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Research Article

Transferability of Barley and Wheat EST-Microsatellite Markers in some *Poaceae* Members

Hülya Sipahi [a*], Yeliz Aslan [a], Ayşen Yumurtacı [b]

[a] University of Sinop, Faculty of Arts and Sciences, Department of Biology, 57000 Sinop, Turkey,
[b] University of Marmara, Faculty of Science and Letters, Department of Biology, 34722 İstanbul, Turkey

Abstract

The cross species transferability of barley and wheat microsatellite markers developed from expressed sequence tag (EST) libraries constructed under *Fusarium* infection conditions were detected among 17 species including 8 from *Aegilops*, 6 from *Triticum*, *Zea mays*, *Avena sativa*, *Oryza sativa*.

Transferability rates of barley microsatellite primer pairs ranged from 29% to 100%. A maximum of 100% cross-genera transferability noticed with *Avena* followed by *Zea* (92%), *Triticum* (83%), *Aegilops* (68%), and *Oryza* (8%). Primer pairs were highly transferable within species of *Triticum* (100% in *T. turgidum durum durum*, 92% in *T. turgidum durum dicoccon* and *T. monococcum aegilopoides*, 83% in *T.timopheevii timopheevii* and *T. turgidum dicoccoides*, 67% in *T. timopheevii armeniacum*). Only one primer pair (contig624) showed 100 % cross-species/genera amplification in all materials studied.

Considering wheat microsatellites, the microsatellite primer pairs were highly transferable within species of *Triticum* (ranged from %100 to %70) and but low transferable in the allied cereals (15% in *Avena*, 50% in *Oryza*, 45% in *Zea*, 60% in *Hordeum*). Two primer pairs have shown transferability only in some *Triticum* species, while two others showed amplication only in species of *Aegilops* and *Triticum*. Only one primer pair showed 100 % cross-species/genera amplification in all materials studied.

This study indicated that 12 barley and 20 wheat microsatellite markers showed a high level of transferability across distantly related species. As a result of that, these markers may be expected to be useful markers for comparative genome mapping and for following gene introgressions from wild species and analyzing genetic diversity and phylogenetic in *Poaceae*.

Key words: Microsatellite markers, EST, transferability, cereals.

* Corresponding Author
e-mail:hulyasipahi@hotmail.com

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Bazı *Poaceae* Bireylerinde Arpa ve Buğday EST-Mikrosatellit Markörlerinin Aktarılabilirliği

Öz

Fusarium enfeksiyon koşulları altında oluşturulmuş ifade olan dizi (EST) kütüphanelerinden geliştirilen arpa ve buğday mikrosatellit markörlerinin türler ve cinsler arası aktarılabilirlikleri, 8 *Aegilops,* 6 *Triticum* türü ile, *Zea mays, Avena sativa* ve *Oryza sativa* 'yı içeren 17 türde belirlenmiştir. Arpa mikrosatellit primerleri için aktarılabilirlik oranı %29 ile %100 arasında değişmiştir. Cinsler arası en yüksek (%100) aktarılabilirlik *Avena* sativa' da not edilmiş, bunu *Zea mays* (%92) , *Triticum (*%83), *Aegilops* (68%) ve *Oryza sativa* (%8) takip etmiştir. Primer çiftleri, *Triticum* türleri içerisinde oldukça yüksek aktarılabilirdir (*T. turgidum durum durum*' da %100, *T. turgidum durum dicoccon* ve *T. monococcum aegilopoides*' da %92, *T.timopheevii timopheevii ve T. turgidum dicoccoides*' de %83, *T. timopheevii armeniacum*' da %67). Yalnızca bir primer çifti (contig624) çalışılan tüm materyallerde, %100 tür/cinsler arası çoğaltım göstermiştir.

Buğday mikrosatellitleri değerlendirildiğinde, mikrosatellit primer çiftleri *Triticum* türleri içinde oldukça aktarılabilirdir (%70 ile %100 arasında) ancak akraba tahıllarda düşük aktarılabilirdir (*Avena sativa*' da 15%, *Oryza sativa*' da 50%, *Zea mays'* da 45%, *Hordeum*' da 60%). İki primer çifti yalnızca bazı *Triticum* türlerinde aktarılabilirlik göstermişken, diğer iki primer çifti sadece *Aegilops* ve *Triticum* türlerinde çoğaltım göstermiştir.

