Short Communication

Resistance gene analog polymorphism (RGAP) markers co-localize with the major QTL of fusarium head blight (FHB) resistance, *Qfhs.ndsu-3BS* in wheat

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Abstract
Resistance gene analog polymorphism (RGAP) markers linked to Fusarium head blight resistance (FHB) and co-localize with Qfhs.ndsu-3BS were identified using F_3 plants and $F_{3:5}$ lines derived from a 'Wangshuibai' (resistant) / 'Seri82' (susceptible) cross. The mapping populations were genotyped using 50 degenerate primers designed based on the known R genes. Out of the 50 designed primer combinations, eight showed polymorphism and produced 16 RGAP markers. Out of the 16 RGAP markers, two were integrated into the major QTL for FHB resistance, *Qfhs.ndsu-3BS*. Composite interval mapping (CIM) analysis detected two QTLs in a genomic region that were coincident with *Qfhs.ndsu-3BS*, thus explaining up to 12.5% of the phenotypic variations. The nucleotide sequence analysis of the positive subjected RGAP markers showed that known R-genes, namely *Pto* and *Pto*-like genes, may be considered as FHB candidate resistance genes underlying *Qfhs.ndsu-3BS* and may be used in future studies.
Keywords: Fusarium head blight; *Qfhs.ndsu-3BS*; RGAP markers; resistance genes*; Triticum aestivum* L.

Fusarium head blight (FHB or scab), commonly caused by *Fusarium graminearum Schwabe* and *Fusarium culmorum* (*W.G. Smith*) is one of the most destructive diseases of wheat, *Triticum aestivum* L., in humid and semi-humid areas worldwide. The development of cultivars with high FHB resistance is the most economical and effective method to control the disease. Although diverse sources of resistance to FHB are available in wheat, breeding for FHB resistance with classical selection methods is costly and time-consuming (Mardi *et al*., 2004). Mapping of quantitative trait loci (QTL) associated with FHB resistance and application of marker-assisted selection (MAS) can be used to accelerate the selection and development of FHB resistant cultivars (Anderson *et al*., 2007). DNA markers linked to FHB resistance have been identified in several studies using different mapping populations (Anderson *et al*., 2007; Mardi *et al*., 2006; Mardi *et al*., 2005; Lin *et al*., 2004; Buerstmayr *et al*., 2003).

Recent advances in molecular characterization of plant resistance genes (R-genes) have led to development of direct markers known as resistance gene analog polymorphism (RGAP) markers. RGAP markers are based on designing primers from the conserved domains including nucleotide-binding site (NBS), leucine-rich repeat (LRR) and protein kinase of resistance genes (Chen *et al*., 1998). Several RGAP markers have been used successfully to develop molecular markers linked to resistance genes in wheat (Chen *et al*., 2006; Xie *et al*., 2004; Yan *et al*., 2003), barley (Yan *et al*., 2006) and maize (Wenkai *et al*., 2006). The objective of this study was to identify RGAP markers that co-localize with the major QTL for FHB resistance, *Qfhs.ndsu-3BS*, in wheat using a 'Wangshuibai'

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Table 1. Sequences of the resistance gene analog (RGA) primers used to identify resistance gene analog polymorphism (RGAP) markers between parental lines.

