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Validation of EST-derived STS markers localized on *Qfhs.ndsu-3BS* for Fusarium head blight resistance in wheat using a 'Wangshuibai' derived population

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Abstract

A few EST-derived STS markers localized on *Qfhs.ndsu-3BS*, a major QTL for resistance to Fusarium head blight (FHB) in wheat, have been previously identified in the 'Sumai 3'/'Stoa' population. In this study, we used a 'Wangshuibai' (resistant) /'Seri82' (susceptible) derived population, linkage group, QTL, and quantitative gene expression analysis to assess the genetic background dependence and stability of the EST-derived STS markers for use in marker aided selection to improve FHB resistance in wheat. Based on our results, a QTL in the map interval of *Xsts3B-138_1–Xgwm493* on chromosome 3BS was detected for FHB resistance, which accounted for up to 16% of the phenotypic variation. BLASTN analysis indicated that *Xsts3B-138_1* sequence had significant similarity with the resistance gene analogue. Real-time quantitative PCR showed that the relative expression of *Xsts3B-138_1* in 'Wangshuibai' at 96 h after inoculation was 2.6 times higher than 'Seri82'. Our results underlined that EST-derived *STS3B-138* markers could be predominantly used in marker aided selection to improve FHB resistance in wheat.

Keywords: EST-derived STS markers; *Qfhs.ndsu-3BS*; Fusarium head blight; wheat

Introduction

Fusarium head blight (FHB) caused by [*Fusarium graminearum* Schwabe (telomorph *Gibberella zeae* (Schwein) Petch] is an economically important disease in wheat (*Triticum aestivum* L.) growing regions of the world. Disease severity causes major losses of yield and reduces grain quality. Plants infected by *F. graminearum* have shriveled grains with significantly lower kernel weight (Bai and Shaner, 2004). Additionally, mycotoxins produced by *F. graminearum* in the infected grains are potentially toxic to humans and animals (Mardi et al., 2004). Controlling FHB with chemicals is not very effective and, therefore, exploiting genetic resistance to develop wheat varieties with resistance to FHB has been a priority in wheat breeding programs worldwide. 'Sumai3' and 'Wangshuibai', a Chinese cultivar and landrace, respectively, are used as sources of genetic resistance in different studies. In addition, 'Frontana', a spring Brazilian cultivar, is a moderately resistant genotype. FHB resistance is quantitative in nature with multiple resistance components. Several efforts were made to investigate molecular markers linked to quantitative trait loci (QTLs), conferring resistance to FHB in wheat (Bai et al., 1999; Walderon et al., 1999; Anderson et al., 2001; Burstmayer et al., 2002, 2003, Somers et al., 2003; Pailard et al., 2004; Steiner et al., 2004; Zhang et al., 2004; Mardi et al., 2005, 2006; Bai et al., 2007; Shi et al., 2008). A common QTL for FHB resistance on chromosome 3BS (*Qfhs.ndsu-*

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3BS) was detected using both 'Sumai 3' and 'Wangshuibai' derived populations (Waldron et al., 1999; Anderson et al., 2001; Burestmayer et al., 2002; Mardi et al., 2005, 2006).

Recently, sequence information produced by worldwide wheat expressed sequence tag project provided an access of 500,000 ESTs to the public domain. ESTs provided a useful resource for gene mapping (Liu and Anderson, 2003), identifying candidate gene controlling trait variation (Chee et al., 2004; Shen et al., 2006), and producing functional gene markers (Chee et al., 2004). Real-time reverse transcriptase PCR (Real-time RT-PCR) assay has allowed the accurate expression profiling of RNA transcripts and has become the most useful method for characterizing gene expression in plant and human research (Pritsch et al., 2000; Kong et al., 2005).

Identifying DNA markers linked to genes governing resistance to FHB provides an effective tool for breeding programs. The DNA marker results need to be independently validated before one can recommend their application in cultivar development. The major QTL, *Qfhs.ndsu-3BS*, from 'Sumai-3' or its derivatives was validated in several studies (Waldron et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2002, 2003). Liu and Anderson (2003) increased the marker density in the vicinity of *Qfhs.ndsu-3BS* and localized 28 STS markers developed from wheat ESTs in 3BS chromosome. They mapped six EST-derived STS markers localized on *Qfhs.ndsu-3BS* in the *T. aestivum* 'Sumai 3'/*T. aestivum* 'Stoa' population with higher R^2 and *LOD* compared with the most significant markers reported previously.

The aim of this study was therefore to map previous EST-derived STS markers localized on *Qfhs.ndsu-3BS* using an independently derived 'Wangshuibai'/'Seri82' population in order to assess the genetic background dependence and stability of EST-derived STS markers for marker aided selection to improve FHB resistance in wheat. The ultimate goal was to determine expression of determined EST-derived STS markers involved in FHB resistance using quantitative gene expression.