Bu çalışma 12 arpa ve 20 buğday mikrosatellit markörünün uzak türler arasında oldukça yüksek aktarılabilirlik gösterdiğini belirtmiştir. Sonuç olarak, bu markörlerin, karşılaştırmalı genom haritalaması, yabani türlerden gen geçişinin izlenmesi ve *Poaceae*' de genetik çeşitlilik ve filogenetik analizlerde faydalı olabilecekleri beklenmektedir.

Anahtar kelimeler: Mikrosatellit markör, EST, aktarılabilirlik, tahıllar

Introduction

Genome rearrangements are ongoing process during plant breeding and may cause major or minor changes on genetic material. Chromosomal rearrangements can lead to emergence of either a new plant line or a novel allelic composition [1]. The translocation and deletion/insertion events may change the sequence and the order of the gene/gene related regions in crops. However, the extensive conservation of gene sequence and order among cereals is essential to establish the extended genetic maps of cross-species [2]. Thus, the collinearity between the homoeologous and heterologous plant genomes can be used to facilitate the integration of molecular markers to the genetic maps.

The saturated genetic maps with feasible molecular markers can help to identify the common alleles or non-allelic regions. In this context, numerous types of molecular markers have been introduced into the plant researches. Microsatellites are one of the most commonly used molecular markers because of their co-dominant natures and their locus specificity [3]. Expressed sequence tag (EST) libraries are crucial source for providing and enriching the alternative molecular markers [4] and the increase in the number of EST libraries are accelerated with high-throughput genomic technologies. In cereals, the use of

ESTs for development of microsatellites was investigated [5 - 9]. Since flanking sequences of genic microsatellites are highly conserved, microsatellite primers from a species are easily transferable among closely related species [10 - 15].

Microsatellites developed for some cereals were examined for their transferability to *Triticum* species, barley, oat, rye, rice, maize, ryegrass [16 - 20]. Barbara et al. **[**21**]** tested 64 EST-microsatellites for their transferability among dicotyledone and monocotyledon plants. In addition to the crops, different taxons were also used to test the transferability levels of EST SSRs. For example, apple EST-SSR's transferability was investigated in some *Rosaceae* members **[**22**]**. In another study, *Helianthus* EST-SSRs were deeply examined in *Carthamus tinctorius* L. and *Lactuca sativa* genomes and transferability were determined according to the amplicons **[**23**]**., Microsatellites markers are useful for estimating genetic parameters in natural populations such as gene flow, parentage and paternity analysis, Transferable microsatellite markers are also good sources for plants which have limited numbers of molecular markers are available. Thus, they have been used for the study of natural plant populations and contributed to plant diversification studies by enlightening the origin of species **[**24**]**. EST- microsatellites were used for diversity analysis in Gossypium **[**25**]** and discrimination of Tibetan annual wild barley genotypes **[**26**]**. With the same approach, Zhou et al **[**27**]** developed 204 novel EST-SSRs in alfafa. Also, microsaatellite marker from a gene with known function can be used for homologous gene identification and cloning in related species **[**28**]**.

The aim of this study was to evaluate the transferability rate of 32 polymorphic barley and wheat microsatellite markers recently developed by Sipahi et al **[**29**]** and Yumurtaci et al. [Computational Biology and Chemistry, in press] to 8 *Aegilops* species, 6 *Triticum* species, *Zea mays, Avena sativa, Oryza sativa, Hordeum vulgare.*

Material and methods

Plant materials

18 species including 8 from *Aegilops*, 6 from *Triticum*, and one each from *Zea, Avena, Oryza, Hordeum* were used to determine transferability of barley and wheat microsatellite markers (Table 1). All seeds of plants were obtained from United States Department of Agriculture seed bank.

