 and F_{10} generations, respectively. The marker BARC017 was located on chromosome bin 1AL-3 through genetic and physical mapping (Song et al., 2005), suggesting that this linkage group is most likely on chromosome 1AL (Fig. 3). Interval mapping indicated that these markers were linked to a minor QTL for FHB resistance in Ning 7840 (Fig. 3). The chromosome locations for markers RGA14-339 and RGA18-754 are unknown because linked SSR markers were not found.

Sequence analysis of RGAs

Five RGAs were selected to amplify DNA fragments in Ning 7840 for further sequencing. For each RGA marker, 16 colonies were sequenced and 13, 12, 7, 13 and 6 identical sequences were obtained for fragments RGA14-310, RGA14-339, RGA16-462, RGA18-356 and RGA18-754, respectively. Those identical sequences from each RGA were selected for TBLASTx similarity searches in the NCBI GenBank database. The results indicated that RGA14-310 is similar to a

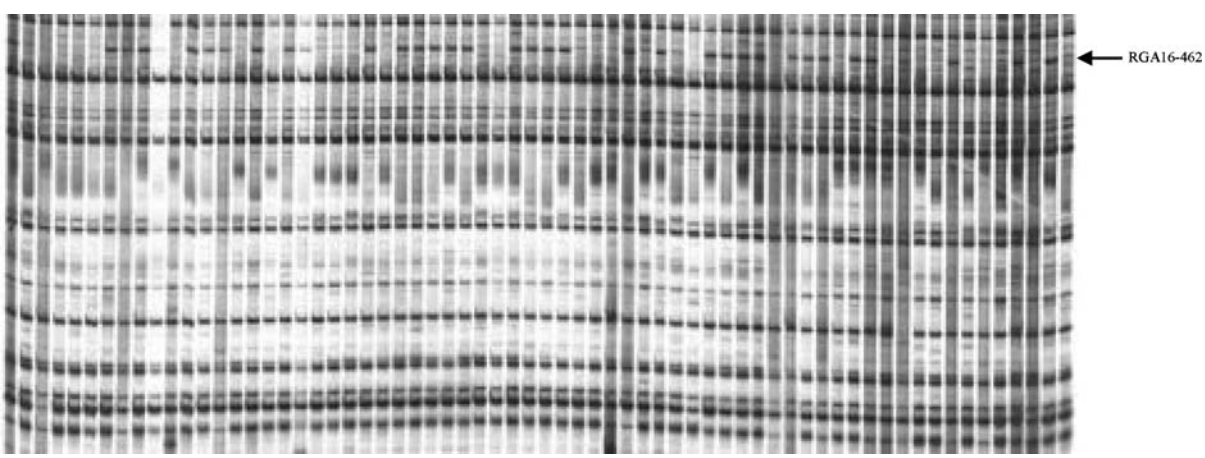

Fig. 2 A portion of ^{33}P denaturing polyacrlamide gel showing polymorphic bands segregating among Clark (first lane from right), Ning7840 (second lane from right) and their 64 F10 RILs (lanes 1 to 64 from left). DNA was amplified with the RGA16 primer pair

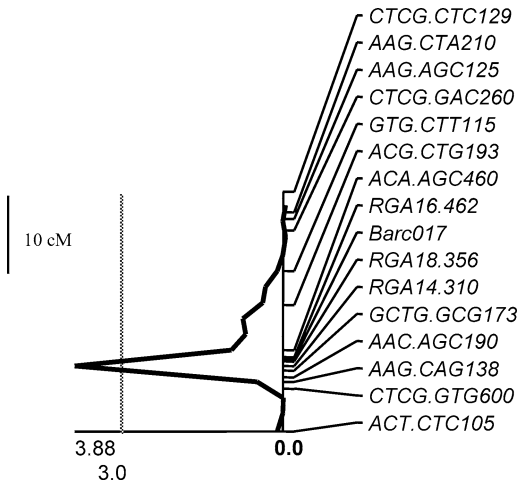

Fig. 3 The linkage group harboring a minor QTL for FHB resistance. The marker names are shown at the right side of map. The LOD (log 10 of the odds ratio) plot at the left side of map indicates the most likely position of the minor QTL relative to RGA markers for FHB resistance identified in F_7 RILs using the QGene program (Nelson 1997)

