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

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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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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)

3‘) was added as an extension to enable their labeling with IRD700 fluorochromes (LI-COR Bioscience, Lincoln, NE). For the PCR amplification, a 10 μl master mix was consisted of 4.3 μl of H_2O, 2 μl of 5X Go Taq buffer, (Promega, Madison, USA), 0.6 μl of 2 mM $MgCl_2$, 0.2 μl of 10 mM dNTPs (Promega, Madison, USA), 0.3 μl of 50 μM IRD700 labelled M13 primer, 0.3 μl of 50 μM reverse primer and 0.1 μl of 50 uM forward primer, 0.2 μl of 5U/ul Taq polymerase (Promega, Madison, USA) and 2 μl of template gDNA (concentration ~25 ng/μl). The PCR amplification was carried out using GeneAmp PCR System 9700 (Applied Biosystems, Malaysia). The PCR was performed using a touchdown program with the following: 95 °C for 5 min, 20 cycles of 95 °C for 20 s, 65 °C for 30 s, decreasing 0.5 °C/cycle, 72 °C for 30 s followed by 20 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and final extension step at 72 °C for 10 min. After PCR, 4 μl of Blue Stop Solution (LI-COR Bioscience, Lincoln, NE) was added immediately to each reaction before storage at −20 °C. The PCR products (1.5 μl) were analyzed for their size on a 6% polyacrylamide gel after mixing with 0.5 ul stop solution (LI-COR Bioscience, Lincoln, NE) using LI-COR DNA bioanalyzer (Model 4300, LI-COR, Inc. Lincoln, Nebraska, USA).

The PIC values were measured for the informativeness of a marker. The PIC was calculated according to the formula: where k is the total number of alleles detected for a marker locus and p_i is the frequency of the *i*th allele. EST-SSR alleles were scored for the presence (1) or absence (0) profile of a fragment in ten wheat cultivars. Genetic similarity was calculated by Dice (1945). UPGMA dendrogram was drawn using the NTSYSPC 2.0 software package (Rohlf, 1998).

$$PIC = 1 - \sum_{i=1}^{k} P_i^2$$

3. Results

3.1. Frequency and distribution of EST-SSRs

The frequency and distribution of various repeat motifs were analyzed in seven wheat EST libraries. According to results, 670 EST-derived SSRs in contig and singleton sequences were covered the 3.7% of all ESTs (Table 2). The percentage of assembled ESTs was 71.1% and covered a total of 3644 contigs. The length of contigs was varied from 82 bp to 967 bp, and about 97 (14.5%) SSR-containing ESTs had repeats in imperfect form. The most repeated motifs and numbers were $(CA)_{43}$, $(AGA)_{14}$ and $(GACG)_6$-$(AAGG)_6$. The number of SSR containing contigs was higher than that of SSR containing singletons.

3.2. Polymorphism and annotation analysis

Among 670 SSR containing ESTs, a hundred and one sequences were defined as suitable to design PCR primers. Primer pairs were designed from flanking regions of simple repeat containing sites and used to evaluate the allelic variations in ten wheat cultivars (Supplementary Table 1). The fifty-five primer pairs were successful in producing different size of amplicons and they were distributed on the 21 wheat chromosomes. Nine primer pairs were associated with chromosome group 1, ten with chromosome group 2, eight with chromosome group 3, ten with chromosome group 4, six with chromosome group 5, seven with chromosome group 6, and five with chromosome group 7. Sixteen loci were located on the D genome, 23 on the B genome, and 16 on the A genome. No markers were found on chromosomal arms of 3AL, 3DL, 5AL, 5BS, 5DS, 7AS and 7BL (Supplementary Table 2).

The distribution of EST sequences assigned to different functional categories and the estimation of their chromosome locations were given in Supplementary Table 2. Out of the 55 EST sequences used, 49 showed homology with known proteins, while only 6 sequences did not possess homology with any known proteins. The putative functions of these EST-SSRs were classified into different categories such as metabolic enzymes, structural proteins, stress response proteins, transcription and post-

Table 3
The list of 23 polymorphic EST-SSRs and allele numbers. Expected heterozygosity (He), observed heterozygosity (Ho), Polymorphism Information content (PIC) values.

