Computational Biology and Chemistry 68 (2017) 22–28

Contents lists available at ScienceDirect

Computational Biology and Chemistry

journal homepage: www.elsevier.com/locate/compbiolchem

Construction of new EST-SSRs for *Fusarium* resistant wheat breeding

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ARTICLE INFO

Article history:
Received 9 May 2016
Received in revised form 12 December 2016
Accepted 2 February 2017
Available online 11 February 2017

Keywords:
Wheat
EST
Fusarium
Genetic diversity
Resistance
SSRs

ABSTRACT

Surveying *Fusarium* resistance in wheat with easy applicable molecular markers such as simple sequence repeats (SSRs) is a prerequest for molecular breeding. Expressed sequence tags (ESTs) are one of the main sources for development of new SSR candidates. Therefore, 18.292 publicly available wheat ESTs were mined and genotyping of newly developed 55 EST-SSR derived primer pairs produced clear fragments in ten wheat cultivars carrying different levels of *Fusarium* resistance. Among the proved markers, 23 polymorphic EST-SSRs were obtained and related alleles were mostly found on B and D genome. Based on the fragment profiling and similarity analysis, a 327 bp amplicon, which was a product of contig 1207 (chromosome 5BL), was detected only in *Fusarium* head blight (FHB) resistant cultivars (CM82036 and Sumai) and the amino acid sequences showed a similarity to pathogen related proteins. Another FHB resistance related EST-SSR, Contig 556 (chromosome 1BL) produced a 151 bp fragment in Sumai and was associated to wax2-like protein. A polymorphic 204 bp fragment, derived from Contig 578 (chromosome 1DL), was generated from root rot (FRR) resistant cultivars (2–49; Altay2000 and Sunco). A total of 98 alleles were displayed with an average of 1.8 alleles per locus and the polymorphic information content (PIC) ranged from 0.11 to 0.78. Dendrogram tree with two main and five sub-groups were displayed the highest genetic relationship between FRR resistant cultivars (2–49 and Altay2000), FRR sensitive cultivars (Seri82 and Scout66) and FHB resistant cultivars (CM82036 and Sumai). Thus, exploitation of these candidate EST-SSRs may help to genotype other wheat sources for *Fusarium* resistance.

1. Introduction

Fungal diseases are one of the limiting biological factors for wheat farming by reducing quality and grain yield, also endangering consumers' health due to the mycotoxin product. In wheat ecosystems, fungi cause seed, spike, green part, root and crown rot diseases. Especially, diseases such as root-crown rot and head blight in wheat belong to various fungal species derived from *Fusarium* genus. *Fusarium graminearum* (*Gibberella zeae)* and *Fusarium culmorum* are two widely encountered factors of head blight, root and crown rot diseases (Miedaner et al., 2008).

In the last decade, severe epidemics caused by *Fusarium* ssp. have been detected in Turkey (Hekimhan and Boyraz 2011), Germany (Talas et al., 2011), UK (Jennings et al., 2004), Canada (Guo et al., 2008) and USA (Ward et al., 2008). Up to 100% yield loss was recorded under optimal disease conditions. So far, agronomical and chemical applications for preventing disease and mycotoxin contamination caused by *Fusarium* have been insufficient (Hollingsworth et al., 2008; Lehoczki-Krsjak et al., 2010)

The most reliable and effective way to combat with diseases is to breed disease resistant varieties. In wheat, resistance to *Fusarium* head blight (FHB) and root rot (FRR) is quantitatively inherited. Quantitative trait loci (QTL) for FHB resistance have been mapped on almost all wheat chromosomes using different mapping populations explaining from 3% to 92.6% of the total phenotypic variation (Buerstmayr et al., 2009). Two to five major and a few minor genes from several source of FHB resistance have been reported (Buerstmayr et al., 2003; Somers et al., 2003). In Sumai3 wheat, QTLs associated to FHB resistance have been

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http://dx.doi.org/10.1016/j.compbiolchem.2017.02.003
1476-9271/

determined on 3BS, 5AS, 6AS, 6BS and 3BS chromosomes (Anderson et al., 2001; Buerstmayr et al., 2003). The 3BS major QTL of Sumai3 has been widely used in different breeding programs and was designed as source of *Fhb1* gene (Cuthbert et al., 2006). Also, QTLs on chromosomes 2DL and 4B for FHB resistance have been mapped in wheat variety "Wuhan1" (Somers et al., 2003). QTLs for FHB on 2A, 2B, 3A, 3B, 5D, 6D, 5B, 4A, 1B and 7A chromosomes have been also recorded from wheat genetic resources derived from European, Asian and Brazilian wheat resources (Jayatilake et al., 2011; Paıllard et al., 2004; Zhang et al., 2012). Previously, Waldron et al. (1999) detected there markers (*Xgwm493*, *Xgwm533*, *Xbcd907*) linked to the major gene *Fhb1* on the short arm of 3B chromosome in Sumai3. Li et al. (2010) was detected a QTL controlling *Fusarium* root rot (FRR) on 3B chromosome. Some markers linked to a QTL for FRR resistance on bread wheat have been tagged on chromosomes 1A, 1D, 2B, 2D, 4B, 5D (Bovill et al., 2006).