maize homeodomain protein (55%, 19/34 amino acid, X92428.1, GI1648930) that was reported to play a role in disease resistance (Korfhage et al., 1994). RGA14-339 had homology with a putative late blight resistance protein of *Solanum demissum* (44%, 47/105 amino acids, AAT39281.1, GI47900682). RGA16-462 exhibited a sequence homology with a rice NBS-LRR type resistance protein (39%, 41/103 amino acids, AAB96999.1, GI2792248). RGA18-356 was similar to a leucine-rich repeat-containing protein (48.5%, 51/105 amino acids, XP_538968.1, GI57095028) and RGA18-754 showed high similarity to *Lr21*, a wheat leaf rust resistance gene (81%, 70/86 amino acids, AF532104.1, GI32141362).

The DNA nucleotide sequence motifs of the five RGAs were analyzed in GenomeNet using Sequence Motif Search (http://motif.genome.jp) with 95% of cutoff score and zero of gap penalty. All five RGAs contain a heat shock factor (HSF) that has been found in many RGAs such as Pto-like serine/threonine kinases and NBS/LRR resistance protein-like proteins. Three RGAs (RGA14-310, RGA16-462 and RGA18-356) contain the activator of nitrogen-regulated genes (*NIT2*), and two (RGA14-339 and RGA16-462) have alcohol dehydrogenase gene regulator 1 (ADR1, Table 2). Environmental stresses can induce heat shock protein and alcohol dehydrogenase (Conley et al., 1999; Queitsch et al., 2000), and nitrogen starvation can induce nitrogen-regulated genes (Liu et al., 2003). These conserved motifs are *cis*-acting regulatory elements for activating these genes during environmental stresses, suggesting these RGAs may be involved in responses to stress environments.

A STS marker developed from RGAs

Three RGA markers (RGA14-310, RGA16-462 and RGA18-356) on 1AL that possessed a significant correlation with FHB resistance were selected to develop STS markers. Two primer pairs per RGA were designed to amplify genomic DNA from Ning7840 and

Table 2 DNA motifs of five selected RGA markers

Marker Name**	Transfac number	Position in sequence	Motif sequence	Motif name	Function
RGA14-310	M00028	90..94, 255..251	AGAAN	HSF	Heat shock factor
	M00142	296..291	TATCTM	NIT2	Activator of nitrogen-regulated genes
RGA14-339	M00028	292..296	AGAAN	HSF	Heat shock factor
	M00048	83..88	NGGRGK	ADR1	Alcohol dehydrogenase gene regulator 1
RGA16-462	M00028	151..147, 346..342	AGAAN	HSF	Heat shock factor
	M00048	3..8, 52..47	NGGRGK	ADR1	Alcohol dehydrogenase gene regulator 1
	M00142	171..166	TATCTM	NIT2	Activator of nitrogen-regulated genes
RGA18-356	M00028	12..8, 75..71, 85..81, 130..126, 196..200, 231..227, 322..318	AGAAN	HSF	Heat shock factor
	M00142	55..60	TATCTM	NIT2	Activator of nitrogen-regulated genes
RGA18-754	M00028	11..7, 476..472, 603..599	AGAAN	HSF	Heat shock factor

*K = T/G; N = A/T/G/C; M = A/C; R = A/G

**The molecular size for each RGA is the number after the name of each RGA primer combination.

TGCTACGTTCTCCGGGGCACCAAGGTCGTCACCTCTTTTTACTGTCTTCT
TGTTATCTCGGAGAGTCCCTTTCTCTTGCTTTCTCGTGGTATTTGAAATT
CTAATCCATGGTATGCTCTAGTTAGTTCTTGGACTGTGTCATCTTGTAAA
AATTATGCACTCCATGTGGGAACAGCGCTGTTATTTCAATCTGAAGAAAT
AATAATGTTGTTGATCCTTGTTTCATTTCTGTGCAAATTTAGTTTGGAAG
GTGTTCGTATTTCCTAGTTTGGTCTATTATGATGCAATCTTCTCTCTTGT
GTGCTCTAGTGTGACGTTTCTTAAATCTCTTCTGTTAAATATTTTTCACG
GCCTGA