Sequence ID	Allele number	He	Ho	PIC
BE585853	2	0.10	0.11	0,11
Contig 226	2	0.46	0.71	0,46
Contig 578	6	0.78	0.44	0,78
Contig 555	3	0.54	0	0.54
Contig 858	4	0.68	0.25	0.73
Contig 122	2	0.50	1	0,38
Contig 2221	2	0.32	0	0,32
Contig 989	4	0.52	0	0.52
Contig 556	2	0.42	0	0,42
Contig 1207	2	0.42	1	0,42
Contig 2353	2	0.32	0.2	0,32
Contig 1137	2	0.32	0	0,32
BM138501	2	0.46	0.7	0,46
WHE3876-A05-A10Z	2	0.32	0.4	0,32
WHE3896-F09-K18ZS	2	0.38	0.5	0,38
Contig 1288	4	0.67	0	0,67
Contig 1836	4	0.7	0	0,7
Contig 1006	3	0.64	0	0,64
Contig 545	2	–	–	–
BQ902548	4	0.48	0	0,48
BQ903543	2	0.35	0	0.35
Contig 190	5	0.78	0	0.78
BQ903999	3	0.54	0	0.56

Table 2
Fusarium infected wheat EST libraries and number of SSRs

No	Library name	Total EST number	Contig number	Singleton number	Contigs including SSRs	Singletons including SSRs
1	TA006G1X	730	93	408	3	24
2	TA009G1X	1586	298	687	17	24
3	TA010G1X	1230	284	360	12	13
4	TA011G1X	1697	257	543	11	22
5	TA012G1X	1724	319	947	14	25
6	TA027G1X	89	–	89	–	1
7	TA09XXX	11236	2393	2242	358	146
Total	–	18292	3644	5276	415	255

(http://wheat.pw.usda.gov.db_EST).

transcription, protein destination and storage, metabolism, signal transduction, energy, intracellular trafficking, transport, cell structure, cell growth and division, secondary metabolism, uncategorized, and unknown functions.

According to allele scoring (Table 3), two polymorphic loci were available on different wheat chromosomes. Two of them, contig 556 and contig 1207, were located on chromosome 1BL and both of them generated descriptive fragments for only *Fusarium* resistant cultivars (Fig. 1). According to Blast results (Supplementary Table 2), Contig 556 showed a similarity with "wax2-like protein", while contig 1207 was similar to "pathogen related proteins". PCR amplification of contig 1288 sequence produced a clear 350 bp fragment, which was separated the FHB and FRR resistant cultivars from sensitive ones. Interestingly, contig 545 generated two amplicons of 416 bp and 420 bp fragments in FRR resistant cultivars (2–49; Sunco and Altay2000) and FRR sensitive cultivars (Kate and Seri82), respectively. In the current study, different EST-SSRs belong to D genome were only observed in *Fusarium* resistant wheat cultivars. Contig 578 was one of them and generated a polymorphic 204 bp fragment for FRR resistant cultivars (2–49, Altay2000 and Sunco). On the other side, none of the A genome derived EST-SSR loci were discriminative for *Fusarium* resistance. Exceptionally, BQ903543, annotated to wheat chromosome 6AL, was amplified a 900 bp PCR product only for Kate (FRR sensitive), Bezostaya (FHB sensitive) cultivars (Fig. 1). On the D genome, there were two loci (BQ393399, BM138501), which were scored as discriminative fragments for *Fusarium* sensitive cultivars.