RGA1 primer	Sequence (5´-3´)	Gene	Reference
PtoF	GTTTACAAGGGTGTTTTGCG	*Pto*	Wang *et al*. (1998)
PtoR	TATTATGCGACTCCACTGCC	*Pto*	Wang *et al*. (1998)
Xa21F	ATAGCAACTGATTGCTTGG	*Xa21*	Wang *et al*. (1998)
Xa21R	CGATCGGTATAACAGCAAAAC	*Xa21*	Wang *et al*. (1998)
RPS2F	ACCCATCAAGTCCGTTGTCG	*RPS2*	Wang *et al*. (1998)
RPS2R	CAGTGCTTTCCACCATTTCTCC	*RPS2*	Wang *et al*. (1998)
L6F	CTCTGGTTCATTTCCCTCCG	*L6*	Wang *et al*. (1998)
L6R	CAATCATCAGCCGTTATGTGG	*L6*	Wang *et al*. (1998)
NF	ATGGTGTTCGGATTATGGGG	*N*	Wang *et al*. (1998)
NR	TTGTGTCGGAGTTGGAGGTG	*N*	Wang *et al*. (1998)
Pto kin1 IN	AAGTGGAACAAGGTTACG	*Pto*	Shi *et al*. (2001)
Pto kin2 IN	GATGCACCACCAGGGGG	*Pto*	Shi *et al*. (2001)
XLRR Rev	CCCATAGACCGGACTGTT	*Xa21*	Chen *et al*. (1998)
Pto kin1	GCATTGGAACAAGGTGAA	*Pto*	Chen *et al*. (1998)
Pto kin2	AGGGGGACCACCACGTAG	*Pto*	Chen *et al*. (1998)
Cre3LR-R	CAGGAGCCAAAAATACGTAAG	*Cre3*	Yan *et al*. (2003)
RLK-Rev	TCYGGYGCRATRTANCCNGGITGICC	*LrK10*	Feuillet *et al*. (1997)
XalNBS-F	GGCAATGGAGGGATAGG	*Xal*	Shi *et al*. (2001)
XalLR-F	CTCACTCTCCTGAGAAAATTAC	*Xal*	Yan *et al*. (2003)
Pto kin4	AGTGTCTTGTAGGGTATC	*Pto*	Shi *et al*. (2001)

[1]Resistance gene analog.

derived population. The ultimate goal was to find out the possibility of identifying FHB candidate resistance genes that to be investigated in future studies.

Hundred and seventy one F_3 individual plants, one from each F_2 individual, and their derived $F_{3:5}$ obtained from a cross between 'Wangshuibai', a highly resistant Chinese landrace, and 'Seri82', a susceptible Mexican spring cultivar, were used in this study. The pedigree of 'Seri82' is 'Kavkaz'/'Buhosib'//'Kalyansona'/'Bluebird'. The 171 F_3 plants and their derived $F_{3:5}$ were assessed for disease by evaluating the area under the disease progress curve (AUDPC) in field experiments carried out by Mardi *et al.* (2005). Total genomic DNA was isolated using the cetyltrimethyl-ammonium bromide (CTAB) method (Saghai-Maroof *et al*., 1984) with minor modifications. DNA quantity and quality were measured with a DU- 530® UV-Photometer (Beckman, USA). Fifty degenerate primers based on the sequence of known R-genes were used (Table 1). PCR analysis was performed as described by Chen *et al*. (1998). Amplification was performed in a thermocycler (BioRad Laboratories Inc., USA) programmed for 5 min at 94ºC for initial denaturation and 45 cycles each consisting of 1 min at 94ºC, 1 min at 45ºC and 2 min at 72ºC, followed by a final 7 min extension at 72ºC. The PCR products were separated on 6% (w/v) denaturing polyacrylamide gel followed by silver-staining as described by Bassam *et al*. (1991). The RGAP markers were integrated into linkage maps constructed with 421 simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers covering a genetic distance of 2554 cM (Mardi *et al*., 2005), considering a minimum logarithm of the odds ratio (LOD) of 5 and a maximum genetic distance of 30 cM. The Kosambi mapping function (Kosambi,

Figure 1. Silver-stained denaturing polyacrylamide gel showing the resistance gene analog polymorphism (RGAP) locus *Xrga1* (228 bp) co-localize with Qfhs.ndsu-3BS using F3 and F5 generations derived from ' 'Wangshuibai' (W) / 'Seri82' (F).

Table 2. Amplification of RGAP markers with primer pairs, showing loci names as integrated into the linkage maps, and size of the markers.