Fig. 4 DNA sequence of RGA18-356 derived from the resistant cultivar Ning7840. Underlined nucleotides are the sequences used for designing the new STS primers, in which forward and reverse sequences from 5′ to 3′ are CCAAGGTCGTCACCTCTTTTTAC and CCCACATGGAGTGCATAATTTTTAC, respectively

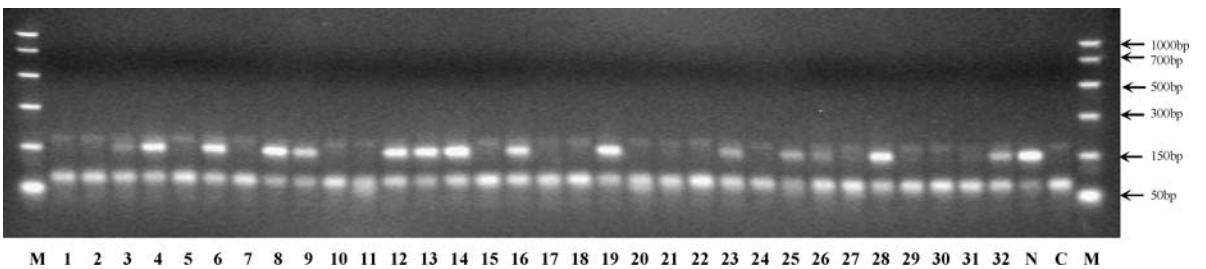

Fig. 5 DNA fragment patterns for marker FHBSTS1A-160. Lanes 1 to 32 are the F_{10} RILs from the cross of Ning7840/Clark; 'N' Ning7840; 'C' Clark; 'M' is the molecular size marker. The PCR products were electrophoresed on a 3% agarose gel

Clark. Five primer combinations amplified a monomorphic band between the two parents. Only one primer pair from RGA18-356 (Fig. 4) amplified a single unambiguous band that was polymorphic between two parents (Fig. 5). This primer combination amplified the same banding pattern in the RILs as that of RGA18-356; therefore, it is most likely the true representative of RGA18-356. This STS marker, named FHBSTS1A-160, explained 5.84% and 4.55% of the phenotypic variations reflected by the proportion of scabbed spikelets (PSS) in inoculated spikes in the F_7 and F_{10} generations, respectively. A very small difference in R^2 value between the marker FHBSTS1A-160 and the original RGA is probably due to one missing RGA data point for one RIL.

The interaction between the newly identified QTL and the major QTL on 3BS for FHB resistance was analyzed using the marker FHBSTS1A-160 and SRSY.3B1 linked to the major QTL on 3BS previously developed from an AFLP (Guo et al., 2003). The results indicated that 133 RILs could be separated into four genotypes based on combinations of four marker alleles at the two marker loci. Table 3 shows that the alleles from Ning7840 at SRSY.3B1 locus have a major effect on FHB resistance, whereas the Ning7840 allele at FHBSTS1A-160 locus alone contributes only a trivial effect on FHB response. However, RILs carrying Ning 7840 alleles at both the 3BS and 1AL loci exhibited a significantly higher level of FHB resistance than those with the Ning 7840 allele alone at one of the two loci.