DNA isolation

DNA was isolated from the young leaves of each accession according to the method of Song and Henry **[**30**]**. DNAs were diluted to the 30ng/µl and directly used in amplification reactions.

PCR amplification

12 barley and 20 wheat microsatellite primer pairs were used (Table 2, 3). PCR mixture was included the following contents; 30 ng genomic DNA, 1X Taq Reaction Buffer, 5 units of Taq DNA Polymerase, 0.2 mM dNTPs and 0.25 µM of each primer. PCR cycles were set up as (3 min at 94°C; 1 min at 94°C, 1 min at primer binding temperature, 2 min at 72°C) for 40 cycle; 7 min at 72°C). The amplification products were visualized using an ABI PRISM®3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with GeneScan 500 LIZ size standard according to the manufacturer's recommendations. The sizes of fragments were analyzed using Peak Scanner Software

v1.0 (Applied Biosystems, Foster City, CA, USA).

Table 1: *The list of material representing the different genome sources*

No	GenBank code	Taxon	Genome source	Origin of place
1	PI 542172	*Aegilops comosa*	MM	Turkey, Izmir
2	Clae 70	*Aegilops bicornis*	S^b	unknown
3	PI 170194	*Aegilops biuncialis*	UUMM	Turkey, Kırklareli
4	PI 178821	*Aegilops neglecta*	UMN	Turkey, Balikesir
5	PI 276968	*Aegilops columnaris*	UUMM	Turkey, Konya
6	PI 276976	*Aegilops cylandrica*	DC	Iran, Zanjan
7	PI 276999	*Aegilops ventricosa*	DN	unknown
8	PI298892	*Aegilops crassa*	DM	Afganistan, Kondoz
9	Cltr 14429	*Triticum turgidum durum subsp durum*	AABB	Ethiopia, Shewa
10	Cltr 14637	*Triticum turgidum durum subsp dicoccon* (wild emmer)	AABB	Ethiopia, Harer
11	Cltr 15205	*Triticum timopheevii subsp timopheevii*	AAGG	Greece
12	PI 352269	*Triticum monococcum subsp.aegilopoides* (wild einkorn)	A^u	Germany, Bavaria
13	PI 352327	*Triticum turgidum subsp dicoccoides* (wild emmer)	AABB	Switzerland
14	PI 427361	*Triticum timopheevii subsp armeniacum*		Iraq, Süleymaniye
15	Clav 1122	*Avena sativa*		
16	Clor 1160	*Oryza sativa*		
17		*Zea mays*		
18		*Hordeum vulgare subsp vulgare*		
19		*Triticum aestivum (common wheat)*	AABBDD	

Table 2. *The list of primer sequences of barley EST-microsatellites*

Locus name	Repeat Type	Forward and Reverse Primer Sequence (5'-3')
Contig 624	(CATC)6	F: GCCAGACCAACCAATACC R: GGAGCAGCAACAATAGCA
Contig 381	(TTG)6	F: TGAGCAATAAGGTGGAACAT R: GCAACAACAACAACAACAAG
Contig 608	(TA)6	F: GGCGGAGTGAGGTGTAA R: CCTGCGAAGAAGAGAAGAG
Hv#S12622295 HVSMEl0002A13f	(ATGG)7	F: ACCATCTTCCTTCCTTCCT R: CCTTCCTCCATCCATCCA
Hv#S12624235 HVSMEl0010G24f	(TG)10	F:CCAGGTCCCAGTTGTTCT R:TCCAGTTTCAGCCACCAA
Hv#S12625602 HVSMEl0017O10f	(TGC)7	F: GCTGTGGGTCTGTCTTTG R: CAAGGATGCTGCGAAGTA
Contig 305	(TTC)7	F: GGACTGACTGACGAAGGT R: CGATTAGAGGAGAGGAATAACA
Contig 269	(CAACGG)4	F:ATCATCACCGCCGTCCT R:TTGGAGCCGTTGCCGTT
Hv#S48848420 HVSMEl0003G02r2	(ATC)12	F: AATGTCGCACGCATAGTTA

