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ORIGINAL RESEARCH ARTICLE

From *FHB* Resistance QTLs to Candidate Genes Identification in *Triticum aestivum* L.

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Abstract *Fusarium* head blight (*FHB*) caused by *Fusarium graminearum* is a worldwide destructive disease affecting cereals such as wheat. *FHB* resistance is a quantitative trait, and information for *FHB* resistance QTLs in wheat is available. However, little is known about genes underlying the *FHB* resistance QTL regions. Using a computational approach in this study, we have mined eight *FHB* resistance QTLs in wheat and predicted the candidate genes falling within these QTL intervals based on the available sequences and markers. A total of 18 genomic scaffolds located at chromosomes 2AL, 2DL, 3B and 4BS were prioritized to harbor *FHB*-resistant candidate genes. These genes are mainly involved in plant defense response, immune regulation and cellular detoxification. We believe that our results constitute a starting point for further validation to improve *FHB*-resistant bread wheat varieties.

Keywords Candidate gene · *FHB* resistance · QTL · *Triticum aestivum*

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1 Introduction

Fusarium graminearum produces mycotoxins, among which deoxynivalenol (DON) is harmful to humans and animal [1]. *Fusarium* head blight (*FHB*) commonly affects wheat, barley and other cereal grains causing high-yielding crop losses within a few weeks of harvest [2]. According to FAO, Fusarium scab causes severe production losses worldwide and may be as high as 50 %. Coping with this huge damage, while avoiding fungicides and traditional cultural practices such as crop rotation, has been a major challenge for farmers and pathologists as well. Breeding wheat for resistance to *FHB* is one of the efficient ways to minimize crop and grain quality losses [2]. Developing resistant varieties is the most economic, effective and eco-friendly approach [3].

Quantitative trait loci (QTLs) are regions in the genome statistically associated with a phenotype. A QTL region may contain over hundreds of genes including relevant genes for breeding goals. Mapping QTLs controlling regions is the major task in *FHB* resistance breeding approach. A number of QTLs involved in resistance to *F. graminearum* have been identified in wheat, as reviewed in [4]. Previous transcriptomic studies on *F. graminearum*–wheat interaction identified multiple defense genes including glucanases, NBS-LRR, WRKY transcription factors and UDP-glycosyltransferases [5, 6].

The wheat database established at INRA URGI (http://urgi.versailles.inra.fr/Species/Wheat) enables users to explore the available physical maps and to query the wheat survey sequence assemblies.

Our study aims to identify putative candidate functional genes by investigating *FHB* resistance QTLs regions in wheat using wheat markers flanking the QTL region by applying computational approach. Mapping positions of

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these candidate genes are expected to be useful for wheat breeders to improve *FHB*-resistant bread wheat varieties.

2 Methods

2.1 QTLs Mining

The QTLs were queried and displayed by GRAMENE Mart http://archive.gramene.org/qtl/ [7] against Gramene's QTL database [8]. The query was then filtered by 'species' and 'trait category: biotic stress' to obtain only the QTLs involved in *FHB* resistance in *Triticum aestivum* genome including map position and associated markers.

2.2 Sequence Retrieval

To link genetic map with the genomic sequence, sequence alignment of marker sequences (RFLP, SSR) and ESTs (if available at GrainGenes http://wheat.pw.usda.gov/GG2/index.shtml) was performed with wheat sequences available at (http://urgi.versailles.inra.fr/blast/blast.php). Even though the *Triticum aestivum* genome has not been fully public, sequences provided by the International Wheat Genome Sequencing Consortium (IWGSC) were gathered through ViroBLAST which is a stand-alone Basic Local Alignment Sequence Tool (BLAST) web interface for nucleotide and amino acid sequence similarity searches [9]. Following BLAST analysis, results were parsed to eliminate low-identity sequences with a threshold score >50 [10].

2.3 Sequence Analysis

Sequence annotation was performed using TRiAnnot pipeline [11], with additional manual annotation. Similarity searches performed by BLAST against *Brachypodium distachyon* and *Oryza sativa* annotated genes and proteins, ESTs and unigenes available for *Triticum aestivum, Hordeum vulgare* and all other Poaceae, and finally, against full-length cDNAs available for wheat, barley and *Arabidopsis thaliana* [12].

3 Results

3.1 QTLs mining

By querying GRAMENE database (Release 39) using GRAMENE Mart and by choosing Gramene QTL 38 as dataset and biotic stress as trait category, we found 14 QTLs in *Triticum aestivum*. Only 8 QTLs involved in wheat Fusarium head blight resistance were retrieved including five QTLs located in chromosome 3 as shown in Table 1.