Table 3 Epistatic effect of QTL on 1AL identified by the marker FHBSTS1A-160 to the major QTL on 3BS identified by STS marker SRSY.3B1

Genotype	% of RILs	F_7 PSS	F_{10} PSS
N1AL +N3BS	22.3%	0.35[a]	0.32[a]
C1AL +N3BS	19.2%	0.51[b]	0.48[b]
N1AL +C3BS	21.5%	0.80[c]	0.77[c]
C1AL +C3BS	36.9%	0.82[c]	0.80[c]

N3BS and C3BS refer to presence of Ning7840 and Clark alleles, respectively, at SRSY.3B1 locus on 3BS
N1AL and C1AL refer to presence of Ning7840 and Clark alleles, respectively, at the FHBSTS1A-160 locus
Letters a, b and c represent values that are significantly different ($\alpha = 0.05$) in mean PSS for different genotypes

An additional 14 cultivars with various pedigrees and FHB response were analyzed with FHBSTS1A-160. All cultivars with Sumai 3 or a derivative in their pedigrees showed the same band as that in Ning7840

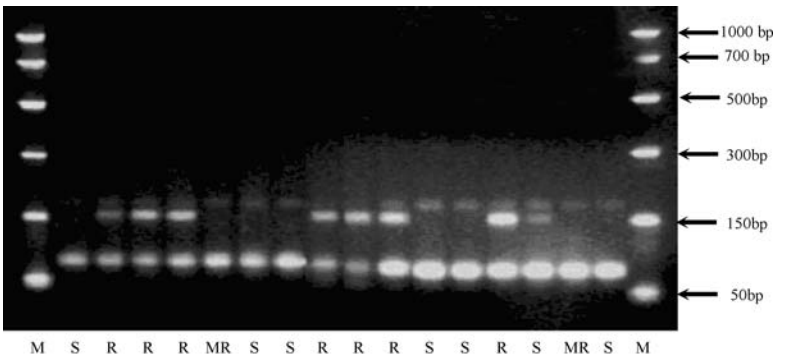

Fig. 6 Banding pattern of the STS marker FHBSTS1A-160 amplified in 16 wheat cultivars. Lanes from left to right are: molecular size marker 'M' (PCR marker from Promega Corporation, Madison, WI, USA), cultivars MO-94-193, Fu5114, Ning8331, Sumai49, IL94-1549, IL94-6280, Clark, Sumai3, Fu5125, Ning8026, Cardinal, NTPHP, Ning7840, Foster, Expert, Pontiac and the molecular size marker. The PCR products were analyzed on a 3% agarose gel. 'R', 'MR' and "S" represent FHB resistant, moderately resistant and susceptible lines, respectively

(Fig. 6 and Table 4), whereas cultivars without Sumai 3 in the pedigree did not amplify the band except for Foster. Therefore, marker FHBSTS1A-160 has potential to be used as a selectable marker for the minor QTL on 1AL.

Discussion

RGAs have been used to identify candidates for resistance genes in several plant species, and are believed to be significantly associated with QTLs conferring resistance to pests (Wisser et al., 2005). In *Arabidopsis thaliana*, a RGA was identified as part of the RPP5 gene (Aarts et al., 1998). In wheat, RGA markers were tightly linked to *Yr5*, *Yr9* and *Yr17* for stripe rust resistance, *Lr21*, *Lr26* and *Lr37* for leaf rust resistance, *Sr30*, *Sr31* and *Sr38* for stem rust resistance, and *Pm31* for powdery mildew resistance (Koebner, 2003; Yan et al., 2003; Xie et al., 2004). However, no research has associated RGAs with FHB resistance in wheat. In this study, five RGAs linked to FHB resistance were

Table 4 Pedigree, proportion of symptomatic spikelets (PSS), and deduced FHB responses for the sixteen wheat cultivars or lines analyzed with the STS markers FHBSTS1A-160 and SRST.3B1