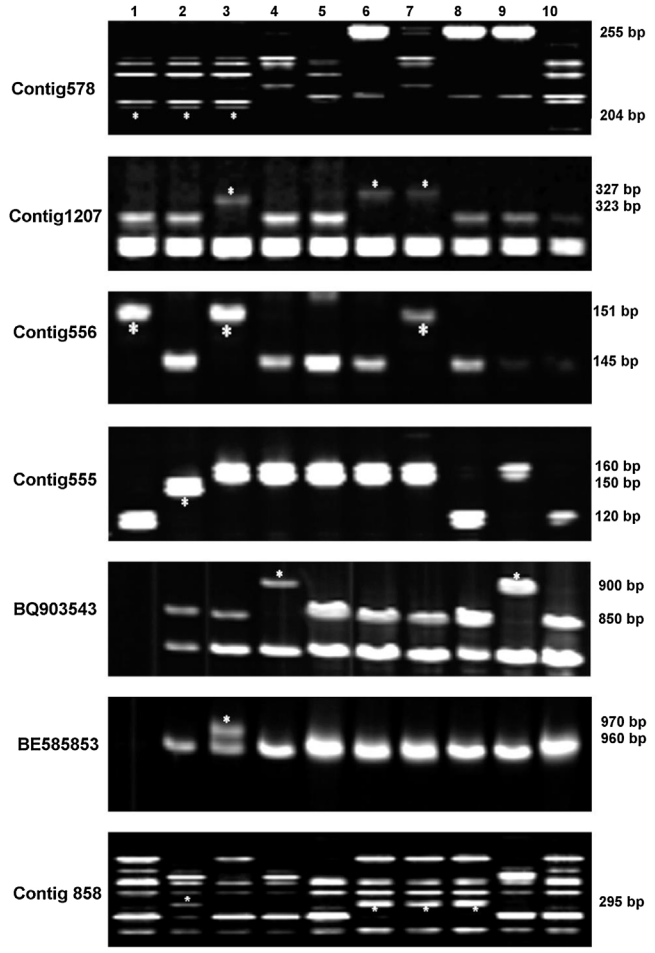

Fig. 1. Fragment profiles of seven polymorphic EST-SSR primer sets. Corresponding sample names of wheat cultivars were listed as 1; 2-49 (FRR resistant), 2; Altay2000 (FRR resistant), 3; Sunco (FRR moderately resistant), 4; Kate (FRR sensitive), 5; Seri82 (FRR sensitive), 6; CM82036 (FHB resistant), 7; Sumai (FHB resistant), 8; Renan (FHB moderately resistant), 9; Bezostaya (FHB sensitive), 10; Scout66 (FHB sensitive). FRR; *Fusarium* root rot, FHB; *Fusarium* head blight. Fragment sizes (bp) were placed on the right and polymorphic fragments associated to putative *Fusarium* resistance and sensitivity were indicated with asterix.

3.3. Genetic diversity analysis

A hundered one EST-SSR primers were screened for polymorphism among ten bread wheat cultivars. Out of them, fifty-five EST-SSR derived primer pairs produced clear fragments. A total of 66 polymorphic alleles from twenty-three EST-SSRs were produced used in phylogenetic analysis (Table 3). The remaining 32 EST-SSR primers produced monomorphic fragments were excluded (Supplementary Table 1). Base on the visual screening, polymorphic allele numbers were varied from 2 to 6 and 56.5% of 23 polymophic loci were represented with at least two alleles. While the highest PIC value of 0.78 was obtained from two different primer pairs (Contig 578 and Contig 190) that were produced six and five polymorphic alleles, respectively, Contig 858, having the third highest PIC value (0.73), was produced four polymorphic alleles. According to dendrogram tree (Fig. 2), FRR resistant cultivars, 2–49 and Altay2000 displayed the highest genetic relationship. It was also pointed out that the FRR moderately resistant cultivar Sunco was placed on the same group with other FRR resistant genotypes. Another significant relationship was detected between Seri82 and Scout66 which were considered sensitive cultivars under *Fusarium* infection. On the second main root of dendrogram tree, two FHB resistant cultivars (CM82036 and Sumai) were clustered on the same root. However, Renan (FHB moderately resistant) and Bezostaya (FHB susceptible) genotypes have been reported to show different responses against to *Fusarium* pathogen. They were displayed closed relatedness (Fig. 2).

4. Discussion

Newly developed wheat EST-SSR markers were validated among *Fusarium* resistant and sensitive wheat cultivars. Before allele scoring, ESTs were mined to detect for the repeats and their distribution on contig and singleton sequences. The SSR frequency in contig sequences was 1.6 times higher than singletons and the SSR frequency was 3.7%. Kantety et al. (2002) reported a SSR frequency of 3.2% in 38.233 wheat ESTs.

Tri-repeats showed the highest percentage of 30.7%, followed by tetra- 10.9%, di- 8.9%, penta-5% and hexa- repeats 1%. The high frequency of tri-repeats was consistent with previous studies in wheat (Chen et al., 2005; Li et al., 2008) and it was explained by a selection against non-trimeric SSRs in coding regions due to possible frame shift mutations (Metzgar et al., 2000). However, the less frequency of hexa-repeats in the present study is in disagreement with Chen et al. (2005), Li et al. (2008). Thirty four types of tri-nucleotide repeats were identified. The most frequent tri-repeats were GCA (1.8%) and GCC (1.8%), and they were followed by CGC, CGG, GGC, GCG (5.9%). In the previous reports, the CAA, CCG, AAC, AGC, CGG, and CGC motifs were favored in wheat (Chen et al., 2005). Significantly, the high frequency of GC content found in the identified repeats might be due to the specific genome feature of monocots (Morgante et al., 2002). In this context, Lucas et al. (2012) surveyed the wheat 1A chromosome with BAC end sequencing and found a large proportion of GC content which was comprising 90% of 1A chromosome in wheat.