3.2 Identification of FHB Resistance Candidate Genes

Flanking markers of QTLs are shown in Fig. 1. We have used flanking marker sequences when available, as queries to perform a BLASTN search against WHEAT sequence survey databases by specifying also corresponding chromosome. In chromosome 3B, only AQFI001 and AQFO003 QTLs have flanking sequence markers available. We have noticed that AQFI001 QTL, ranging from 0 to 50 cM, covers AQFG001 and AQFI002 QTLs. The latter is also covered by AQFO003 QTL. Therefore, only AQFI001 and AQFO003 QTLs were selected in this report. As a result, 18 genomic scaffolds, located at chromosomes 2AL, 2DL, 3B and 4BS, were retrieved for further analysis (Table 2).

Table 1 QTLs involved *FHB* resistance in wheat retrieved from GRAMENE database

QTL accession ID	Published symbol	Chr	Start[a]	Stop[a]	LOD[b]	R2[c]	References
AQFG001	QFhs.ndsu-3B	3B	1.00	27.00	3	15.4	[13]
AQFG002	QFhs.ndsu-2A	2A	29.00	33.00	3	15.4	
AQFI001	QFhs.ndsu-3BS	3B	0.00	50.00	9.9	35.5	[13–15]
AQFI002	QFhs.ndsu-3BS	3B	0.00	32.00	9.9	35.5	
AQFO001	QFhs.crc-2D	2D	92.00	140.00	2.5	9	[16]
AQFO003	QFhs.crc-3B.1	3B	0.00	37.50	2.5	13	
AQFO004	QFhs.crc-3B.2	3B	58.50	66.50	2.5	4	
AQFO005	QFhs.crc-4B	4B	0.00	31.00	2.5	12	

[a] Position (cM)

[b] Likelihood of odds

[c] Phenotypic R2: This value indicates the relative importance of QTL influencing a trait. It is the percent of a total phenotypic variance for the trait that is accounted for by a marker

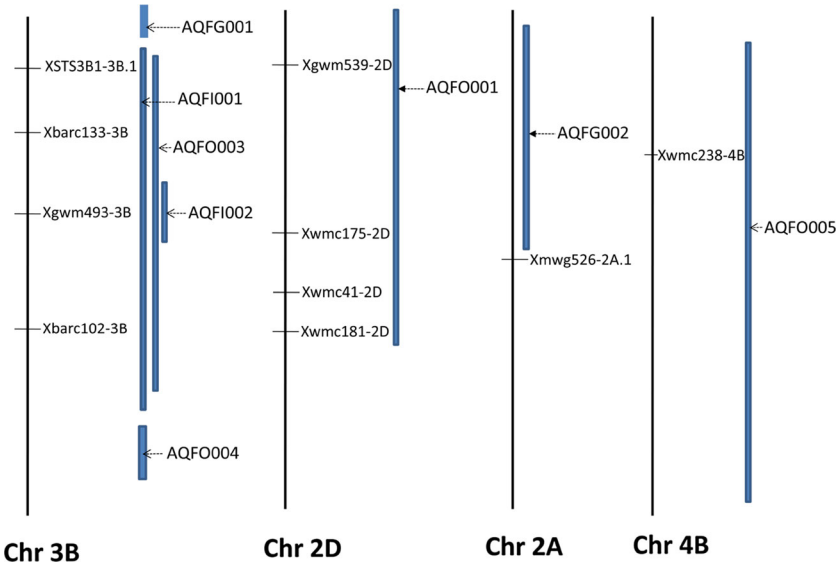

Fig. 1 Schematic representation of FHB QTLs and corresponding flanking markers retrieved from GRAMENE database. Genetic markers are indicated on chromosomes. *Blue vertical boxes* located on the *right side* of the chromosomes depict QTLs

The genomic sequences are predicted to encode 32 proteins involved mainly in defense response, immune regulation, DNA repair, polymerase activity, transcription regulation, cellular detoxification, amino acid biosynthesis and protein and DNA bindings (Table 2).

4 Discussion

The overall aim of this study was to predict the candidate genes of the targeted QTLs underlying *FHB* resistance in wheat. For this purpose, we employed computational approach based on wheat DNA sequences and markers linked to the QTL region available so far. QTL regions typically contain tens to hundreds of genes. For this reason, gene function prediction has been performed to reduce the initial gene numbers and to prioritize relevant candidate genes.

Based on this approach, we have noticed that our results are consistent with previous reports. First, serin proteinase inhibitors (serpin) may play important role in defense responses against insects and pathogens [17]. Moreover, phosphorylated protein analyses have demonstrated that serpin, AGPase, sucrose synthase and Hsp 90 are involved in the wheat grain development and stress defense [18].

In addition, the ethylene-responsive factor (AP2/ERF), under stress, activates the defense-related genes such as the pathogen-related (PR) genes, osmotin, chitinase and β-1, 3-glucanase. Therefore, these genes have been considered as an attractive target for breeders [19].

Furthermore, retrotransposon, Ty3–gypsy subclass proteins and LTR-retroelements were identified. In addition to their role in evolution, the retrotransposon proteins are activated under stress and play possible roles in disease responses and in floral and early meiotic development in wheat [20]. In addition, it was suggested that MAPK phosphatase 2 dual-specificity protein phosphatase may be the key regulator of MPK3 and MPK6 networks controlling specific pathogen responses in plants [21].