		R: GTGACCACACAAGAAGAAGAA
Hv#S48848649 HVSMEl0006F02f2	(CT)15	F: AGACAGCAAAAGGAAAAGTG
		R: GACAGGAGGGGTGGAGAC
Hv#S48849653 HVSMEl0017O10f2	(AGC)7	*F*: GTCGCACAACTTCCTGTC
		R: TACTTCAAACTTCTTGCCTGT
Hv#S48849571 HVSMEl0016A02f2	(TC)10	F: ATTGCTCATCTCATCCATACA
		R: GAGGGGAAGGAAGGAACT

Table 3. *The list of primer sequences of wheat EST-microsatellites*

Locus name	**Repeat type**	**Forward and Reverse Primer Sequence (5'-3')**
Contig 578	$(TC)_{19}$	F:GCATAGTCCGTCCTCAGA
		R:CGCTCCTTGTTCACATCA
BQ903543	$(GCT)_7$	F:ACTATCGGTAATCTGTGGAT
		R:GAACTTCTCCCTCCTCAG
Contig 555	$(AGA)_{14}$	F:AATCTTGTCTGCGTGAATG
		R:TCCTCCACCACACCATAA
Contig 989	$(GCC)_6$	F:GAATCAGGAGGTAGGTAA
		R:CGTGAGTGCTACAAGT
Contig 556	$(TAC)_7$	F:AGCCAAGCCAGTCCAA
		R:AGCATCGTCTCGTCAGA
Contig 1207	$(TA)_{11}$	F:AACGTGCATGAATCCTTG
		R:CTGTGGGTGGACGAGAAGA
WHE3876_A05_A10ZS	$(CATG)_5$	F:GGTAACAGTGCGTGCTT
		R:GCCTCGTCCTCAACAAC
Contig 122	$(GAA)_6$	F:CGTCGCAAGAGGAATCG
		R:CGTCACCAGAACCATCAG
Contig 2305	$(CT)_{11}$	F:GTCACTTGGATGAGTCTGGAAG
		R:CCTGAACTGAAAGGAGCAACAT
Contig 1270	$(TCT)_6$	F:AAGTCTCCTCCTCCATCG
		R:CAGTTCGTGTCCACTAGG
Contig 883	$(GGC)_8$	F:ATCGGAAGCACCAACCA
		R:TCCATGTGGAGCCAGTC
BE585853	$(CA)_5$	F:GCAGAGCATCATCCATCC
		R:CCACAGCCTTCACCATTG
Contig 196	$(CAG)_6$	F:CACAAGACCAGACGAGGA
		R:AGCCGACTACAACATCCA
Contig 210	$(AAGAG)_5$	F:GTCATCAGTAGAGGATAGA
		R:ATCACCGAGTTCTGTAAGA
Contig 267	$(CGG)_6$	F:GCCATCCCTATCCATAAG
		R:ATACGGTTCTTGCACTG
Contig 858	$(CGC)_5$	F:GAGGTAGTTCATGTGCT
		R:CCCAATTCCCGATCTC
BM138501	$(GCGA)_5$	F:CCTTGGTAACGGCTTGG
		R:GTAGTTCTTGTTGATGGAGTC
WHE3896_F09_K18ZS	$(AGA)_8$	F:AGAGCAGTGAATAGCCATC
		R:GGAGAAGAGAAGCAGCAA
Contig 2221	$(TA)_{28}$	F:ACGTTTGATTTGACAT
		R:GGACCTTGCTCCAGAC
Contig 545	$(GCC)_7$	F:CCGACCATCATCATCAA
		R:CACCTCCACGATCTTG

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Statistical Analysis

Transference is defined as the positive amplification of a PCR band of the expected size. Transferability of the barley and wheat microsatellite markers to the related species was computed as the percentage of markers giving an amplification product on the species examined.