As versatile biotechnological tools, molecular markers have been introduced to identify the source of disease resistance in plants (Torres et al., 2010). In addition, different types of molecular markers were used for assessment of molecular diversity in plants. Co-dominant markers such as SSRs have mostly been preferred during determination of stress related gene/genome regions due to their multiple allelic structure, codominant inheritance, abundance and reproducibility. Moreover, SSR sequences have high mutation rate while their flanking regions showed unusual stability (Joukhadar and Jighly 2012). SSR markers derived from expressed sequence tags (ESTs) have received a lot of attention because of the increasing number of ESTs in databases and their easy availability at low cost. Also, EST-SSRs are known to be in comparatively conserved expressed regions, therefore they can lead to the development of gene-based maps which may increase the efficiency of marker-assisted selection (MAS) (Varshney et al., 2005).

The first microsatellite map of wheat contained 279 markers (Röder et al., 1998). Eujayl et al. (2002) used EST-SSRs derived from EST databases for genetic diversity analysis of the A and B genome. Somers et al. (2003) mapped 1.235 SSR loci, covering 2569 cM with an average interval distance of 2,2 cM. Later, 2.038 EST-SSRs, were identified from 151.695 wheat ESTs (Chen et al., 2005). They detected 93 EST-SSR primer pairs and 193 EST-SSR loci were located on 19 wheat chromosomes by using Chinese Spring nullitetrasomic lines. The lack of adequate polymorphic marker flanking QTLs limits QTL/gene mapping and transfer of QTLs between genetic backgrounds. Thus, more new markers are needed to be developed.

This study was aimed to provide integrable candidate marker source for *Fusarium* resistant wheat breeding. In this frame, the main objectives were outlined to mine SSRs from wheat EST sequences expressed under *Fusarium* infection, to design primers for them, to analyze their functional annotations and to test their validation in different levels of *Fusarium* resistant bread wheat genotypes.

2. Materials and methods

2.1. Plant material

Ten bread wheat (*Triticum aestivum*) cultivars were used to determine SSR marker polymorphism. These were chosen based on their response to diseases which were reported in the previous studies (Table 1).

2.2. Sequence mining and primer design

Seven differrent EST libraries including 18.292 wheat sequences were downloaded in fasta-formatted raw data files (http://wheat.pw.usda.gov/db_EST). These ESTs were specifically constructed from *Fusarium* infected wheat leaf tissues. For *in silico* analysis, Vector NTI 10.0 (Bethesda, USA) was used for trimming contaminated sequence sites and filtered ESTs were assembled into contig tags by using DNASTAR Lasergene 11 program. For SSR classification, Tandem Repeats Analyzer 1.5 (TRA1.5) program (Bilgen et al., 2004) was used under the following parameters: di, tri- and tetranucleotide SSRs with a minimum length of 20 bp. 101 out of 670 wheat unigenes carrying simple sequence repeats were selected due to availability of suitable primers for further analysis. Based on EST-SSR sequences, primers were designed with Primer premier 6.0 program according to minor parameter modifications. In this context, primer pairs were selected to produce amplicon size of at least 100 bp. Blast2Go program was used to find out the wheat genome annotations for EST-SSRs that were showed succesful amplification.

2.3. DNA isolation

For total genomic DNA (gDNA) extraction, wheat plants were grown in small pots under controlled conditions with a temperature range between 22 and 24 °C and short-day photoperiod (10 h light). Total gDNA was extracted from leaf tissue collected from two-week-old seedling of each genotype using the CTAB (cetyltrimethylammonium bromide) method as described in Winnepenninckx et al. (1993).

2.4. PCR and fragment analysis

To the 5' end of each forward EST-SSR primer (Supplementary Table 1), the M13 DNA sequence (5'-GGGTTTTCCCAGTCACGACGTT-

Table 1
Pedigrees and disease resistance level of wheat cultivars. (FHB: *Fusarium* head blight and FRR: *Fusarium* root rot).

Genotype	*Pedigree*	*Fusarium* resistance level	Reference
2-49	Gluyas Early/Gala	FRR resistant	Collard et al. (2005), Mitter et al. (2006)
Altay-2000	Es14//Ykt/Blueboy2	FRR resistant	Hekimhan and Boyraz (2013), Nicol et al. (2006)
Sunco	SUN-9-E-27*4/3-AG-14//WW-15/3/3*COOK	FRR moderately resistant	Personal communication with CIMMYT
Kate A-1 57	Hebros/Bezostaya-1	FRR susceptible	Arslan and Baykal (2002)
Seri-82	Kavkaz/(SIB)BUHO//KALYANSONA/BLUEBIRD	FRR susceptible	Arslan and Baykal (2002)
CM82036	Sumai3/Thornbird	FHB resistant	Steiner et al. (2009)
Sumai3	Funo/Taiwan	FHB resistant	Anderson et al. (1998)
Renan	Mironovskaya 808/MarisHuntsman//VPM/Moisson/3/ Cour-tot	FHB moderately resistant	Gosman et al. (2010)
Bezostaya-1	Lutescens17/Skorospelka2	FHB susceptible	Mesterházy (1986)
Scout66	Nebred//Hope/Turkey/3 Cheyenne/Ponca	FHB susceptible	Jin et al. (2013)