Cultivar	Pedigree	PSS	Deduced FHB response
Fu5114[a,b]	LongXi18/Ning8017	0.06	R
Fu5125[a,b]	Fufan904/Ning8017	0.05	R
Ning7840[a,b]	Aurora /Anhui11//Sumai3	0.08	R
Ning8026[a,b]	Aurora/ Sumai3//Yangmai2	0.28	R
Ning8331[a,b]	Yangmai4/ Ning7840	0.12	R
NTPHP[a]	Chinese Landrace	0.58	R
Sumai3[a,b]	Funo/Taiwanxiaomai	0.08	R
Sumai49[a,b]	N7922/Ning7840	0.08	R
Foster[b]	Coker65-20/Arthur/4/Chul*8CC//VA68-2-7/Abe/3/Va72-54-14/Tyler//Suwon92/Arthur//Arthur/VA 70-52-2	0.71	S
Cardinal	Logan *2/3/Va63-5-12/Logan//Blueboy	0.76	S
Clark	Beau//65256A1-8-1/67137B5-16/ Sullivan/ Beau// 5517B8-5-3-3/Logan	0.94	S
Expert	Extrem/Mexico4040//Neuhof1/3/Extrem/HP35719	0.37	S
IL94-1549	Auburn/Ark38-1/Arthur/Blueboy	0.30	S
IL94-6280	IL87-3721/Cardinal//P808801-4-2-4-107	0.94	S
MO-94-193	MO 11728/Becker	1.00	S
Pontiac	Magnum/Auburn	0.71	S

Note: 'a' and 'b' represent the same banding pattern as Ning7840 when analyzed by SRST.3B1 and FHBSTS1A-160, respectively

identified in the RIL population of Ning7840/Clark. Among them, RGA14-310 showed sequence similarity to a maize homeodomain protein and explained 12.7% and 10.4% of the phenotypic variation for FHB resistance in F_7 and F_{10} RIL populations, suggesting this RGA may be located in the region tightly linked to the QTL on 1AL. Two other RGAs were identified in the same linkage group, but with much smaller effects on FHB response. One of them (RGA16-462) showed DNA sequence homology with an NBS-LRR type resistance protein, the other (RGA18-356) is similar to a leucine-rich repeat-containing protein. These two RGAs are closely linked to the 1AL QTL. Two other unmapped RGAs (RGA14-339, and RGA18-754) showed sequence similarity with a putative late blight resistance protein, and wheat leaf rust resistance gene *Lr21*, respectively. These two RGAs explained 3 to 5% of the phenotypic variation for FHB response; and therefore, may be closely linked to the other minor QTLs. Searches for conserved nucleotide sequences (motif) indicated that all five RGAs contained the same motif of HSF (Table 2). HSF initiates transcription of heat shock proteins when a plant undergoes various environmental stresses such as heat, cold and oxygen deprivation (Queitsch et al., 2000), and is believed to play an important role in signaling and activating HSP transcription under pathogen attack (Cheong et al., 2002). Therefore, HSF in these RGAs may play an important role in plant defense against FHB infection.

RGAs have been used as markers to tag disease resistance genes in plants (Chen et al., 1998; Seah et al., 1998; Mago et al., 1999; Yan et al., 2003; Huang et al., 2004). However, RGA markers are not practical for marker-assisted selection in breeding programs due to their complexity. STS and SSR markers are simple, rapid and inexpensive, thus are suitable for screening of breeding materials in MAS programs. In the present study, SSR marker BARC017 was linked to the minor QTL on chromosome 1A, and it could be used for MAS. However, this SSR marker explained less phenotypic variation for FHB resistance than the RGA markers, and therefore developing STS markers linked to the QTL would be beneficial to MAS in breeding programs.

Converting RGAs into STS markers is still a technical challenge in wheat. One of the obstacles is that target markers may be contaminated with DNA fragments from adjacent bands when they are excised from the gel for sequence analysis. Selecting the right fragment for sequencing and designing the right primers for fragment amplification are critical steps for successful cloning of the target fragment. The primers have to amplify the polymorphic band with the same segregating pattern as the original RGA marker (Bradeen & Simon, 1998). A few cases of converting complicated PCR markers into simple STS markers were successful in wheat (Parker & Langridge, 2000; Guo et al., 2003; Xu & Ban, 2004). In this study, the conversion of either RGA14-310 or RGA16-462 into STS markers failed due to lack of polymorphism between the parents. Only RGA18-356 was successfully converted into a STS marker. Currently, many markers for the major QTL on 3BS have been developed, but markers for minor QTLs are limited. Nevertheless, markers for additional minor QTLs are urgently needed for MAS to pyramid minor QTLs with the major QTL on 3BS to enhance resistance to FHB in commercial cultivars. Although marker FHBSTS1A-160 explained only a small portion of the phenotypic variation for FHB resistance in F_7 and F_{10} RILs, it significantly enhanced wheat FHB resistance when it was combined with markers for the QTL on 3BS. The marker FHBSTS1A-160 was validated across an additional 14 cultivars or lines and showed that it was present in Sumai 3 and, with one exception, its derivatives and absent in cultivars not related to Sumai 3. The result suggests that the marker FHBSTS1A-160 linked to the minor QTL can be used for MAS in combination with markers for the major QTL on 3BS.