Materials and methods

Plant materials and phenotypic data

One hundred and eighty F_3 individual plants, one from each F_2 individual, and their derived F_5 lines obtained from the cross between 'Wangshuibai', a highly resistant Chinese landrace, *T. aestivum* L., and 'Seri82', a susceptible Mexican spring cultivar, were used in this study. The pedigree of 'Seri82' is 'Kavkaz'/'Buho-sib'//'Kalyansona'/ 'Bluebird'. Three heads in each F_3 plant and three blocks in each F_5 lines were inoculated with a pathogenic strain of *F. graminearum*. The percentage of infected spikelets was estimated 14, 18, 22, and 26 days after inoculation. The area under the disease progress curve (AUDPC) was calculated for each inoculated spike in F_3 plants and each block in F_5 lines. Mardi et al. (2005) developed the phenotypic data.

EST-derived STS markers analyses

Healthy leaves harvested from the parents ('Wangshuibai' and 'Seri82') and 180 F_3 individual plants were used for DNA extraction. Total genomic DNA was isolated using CTAB method (Saghai-Maroof et al., 1984). Nine EST-derived STS markers in *Qfhs.ndsu-3BS*, STS3B-15, 49, 52, 55, 58, 66, 80, 138 and 142, were used to assay parental polymorphism as described by Liu and Anderson (2003). The polymorphic EST-derived STS marker was used for genotyping of the entire population. PCR products were visualized on a 6% denatured polyacrylamide gel as described by Bassam and Caetano-Anollés (1993). The polymorphic bands in 'Wangshuibai' were isolated, purified, and sequenced by MWG-BIOTECH AG (MWG-BIOTECH AG, Germany).

Linkage and mapping analyses

The polymorphic bands (loci) were integrated into constructed linkage maps by Mardi et al. (2005) using the computer program MAPMAKER 3.0b (Lander et al., 1987). A minimum logarithm of the odds ratio (*LOD*) score of 3 and a maximum genetic distance of 30 cM were used for pair wise linkage analysis. The Kosambi mapping function (Kosambi, 1944) was used to convert recombination frequencies into genetic distances. The QTL analysis was carried out using genotypic data, from the 180 F_3 plants, and the phenotypic data, obtained from the same F_3 plants, their derived F_5 lines, and combined data. Composite interval mapping (CIM) was carried out using PLABQTL software (Utz and Melchinger, 1996).

Functional analysis of identical EST-derived STS marker

Parental lines were sown in a randomized complete block design with three replications in a controlled growth chamber at Genomics Department, Agricultural Biotechnology Research Institute of Iran during 2007. Each plot consisted of 10 pots (five seeds per pot). The inoculation of plants with *F. graminearum* was carried out using the spray inoculation method as described by Mardi et al. (2005). Conidia concentrations were adjusted to 50,000 conidia/mL. Three heads in each pot were marked with self-adhesive paper labels and artificially inoculated at anthesis by spraying 1 mL of conidial suspension using a manual atomizer. To provide high humidity, the infected heads were covered with a transparent plastic shelter after inoculation. After 20 h, the covers were removed and the

nurseries were irrigated with overhead misters for 48 h using leaf wetness control for switching the mist-irrigation. Total RNA from inoculated spike (combination of three spikes for each pot) and water control were extracted using Trizol reagent (Invitrogen, USA) 0, 24, 48, and 96 h after inoculation.

One microliter of each RNA sample was used for constructing cDNA using a iScript cDNA Synthesis kit (Bio-Rad, USA). Primer pairs were designed using Beacon Designer ver. 4 software (Premier Biosofte, USA) based on a polymorphic EST-derived STS marker. Quantitative gene expression was assayed using the iCycler My IQ, single color Real-Time PCR Detection System (Bio-Rad, USA) and iQ SYBR Green Supermix kit (Bio-Rad, USA). The following PCR thermal profile was used: 2 min at 95°C followed by 45 cycles of denaturation for 10 s at 95°C, annealing for 30 s at 60°C, extension for 1 min at 72°C, and a final polymerization for 5 min at 72°C followed by recording of a melting curve. 18S rRNA was used as the reference gene to normalize the data for difference in input RNA.

Results and discussion

Out of nine EST-derived STS markers, *STS3B-138*, was polymorphic (*Xsts3B-138_1* and *2*) between parental lines as well as resistant and susceptible F_3 plants (Fig. 1). Linkage analysis assigned the polymorphic *Xsts3B-138* to 3BS chromosome between *Xgwm533* and *Xgwm493* loci (Fig. 2). Based on QTL analysis, one QTL in the map interval of *Xsts3B-138_1–Xgwm493* on chromosome 3BS was detected in both generations accounting for approximately 16% of the phenotypic variation (Table 1). The QTL alleles conferring resistance were contributed by 'Wangshuibai' and were tagged with flanking SSR markers.