Primer pair[1]	Locus name	Size (bp)[2]
PtoF/NF	*Xrga1*	228
PtoF/L6R	*Xrga2*	250
	Xrga3	270
Xa21F/Xa21R	*Xrga4*	200
	Xrga5	310
	Xrga6	240
	Xrga7	220
	Xrga8	210
Ptokin1/XLRR-Rev	*Xrga9*	157
	Xrga10	180
Xa21F/L6F	*Xrga11*	210
	Xrga12	180
PtoR/L6F	*Xrga13*	160
RPF2F/RPF2/R	*Xrga14*	250
L6F/L6R	*Xrga15*	160
	Xrga16	175

1 For sequence of primer pairs, see Table 1. 2 Size of *Xrga1* and *Xrga9* were based on sequence data. All others were estimated based on the ruler gene 50.

1994) was used to convert recombination frequencies into genetic distances. QTL analysis was performed based on composite interval mapping using the PLABQTL program (Utz and Melchinger, 1996).Cloning of the PCR product was performed by using the pGEM-T Easy vector cloning kit (Promega, USA). Five positive clones of each amplified fragment were sequenced using the ABI Prism 3130xL Genetic Analyzer. The Basic Local Alignment Search Tool (BLAST) algorithm (Altschul *et al*., 1997) was used for similarity search of the sequenced fragment with known resistance gene sequences.

Eight out of the 50 primer combinations showed polymorphism between parental lines (Table 2) and produced 16 RGAP markers (Fig. 1). Two markers were integrated into the chromosal region containing the major QTL for FHB resistance, *Qfhs.ndsu-3BS* (Fig. 2). Composite interval mapping detected two QTLs in a genomic region that were coincident with *Qfhs.ndsu-3BS* (Fig. 2). A QTL in the map interval of *Xrga1-Xgwm533* with 4.2 cM was detected and accounted for up to 12.5% of the phenotypic variation (Table 3). The second QTL was detected in the map interval *Xrga9-Xgwm493* with 9.6 cM. This QTL explained up to 8.5% of the phenotypic variation (Table 3). Both QTL alleles conferring resistance were contributed by 'Wangshuibai' with additive effects and were tagged with flanking SSR markers. In this study, we found two RGAP markers significantly associated with FHB resistance and co-localizing with the major QTL for FHB resistance on the 3BS chromosome. The major QTL, *Qfhs.ndsu-3BS*, from Sumai3 and its derivatives were consistently observed to have a major effect on FHB resistance in various genetic back-

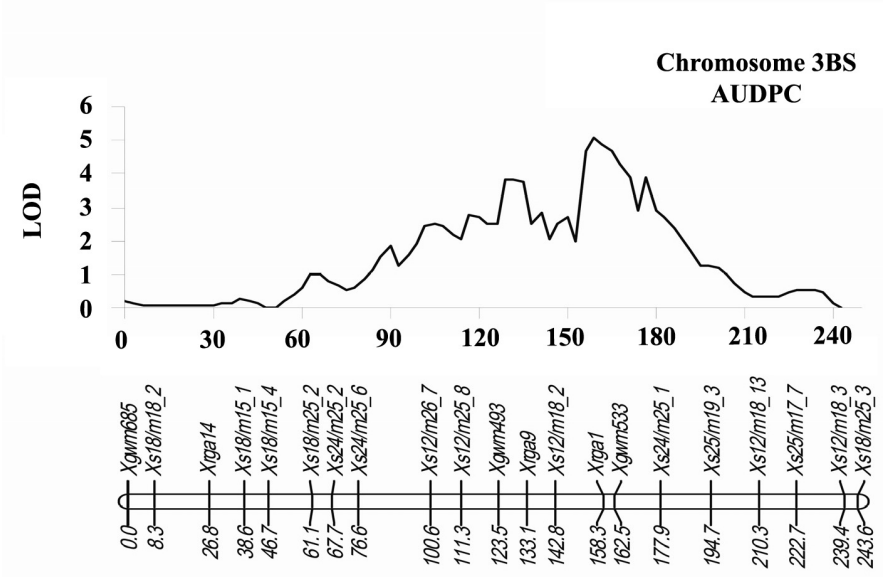

Figure 2. The LOD scores plot of two QTLs for area under the disease progress curve (AUDPC) means over two generations on linkage group corresponding to parts of chromosomes 3BS. LOD values were calculated by composite interval mapping. The AFLP loci names were abbreviated according to the standard nomenclature of AFLPs (http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html).