Primer pairs designed from 55 EST-SSRs were screened for SSR marker validation in ten bread wheat cultivars with different levels of *Fusarium* resistance. According to polymophism patterns, 32 of 55 were identified as monomorphic and 23 were showed polymorphism among wheat genotypes. The proportion of polymorphic primers was high (41.8%) as compared to that in

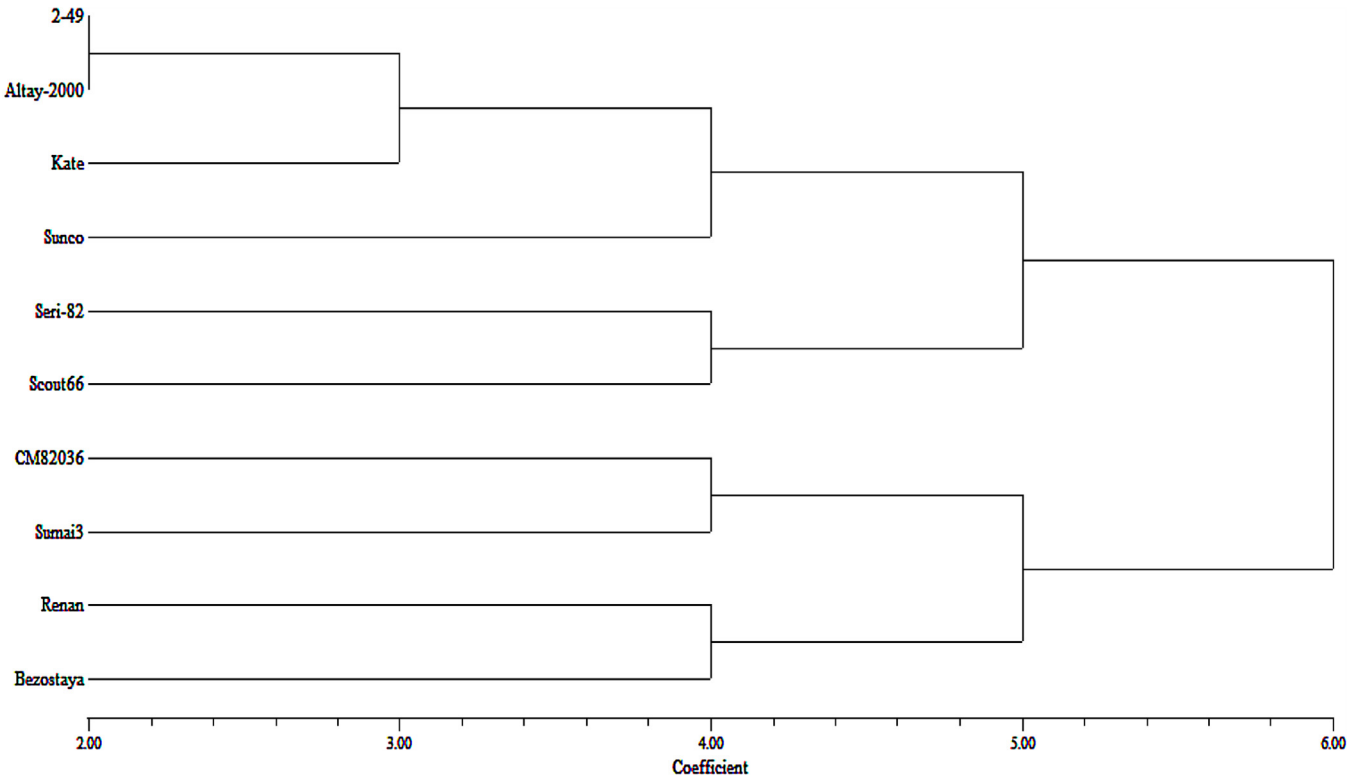

Fig. 2. UPGMA dendrogram of ten wheat cultivars calculated from polymorphic EST-SSR data.

the studies of Hilton et al. (1999) where they found only four primers polymorphic among 15 varieties of bread wheat and Eujayl et al. (2002) where 34 out of 137 EST-SSRs were polymorphic (24.8%) in 64 durum wheat varieties. On the contrary, Gupta et al. (2003) detected 55% polymorphism frequency for their EST-SSR primers among 52 bread wheat genotypes. Of the 55 primers tested in this study, 45 primers yielded almost expected product size, which was quite comparable to earlier reports by Chen et al. (2005) where they reported 133 out of 159, (83.7%) of primer pairs produced expected fragments in wheat. In a recent report of Burt et al. (2015), only two EST-SSR markers showed polymorphic fragment pattern among 26 wheat EST-SSR markers, which were tested on HS (FHB susceptible) and DH81 (FHB resistant) wheat lines and their subsequent F_3 and F_4 inbreds.