The high multiplex ratio of AFLPs and SSRs integrated with the known functional markers provided identical QTL analyses for FHB resistance in independent wheat mapping populations. Liu and Anderson (2003) previously mapped *Xsts3B-138* at the short arm of 3B between *Xgwm533* and *Xgwm493* loci using the *T. aestivum*

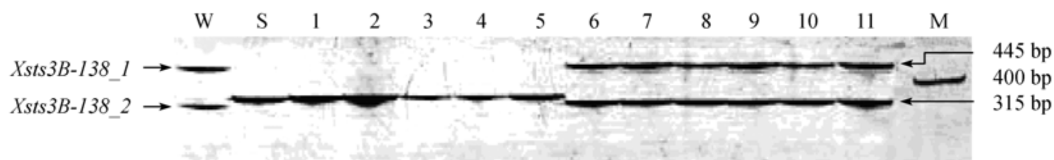

Fig. 1. PCR products of EST-derived STS3B-138 marker amplified from resistant ('Wangshuibai') and susceptible ('Seri82') cultivars and five susceptible (1 to 5) and six resistant (6 to 11) F_3 plants developed from the 'Wangshuibai'/'Seri82'.

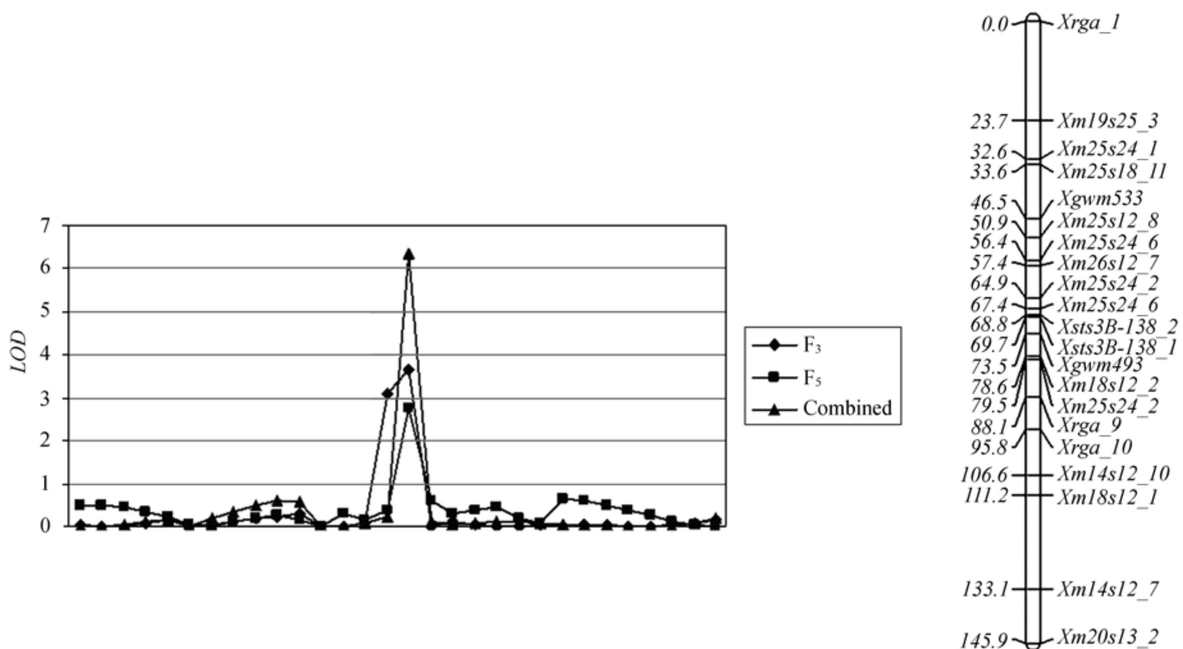

Fig. 2. The linkage group and the LOD (log10 of the odds ratio) plot indicate the most likely position of the QTL relative to *Xsts3B-138* for area under the disease progress curve (AUDPC) identified in the mapping population developed from the 'Wangshuibai'/'Seri82'. AFLP marker names are abbreviated according to the standard nomenclature of AFLPs proposed by KeyGene.