Table 3. The map intervals, chromosomal locations, logarithm of odds (LOD) and the percentage of explained phenotypic variance (V) of quantitative trait loci (QTL) detected for area under the disease progress curve (AUDPC) using F3 and F5 generations derived from 'Wangshuibai' / 'Seri82'. QTL analysis was carried out by composite interval mapping (CIM).

Generations	Map interval	Chr.	LOD	V	Effect explain[1]
F3	*Xrga1- Xgwm533*	3BS	2.36	6.0	-1.090
	Xgwm493-Xrga9	3BS	2.02	5.90	-0.090
F5	*Xrga1- Xgwm533*	3BS	6.67	15.9	-0.896
	Xgwm493-Xrga9	3BS	5.48	13.2	-1.028
Combined analysis	*Xrga1- Xgwm533*	3BS	5.07	12.5	-0.972
	Xgwm493-Xrga9	3BS	3.79	8.5	-0.991

Negative additive effect indicate the direction of the quantitative trait locus (QTL) response on the area under the disease progress curve (AUDPC).

grounds (Anderson *et al*., 2007; Buerstmayr *et al*., 2003). Since *Xrga1* and *Xrga9* loci were coincident with *Qfhs.ndsu-3BS,* they could be converted into sequence-characterized amplified region markers, i.e. single band present/absent markers which may be easily used as a low-cost, high-throughput alternative to conventional phenotypic screening in wheat-breeding programs to improve FHB resistant lines. Guo *et al*. (2006) converted one RGA associated with FHB resistance in wheat to a sequence tagged site (STS) marker and mapped it onto chromosome 1AL.

Of 87 BLAST hits, with up to 97% homology to *Xrga1*, 70 had similarity to *Pto* and *Pto*-like genes in *Lycopersicon* spp. and *Capsicum* spp., 3 to the *N* gene in *Nicotiana* spp. and 1 to the putative resistance protein in *Triticum monococcum*. There are some indications that *Qfhs.ndsu-3BS* may be in a gene-rich region (Liu *et al*., 2003) where protein kinase genes have been found (Feuillet *et al*., 1997). The *Pto* interacts with proteins that bind to the cis-element of pathogenesis-related genes in order to confer resistance in tomato bacterial speck disease (Zhou *et al*., 1997). However the role of *Pto* and *Pto*-like genes in enhancing FHB resistance in wheat remains to be determined. The *Xrga9* sequence was highly similar to the gypsy-type retrotransposon reverse transcriptase and *CBF* transcription factors at the frost tolerance locus *Fr-Am2* (AY951944) in *Triticum monococcum*. However, *Xrga9* sequence did not have homology with any known resistance genes. Previous studies have reported that many RGAP cloned fragments do not have significant sequence homology to known resistance genes because of rearrangement of genome and transposon elements (Shi *et al*., 2001; Leister *et al*., 1996; Yu *et al*., 1996).

As reported by Shi *et al*. (2001); Yan *et al*. (2003) and Xie *et al*. (2004), our results also showed that the RGAP markers could be efficient tools for mapping of resistance genes in wheat. Identifying RGAP markers can reduce linkage drag associated with the QTL to obtain improved FHB resistant lines in wheat breeding programs. Fine resolution of the major QTL for FHB resistance, *Qfhs.ndsu-3BS*, may further be improved by subsequent study of this small interval and the QTL thus be assigned to intervals sufficiently small for physical mapping and map-based cloning. The present study provides that known R-genes, namely *Pto* and *Pto*-like genes, may be considered as FHBcandidate resistance genes underlying *Qfhs.ndsu-3BS* and may be used in future studies.

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