Results and Discussion

EST derived microsatellite markers are a good choice for application in plant breeding, germplasm collection conservation, comparative mapping and evolutionary studies across species [21, 31, 32, 33]. Transferable microsatellite markers facilitate providing a cost-effective markers for distantly related species for which little information is available on microsatellites or ESTs [15]. The present study has been focused on a quantitative assessment of the transferability of wheat and barley EST-SSRs. A total of 32 microsatellite primer pairs from barley and wheat amplified products within 17 species including 8 from *Aegilops*, 6 from *Triticum*, *Avena sativa*, *Oryza sativa* and *Zea mays*. In all tested materials, the primer pairs have showed reliable amplification patterns because they have yielded single-copy amplification products with similar molecular weight to the amplification products obtained in barley and wheat.

Twelve (36.4%) of the 33 barley microsatellite primer pairs tested were transferable among the species of *Aegilops* and *Triticum*, and three allied cereals (Table 4). Thirteen primer pairs failed to amplify products. This could be explained by a mutation in the DNA sequences flanking the microsatellites, creating a null allele, or occurrence of high genomic differentiations on the genomes of species tested. The transferability rates for twelve markers ranged from 29% to 100% (Table 4). A maximum of 100% cross-genera transferability noticed with *Avena* followed by *Zea* (92%), *Triticum* (83%), *Aegilops* (68%), and *Oryza* (8%). All primer pairs amplified products in *T. turgidum durum durum* and *Avena sativa*. Only one marker was amplified in *Ae. neglecta*.

Considering wheat microsatellites, 20 pairs tested were highly transferable within species of *Triticum* (ranged from 70% to 100%), but they showed lower transferable percentage in the other cereals (15% in *Avena sativa*, 50% in *Oryza sativa*, 45% in *Zea mays*, 60% in *Hordeum vulgare*) (Table 5). This finding is consistent with Zhang et al (19) that have demonstrated the transferability of 300 bread wheat (*Triticum aestivum*) EST microsatellites to closely related species carrying A genome (*T. monococcum*), B genome (*Ae. speltoides*) and D genome (*Ae.tauschii*) (85.3%, 79.2% and 76.7%, respectively), ranging from 76.7% for *A. tauschii* to 85.3% for *T. monococcum*. The rates were lower for more distant relative species such as barley (50.4%) or rice (28.3%). Also, this result confirmed the general observation that the transferability rate of EST-microsatellite across species/genera decays as the species/genera are more phylogenetically distant [15].

Table 4. *Absence and presence amplification profiles of barley microsatellites across different species*

Locus name	***Aegilops comosa***	***Aegilops bicornis***	***Aegilops biuncialis***	***Aegilops neglecta***	***Aegilops columnaris***	***Aegilops cylandrica***	***Aegilops ventricosa***	***Aegilops crassa***	***T. turgidum durum durum***	***Triticum turgidum durum dicoccon***	***Triticum timopheevii timopheevii***	***Triticum monococcum aegilopoides***	***Triticum turgidum dicoccoides***	***Triticum timopheevii armeniacum***	***Avena sativa***	***Oryza sativa***	***Zea mays***	**Transferability %**
Contig 624	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**100**
Contig 381	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	**82**
Contig 608	+	+	-	-	+	+	+	+	+	-	+	+	-	-	+	-	-	**59**
Hv#S12622295 HVSMEl0002A13f	+	+	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	**82**
Hv#S12624235 HVSMEl0010G24f	-	-	-	-	+	-	-	-	+	+	-	+	+	-	+	-	+	**29**
Hv#S12625602 HVSMEl0017O10f	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	**88**
Contig 305	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	**82**
Contig 269	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	**76**
Hv#S48848420 HVSMEl0003G02r2	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	**76**
Hv#S48848649 HVSMEl0006F02f2	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	**65**
Hv#S48849653 HVSMEl0017O10f2	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	**94**
Hv#S48849571 HVSMEl0016A02f2	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	**94**
Transferability %	**58**	**58**	**83**	**8**	**92**	**75**	**75**	**92**	**100**	**92**	**83**	**100**	**92**	**75**	**100**	**67**	**92**	

Table 5. *Absence and presence amplification profiles of wheat microsatellites across different species*