QTL on 3BS from Sumai 3 has demonstrated the largest effects on FHB resistance in different genetic backgrounds (Bai & Shaner, 2004). However, RGAs linked to the major QTL on 3BS were not identified in this study. This may be due to insufficient primers being screened. It is also possible that the QTL on 3BS for FHB resistance may have a different structure from currently cloned resistance genes. Map-based cloning of the 3BS resistance QTL will facilitate further understanding of the structure of resistance genes on 3BS.

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References

Aarts MGM, Hekkert BTL, Holub EB, Beynon JL, Stiekema WJ, Pereira A, (1998) Identification or R-gene homologous DNA fragments genetically linked to disease resistance loci in Arabidopsis thaliana. Mol Plant-Microbe Interact 11: 251–258.

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Mitchell FJ, Song QJ, Cregan PB, Frohberg RC, (2001) DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor Appl Genet 102: 1164–1168.

Bai G-H & Shaner G, (1994) Scab of wheat: prospects for control. Plant Dis 78: 760–776.

Bai, GH, Kolb FL, Shaner GE, Domier LL, (1999) Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89: 343–348.

Bai GH, Plattner R, Desjardins A, Kolb F, (2001) Resistance to Fusarium head blight and Deoxynivalenol accumulation in wheat. Plant Breed 120: 1–6.

Bai GH, Shaner G, (2004) Management and resistance in wheat and barley to Fusarium head blight. Ann Rev Phytopathol 42: 135–161.

Bent AF, Kunkel BN, Dahlbeck D, Schmidt KL, Brown R, Giraudat J, Leung J, Staskawicz BJ, (1994) RPS2 of *Arabidopsis thaliana*: A leucine-rich repeat class of plant disease resistance genes. Science 265: 1856–1860.

Bradeen JM, Simon PW, (1998) Conversion of an AFLP fragment linked to the carrot Y2 locus to a simple, codominant, PCR-based marker form. Theor Appl Genet 97: 960–967.

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P, (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). Theor Appl Genet 104: 84–91.

Bulgarelli D, Collins NC, Tacconi G, Dellaglio E, Brueggeman R, Kleinhofs A, Stanca AM, Valè G, (2004) High-resolution genetic mapping of the leaf stripe resistance gene *Rdg2a* in barley. Theor Appl Genet 108: 1401–1408.

Cheong YH, Chang HS, Gupta R, Wang X, Zhu T, Luan S, (2002) Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress and hormonal response in Arabidopsis. Plant Physiol 129: 661–677.

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

Conley TR, Peng-P H, Shih MC, (1999) Mutations affecting induction of glycolytic and fermentative genes during germination and environmental stresses in Arabidopsis. Plant Physiol 119: 599–607.

Guo P-G, Bai-H G, Shaner GE, (2003) AFLP and STS tagging of a major QTL for Fusarium head blight resistance in wheat. Theor Appl Genet 106: 1011–1017.

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

Huang D, Wu W, Lu L, (2004) Microdissection and molecular manipulation of single chromosomes in woody fruit trees with small chromosomes using pomelo (*Citrus grandis*) as a model. II. Cloning of resistance gene analogs from single chromosomes. Theor Appl Genet 108: 1371–1377.

Joyeux A, Fortin MG, Mayerhofer R, Good AG, (1999) Genetic mapping of plant disease resistance gene homologs using a minimal *Brassica napus* L. population. Genome 42: 735–743.