A total of 98 alleles, with 1.8 allele number per locus ranging from 1 to 6 were obtained. The maximum number of alleles (6) was similar to the EST-SSR reports of Wei et al. (2008) where they found maximum of five alleles in *Triticum turgidum* L spp . *turgidum* landraces from China. The average PIC values among all the candidate gene primers was 0.33, which was similar to Gupta et al. (2003) and was very closed to Budak et al. (2005). However, it is less than those detected by Dong et al. (2009) where they detected a considerable amount of genetic variations at EST-SSR loci for *T. dicoccoides* in Israel. This might be due to the partly macro-geographic factors and genome organization. Of 55 microsatellite loci analyzed, 8 (14.5%) were highly informative (PIC $\geq$ 0.50) where the maximum PIC was 0.78 for contig 578 and contig 190 loci, while the lowest value (0.11) was observed for the BE585853 locus. The high PIC values showed the eligibility of the new markers for detecting genetic diversity in bread wheat. The expected heterozygosity (He) ranged from 0.10 to 0.78 with an average of 0.48. Also, the known position of each SSRs that were developed *Fusarium* infected wheat libraries may facilitate the QTL mapping, map based cloning.

Based on the 64 polymorphic alleles, similarity coefficients were investigated to examine the genetic relationships of ten bread wheat cultivars with each other. The similarity coefficient among cultivars ranged from 0.60 to 0.81. The Maximum similarity coefficients were observed between "2-49 and Altay 2000", "Sumai 3′ and CM82036" and Seri82 and Scout66 (Fig. 2). Genetic similarity analysis exhibited the ability to partly differentiation between susceptible and resistant cultivars for FRR and FHB. Considering *Fusarium* head blight, the resistant and moderately-resistant cultivars (CM82036, Sumai 3, Renan,) were grouped into a same group, while susceptible cultivars (Bezostaya and Scout66) were clustered on different roots, showing less similarity to the resistant cultivars. In point of FRR, susceptible cultivars (Seri 82 and Kate) exhibited less similarity to the resistant cultivars (2–49, Altay 2000, Sunco) and were located on different groups. The dendrogram profile indicates that EST-SSR markers were effective in clustering cultivars into their respective response to diseases.

Physiologically, *Fusarium* resistance in wheat was divided into five types; I. resistance to the initial infection, II. resistance to spread within the spike, III. resistance to kernel infection, IV. tolerance to infection, V. resistance to toxin accumulation. Among them, type I and II resistance stages, as major components, were frequently studied using marker assisted selection in wheat (Mesterhazy, 1995). QTLs for type I resistance have been reported in the A genome in some studies with different sets of breeding lines (Buerstmayr et al. 2003; Steed et al., 2005), while most identified type II resistance QTLs were mapped on the B genome (Anderson et al. 2001). On the other hand, Buerstmayr et al. (2009) reported that there were no QTLs for wheat FHB resistance on chromosome 7DL. In the present study, the vast majority of EST-SSRs indicating possible relationship for *Fusarium* resistance were dominantly found on B genome derived sequences. Significantly, a major QTL, "Qfhs.lfl-1BL", associated to FHB resistance in wheat was found on the long arm of chromosome 1BL and verified a

reduction for disease symptoms in Cansas, Biscay, History and Pirat wheat cultivars (Haberle et al., 2009). In our study, chromosome 1 B derived two EST-SSRs (contig 556 and contig 1207) were exhibited clear separation between *Fusarium* resistant and susceptible wheat genotypes. The putative functions and chromosomal positions of validated some EST-SSRs might be used to understand the critical genome regions expressed under pathogen attack. In the past, the discriminative nature of ESTs was also used in one of the *Poaceae* members , *Agrostis* spp., for comparative analysis of transcribed genes (Doganay and Budak 2008). As transferable markers, the importance of EST based SSRs was reported for other crops such as barley (Castillo et al., 2008) and proofed with intra and interspecies analysis between wheat and barley genomes (Castillo et al., 2010).

In summary, the present study has resulted the identification of potentially valuable polymorphic EST-SSR marker candidates not only for *Fusarium* resistance but also for mapping, QTL analysis, diversity analysis and marker-assisted breeding in wheat. The new primer sequences derived from *Fusarium* infected wheat specific EST-SSRs may facilitate to find closely linked markers to QTLs for resistance to *Fusarium*. The continued development of SSR markers from EST data can be expected to advance in understanding biological functions, evolutionary biology, germplasm characterization and breeding applications for plants.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.compbiolchem.2017.02.003.

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