Locus name	***Aegilops comosa***	***Aegilops bicornis***	***Aegilops biuncialis***	***Aegilops neglecta***	***Aegilops columnaris***	***Aegilops cylandrica***	***Aegilops ventricosa***	***Aegilops crassa***	***T. turgidum durum durum***	***Triticum turgidum durum dicoccon***	***Triticum timopheevii timopheevii***	***Triticum monococcum aegilopoides***	***Triticum turgidum dicoccoides***	***Triticum timopheevii armeniacum***	***Avena sativa***	***Oryza sativa***	***Zea mays***	***Hordeum vulgare vulgare***	***Triticum aestivum***	**Transferability (%)**
Contig 578	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	+	**17**
BQ903543	-	+	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	+	+	**50**
Contig 555	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	**50**
Contig 989	-	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	-	-	+	**67**
Contig 556	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**100**
Contig 1207	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	**83**
WHE3876_A05_A10ZS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	**94**
Contig 122	-	+	+	-	+	+	+	+	+	+	+	-	+	+	-	-	-	+	+	**67**
Contig 2305	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**94**
Contig 1270	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	**17**
Contig 883	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	**94**
BE585853	-	-	-	-	-	-	+	+	+	+	+	+	-	+	-	-	-	-	+	**39**
Contig 196	-	+	+	+	+	+	+	-	+	+	+	-	+	+	-	+	-	-	+	**67**
Contig 210	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	**78**
Contig 267	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-	+	**56**
Contig 858	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	**83**
BM138501	-	+	+	-	+	+	+	+	+	+	+	-	+	+	-	-	+	-	+	**67**
WHE3896_F09_K18ZS	-	-	-	+	-	-	-	-	+	+	+	+	-	+	-	-	-	+	+	**39**
Contig 2221	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	**89**
Contig 545	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+	-	+	+	**44**
Transferability (%)	**20**	**75**	**65**	**60**	**75**	**75**	**80**	**70**	**100**	**95**	**80**	**75**	**70**	**85**	**15**	**50**	**45**	**60**	**100**	

In this study, all wheat primer pairs were successfully amplified in *T. turgidum durum durum* (100%) and *T. turgidum durum dicoccon* (95%). Only one primer pair (Contig 556) showed 100% cross-species amplification in all materials studied. There were two wheat EST microsatellites (10%) recorded as cross transferable in all the 8 tested *Aegilops* species. The cross species transferability of the remaining 18 EST–SSR primers was observed in 1 to 8 *Aegilops* species with 104 combinations (primer; + amplification x species) (65%) (Table 5). In an earlier study comprising 64 wheat EST- microsatellites and 18 species of *Triticum–Aegilops* complex, 29 (45%) of the 64 primers gave amplified products in all the 18 species used, the cross species transferability was observed in 963 (84%) of 1152 (64 SSRs × 18 species) combinations [34]. Taken together, these results proved the high level transferability of EST microsatellites. Thiel et al. [32] and Holton et al. [35] had also drawn similar conclusions for barley and wheat microsatellites.

Two primer pairs (Contig 578, Contig 1270) produced amplicons in only some *Triticum* species while 3 of the tested primer pairs (Contig 578, BE585853, Contig 267) amplified product in only some of the *Aegilops* and *Triticum* species.

Limited numbers of molecular markers are available for *Aegilops* species. In the present study, the transferable EST-microsatellite markers of barley and wheat contributed the increased number of genetic markers available for the *Aegilops* genomes which are the secondary gene pool of cultivated wheat. Konstantinos and Bebeli **[**36**]** pointed out that the genus *Aegilops* can play an important role in broadening the genetic base of wheat. They contain unique alleles that are absent in wheat cultivars and have a potential interest for improving yield, quality and resistance to stresses factors in wheat improvement.

Conclusion

Thirty one EST-derived microsatellite markers in the present study have high level of cross-species/genera transferability. As stated in previous studies [17, 32] the high level transferability of these markers may occur due to their conserved nature of DNA sequences belonging to the transcribed region of the genome. Therefore, these markers may be expected to be useful and suitable for comparative genome mapping in *Poaceae*. On the other hand, these markers will provide important tools to evaluate the marker-trait association, QTL mapping and genetic diversity analysis, provided that they will be mapped and integrated into the genomic network of cereal species.

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