Kanazin V, Marek LF, Shoemaker RC, (1996) Families of resistance gene analogs are conserved and clustered. Proc Natl Acad Sci USA 93: 11746–11750.

Koebner R, (2003) MAS in cereals: green for maize, amber for rice, still red for wheat and barley. In Marker Assisted Selection: A Fast Track to Increase Genetic Gain in Plant and Animal Breeding? Session I: MAS in Plants, pp. 12–17. FAO Electronic Forum on Biotechnology in Food and Agriculture: Conference 10, 17 November–14 December 2003.

Korfhage U, Trezzini GF, Meier I, Hahlbrock K, Somssich IE, (1994) Plant homeodomain protein involved in transcriptional regulation of a pathogen defense-related gene. Plant Cell 6: 695–708.

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

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

Liu L-H, Ludewig U, Gassert B, Frommer WB, Wirén von N, (2003) Urea transported by nitrogen-regulated tonoplast intrinsic proteins in Arabidopsis. Plant Physiol 133: 1220–1228.

Mago R, Nair S, Mohan M, (1999) Resistance gene analogs from rice: cloning, sequencing and mapping. Theor Appl Genet 99: 50–57.

McMullen M, Jones R, Gallenberg D, (1997) Scab of wheat and barley: a reemerging disease of devastating impact. Plant Dis 81: 1340–1348.

Nelson JC, (1997) QGENE: software for marker-based genomic analysis and breeding. Mol Breed 3: 239–245.

Parker GD, Langridge P, (2000) Development of an STS marker linked to a major locus controlling flour colour in wheat (*Triticum aestivum* L.). Mol Breed 6: 169–174.

Paterson AH, (1998) Of blending, beans, and bristles: the foundations of QTL mapping. In: A.H. Paterson (ed) Molecular Dissection of Complex Traits. pp. 1–10. CRC Press, New York, USA.

Queitsch C, Hong SW, Vierling E, Lindquist S, (2000) Heat shock protein 101 plays a crucial role in thermotolerance in Arabidopsis. Plant Cell 12: 479–492.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW, (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81: 8014–8018.

Seah S, Sivasithamparam K, Karakousis A, Lagudah ES, (1998) Cloning and characterisation of a family of disease resistance gene analogs from wheat and barley. Theor Appl Genet 97: 937–945.

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB, (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor. Appl. Genet. 110: 550–560.

Spielmeyer W, Huang L, Bariana H, Laroche A, Gill BS Lagudah ES, (2000). NBS-LRR sequence family is associated with leaf and stripe rust resistance on the end of homoeologous chromosome group 1S of wheat. Theor Appl Genet 104: 1139–1144.

Tanksley S, (1993). Mapping polygenes. Ann Rev Genet 27: 205–233.

Van Ooijen JW, Voorrips RE, (2001) JoinMap3.0, Software for the calculation of geneitic linkage maps. Plant Research International, Wageningen, the Netherlands.

Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Frohberg RC, (1999) RFLP mapping of QTL for *Fusarium* head blight resistance in wheat. Crop Sci 39: 805–811.

Wisser RJ, Sun Q, Hulbert SH, Kresovich S, Nelson RJ, (2005) Identification and characterization of regions of the rice genome associated with broad-spectrum, quantitative disease resistance. Genetics 169: 2277–2293.

Xie C, Sun Q, Ni Z, Yang T, Nevo E, Fahima T, (2004) Identification of resistance gene analogue markers closely linked to wheat powdery mildew resistance gene *Pm31*. Plant Breed 123: 198–200.

Xu DH, Ban T, (2004) Conversion of AFLP markers associated with FHB resistance in wheat into STS markers with an extension-AFLP method. Genome 47: 660–665.

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

Zhou T, Wang Y, Chen JQ, Araki H, Jing Z, Jiang K, Shen J, Tian D, (2004) Genome-wide identification of NBS genes in japonica rice reveals significant expansion of divergent non-TIR NBS-LRR genes. Mol Genet Gen 271: 402–415.