Although gene function predictions help to prioritize potentially *FHB*-resistant genes, the genes of unknown function may play important role in *FHB* resistance and should be more investigated.

The identification of candidate genes involved in *FHB* resistance may be useful not only for fundamental interest but, also, these genes could constitute perfect markers for plant breeding. In addition, the prioritizing method applied to rice QTL data indicated statistical significance and biological relevance of the obtained connections between genes and traits [22]. Based on the high synteny between rice and wheat, we believe that applying this approach in wheat could be interesting for breeding programs.

Table 2 Genomic locations and positions and predicted functions of retrieved sequences from *Triticum aestivum L*

Marker	Position[a]	Chr[b]	Genomic location scaffolds	Position	% identity	E-value	Predicted functions
Xmwg526-2A.1	34	2AL	IWGSC_CSS_2AL_scaff_6348063	102–201	91	7e−32	Serin protease inhibitor (Serpin)
			IWGSC_CSS_2AL_scaff_6439106	438–538	89	e−29	Retrotransposon protein
			IWGSC_CSS_2AL_scaff_6349653	1130–1231	78	e−10	Regulator of nonsense transcripts 1-like protein cysteine-rich receptor-like protein kinase 7
Xwmc175-2D	99.2	2DL	IWGSC_CSS_2DL_scaff_9910671	4206–4284	83	5.6e−23	Unnamed protein product
Xwmc41-2D	170.4	2DL	IWGSC_CSS_2DL_scaff_9835990	5116–5420	99	7e−154	Pathogenesis-related transcriptional factor and ERF domain-containing protein, AP2 domain-containing protein. Integrase-type DNA-binding superfamily ethylene-responsive element binding protein 1
			IWGSC_CSS_2DL_scaff_9889531	998–1271	77	2e−54	F-box domain-containing protein. Glucosyl transferase, putative
Xwmc181-2D	183.2	2DL	IWGSC_CSS_2DL_scaff_9886850	9953–10,081	95	1.8e−31	Heat-shock protein Dna J family protein. Ribosomal protein-like
XSTS3B1-3B.1	4	3BS	IWGSC_CSS_3B_scaff_10723589	16,482–16,812	99	8.7e−97	Beta mannosidase/glucosidase/exoglucanase putative 3-isopropylmalate dehydrogenase (o.s)
			IWGSC_CSS_3B_scaff_10487301	3194–3341	75	e−20	SEC 13 WD domain, G-beta repeat domain-containing protein
Xbarc133-3B	16	3BS	IWGSC_CSS_3B_scaff_10615382	13,445–13,635	98	5e−88	Alkylated DNA repair protein alkB, putative, expressed. Similar to decarboxylate transporter/malic acid transport protein SLAH2 SLAC1 homologue 2 (Slow Anion Channel associated1)
Xgwm493-3B	20	3BS	IWGSC_CSS_3B_scaff_10576785	93–351	90	e−93	Polyubiquitin
			IWGSC_CSS_3B_scaff_10612693	968–1213	87	9.9e−39	Polynucleotidyl transferase, ribonuclease H-like
Xbarc102-3B	30	3BS	IWGSC_CSS_3B_scaff_10639254	7768–8210	99	6.9e−91	Putative retrotransposon protein, putative non-LTR retroelement reverse transcriptase
			IWGSC_CSS_3B_scaff_10775100	798–1019	76	3e−34	Retrotransposon protein, putative, Ty3–gypsy subclass
			IWGSC_CSS_3B_scaff_10646950	901–1120	75	2e−30	MAPK phosphatase 2 dual-specificity protein phosphatase 1
Xwmc238-4B	25	4BS	IWGSC_CSS_4BS_scaff_4959211	7983–8075	94	5.8e−40	Retrotransposon protein putative, Ty3–gypsy subclass
			IWGSC_CSS_4BS_scaff_4918669	5059–5307	78	2e−69	Retrotransposon protein putative, Ty3–gypsy subclass
			IWGSC_CSS_4BS_scaff_4936648	762–1010	76	6e−63	Vegetative cell wall protein gp1 precursor. Retrotransposon protein putative

[a] Marker position (cM) according to GRAMENE database

[b] Chromosome arm position of the marker

5 Conclusion

In this study, we have prioritized 18 genomic scaffolds located on chromosomes 2AL, 2DL, 3B and 4BS chromosomes which were predicted to harbor *FHB*-resistant candidate genes encoding proteins mainly involved in plant defense response, immune regulation and cellular detoxification. These genes add to the pool of putative candidate genes underlying *FHB* resistance in wheat. Our results indicate that the candidate gene identification starting from targeted QTL could be an alternative approach of *FHB* resistance gene isolation.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest.

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