Table 1
The map intervals, chromosomal locations, logarithm of odds (*LOD*), the percentage of explained phenotypic variance (*VE*) and effects of quantitative trait loci (QTL) detected for area under the disease progress curve (AUDPC) using F_3 and F_5 generations derived from a 'Wangshuibai'/'Seri82' cross based on integrated EST-derived *STS3B-138* marker

Generation	Map interval	Chromosome	Effect	*VE*	*LOD*
F_3	*Xsts3B-138_1–Xgwm493*	3BS	–1.92	7.3	2.93
F_5	*Xsts3B-138_1–Xgwm493*	3BS	–1.11	5.2	2.05
Combined	*Xsts3B-138_1–Xgwm493*	3BS	–1.18	16	6.36

'Sumai 3'/*T. aestivum* 'Stoa' population. The QTL on chromosome 3BS was consistently detected and seems to be the same QTL, *Qfhs.ndsu-3BS*, reported by Liu and Anderson (2003). However, the known pedigree of 'Sumai-3' indicated that 'Sumai-3' was not related to 'Wangshuibai'. Both lines possess the same alleles at *Qfhs.ndsu-3BS* but both are conferring quantitative FHB resistance (Mardi et al., 2005). The discrepancy of determined R^2 might be because of incomplete map coverage or to different experimental conditions in the two mapping experiments.

BLASTN analysis indicated that *Xsts3B-138_1* and *2* sequences had significant similarity with resistance gene analogue (RGA) (GenBank accession no. AF325198) and nuclear localization signal (NLS) (RKGRKRKE motif, http://cubic. bioc.columbia.edu), respectively. Primer pairs designed for expression analysis of *Xsts3B-138_1* is shown in Table 2. Real-time quantitative PCR revealed that the relative expression of *Xsts3B-138_1* in spike at 0, 24, and 48 h after inoculation was not significant. The induction of *Xsts3B-138_1* in 'Wangshuibai' 96 h after inoculation was 2.6 times higher than 'Seri82' (Fig. 3).

Several genetic analyses have shown association of large number of RGAs with known *R*-gene loci that confer resistance to viruses, bacteria, fungi, or nematodes in crop species (Kanazin et al., 1996; Leister et al., 1996; Yu et al., 1996; Aarts et al., 1998; Shen et al., 1998; Speulman et al., 1998; Spielmeyer et al., 2000). RGAs have facilitated the isolation of functional resistance genes (Collins et al., 1998) and might serve as valuable markers for disease resistance breeding (Lagudah et al., 1997; de Majnik et al., 2003). NLSs have been identified in a variety of nuclear proteins including polymerases (Schreiber et al., 1992), kinases and phosphatases (Chen et al., 1992), and transcription factors and tumor suppressors (Shaulsky et al., 1990). Deletion of an NLS from a nuclear protein leads to its redistribution to the cytoplasm, whereas its addition to a heterologous cytoplasmic protein often results in an accumulation of this protein in the nucleus (Sock et al., 1996).

The validation of molecular markers would facilitate in enhancing the selection efficiency in wheat breeding program. In this study, we used an independently derived 'Wangshuibai'/'Seri82' population, linkage group, QTL, and quantitative gene expression analysis in order to assess the genetic background dependence and stability of *STS3B-138* marker. Our results indicated that this marker can be used predominantly in marker aided selection to enhance FHB resistance in wheat breeding programmes.

Table 2
Primers used in real-time PCR

Primer	Sequence
STS3B-138_1-F	5′-TCATCTGGCCCACAACATACCC-3′
STS3B-138_1-R	5′-CGATGGCTCCACCAATAAGTCC-3′
18S rRNA-F	5′-GTGACGGGTGACGGAGAATT-3′
18S rRNA-R	5′-GACACTAATGCGCCCGGTAT-3′

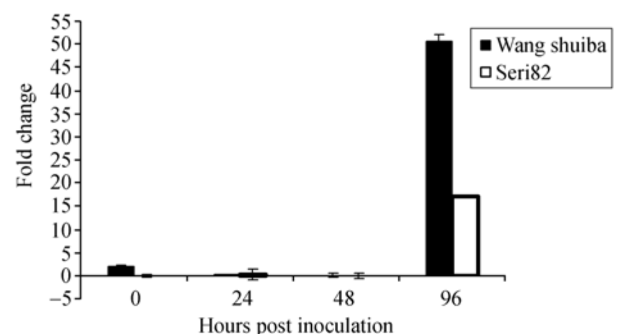

Fig. 3. Fold changes in accumulation of FHB resistance candidate gene *Xsts3B-138_1* encoded RNA in the resistant ('Wangshuibai') and susceptible ('Seri82') wheat at specified hours after inoculation with *F. graminearum*. The relative quantity of target gene transcripts was calculated using the comparative cycle threshold method. The infected samples were quantified relative to the controls (water-inoculated samples) at the same time points. 18S rRNA was used as an endogenous control to normalize the data for input RNA differences between the various samples.

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