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REGULAR ARTICLE

Generation and exploitation of EST-derived SSR markers for assaying molecular diversity in durum wheat populations

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Abstract Durum wheat [*Triticum turgidum* L. subsp. *turgidum* convar. *durum* (Desf.) MK] is an important cereal crop economically and nutritionally in the Central Asia and Caucasian, West Asia, and North Africa (CWANA) regions. Durum landraces and improved lines are largely grown in this region. Its genetic diversity has been studied using different molecular markers. The increasing availability of expressed sequence tags (ESTs) in wheat (*Triticum aestivum*) and related cereals provides a valuable resource of non-anonymous DNA markers to study durum diversity. In this study, a set of 517,319 *Triticum aestivum* EST sequences was employed for the identification of wheat simple sequence repeats called microsatellites (W-eSSRs) with the help of a PERL5 script called MISA. In comparison, barley microsatellites (B-eSSRs) have been used to exploit their transferability to durum wheat. Newly developed W-eSSR markers were probed on the 115 recombinant inbred lines (RIL) of the International Triticeae Mapping Initiative (ITMI) population (Opata 85 × Synthetic 7984). The polymorphic eSSRs were mapped. To examine the potential of the two types of eSSRs markers, 12 W-eSSR markers and 13 B-eSSR markers were used to fingerprint 153 wheat genotypes. Our results indicate that: (1) B-eSSRs show a high level of transferability to wheat, (2) the developed W-eSSRs are significantly polymorphic than those derived from genomic regions, (3) new W-eSSRs were identified and integrated in the ITMI genetic linkage map and, (4) B-eSSR and W-eSSRs are providing additional markers for comparative mapping following gene introgressions from wild species and carrying out evolutionary studies.

Keywords B-eSSRs · ESTs · Genetic diversity · Genetic linkage mapping · Polymorphism Information Content (PIC) · W-eSSRs

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Introduction

Microsatellites or simple sequence repeats (SSRs) are stretches of DNA consisting of tandemly repeated short units 1–6 base pairs in length. The uniqueness and the value of microsatellites arise from their multiallelic nature, codominant inheritance, relative abundance, extensive genome coverage and simple detection by PCR using two unique primers that flank the microsatellite and hence define the microsatellite

locus (Powell et al. 1996). The standard method for developing SSR-markers involves the creation of a small-insert genomic library, the subsequent hybridization with tandemly repeated oligonucleotides, and the sequencing of candidate clones; thus making the process time consuming and labor-intensive (Gupta and Varshney 2000). In wheat, several hundred-microsatellite markers have been developed using this strategy (for review see Röder et al. 2002; Varshney et al. 2006). The increasing amounts of information available in DNA sequence databases make an alternative strategy possible. SSRs can be searched in these databases, in order to reduce the time and the costs required for their development (Varshney et al. 2005a).

In recent years, due to the rapid increase of sequence information, the generation of EST-derived microsatellite (EST-SSR) markers has become an attractive alternative to complement-existing SSR collections. The use of EST or cDNA-based SSRs has been reported for several species including grape (Scott et al. 2000); sugarcane (Cordeiro et al. 2001); durum wheat (Eujayl et al. 2001); hexaploid wheat (Yu et al. 2004a, b; Leigh et al. 2003; Nicot et al. 2004; Gao et al. 2004; Zhang et al. 2005), barley (Thiel et al. 2003; Chabane et al. 2005; Varshney et al. 2006) and rye (Hackauf and Wehling 2002; Khlestkina et al. 2006). Since EST-SSRs are derived from genic parts, a conserved proportion of the genome, EST-SSR markers derived from one species can be used in other related species (Cordeiro et al. 2001; Thiel et al. 2003; Varshney et al. 2005b).

Durum wheat [*Triticum turgidum* L. subsp. *turgidum* convar. *durum* (Desf.) MK] is a tetraploid wheat species, which is mainly grown in the Mediterranean region, Canada, USA, Argentina and India. Demand for durum wheat has grown in recent years. Bread, pastry and pasta quality from durum wheat can be improved using available markers for gluten strength, grain texture, protein content, starch properties, flour and semolina color (Nachit et al. 2001; Maccaferi et al. 2006). Assessment of genetic diversity has been crucial in breeding programs for selection of suitable parents to obtain heterotic hybrids, and for characterization and identification of germplasm (Prasad et al. 2000; Autrique et al. 1996).

In the present study, we attempted to exploit available SSR markers from other crops, since not many are available from durum wheat, to assess the genetic variability present in a collection of durum wheat is from the International Center for Agricultural in the Dry Areas (ICARDA) germplasm bank. We have therefore explored the use of 185 SSR markers developed from barley ESTs at the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany (Thiel et al. 2003; Varshney et al. 2006). We also examined ESTs generated from *T. aestivum* L. (common wheat) database. These markers were searched for occurrence of SSRs. Twenty five EST-SSR markers (13 barley, 12 wheat) were finally used for assessing molecular diversity in 148 accessions of durum wheat and five *T. turgidum* subsp. *dicoccoides* (Körn. ex Asch. et Graebn.) Thell. Finally, four of 12 wheat EST-SSR markers were integrated to the reference ITMI wheat genetic map.

Materials and methods

Plant materials

For detection of SSR polymorphism, a total of 153 wheat accessions were used (Table 1a, b). The wheat landrace germplasm used originated mainly from North Africa (Algeria, Morocco and Tunisia), the Fertile Crescent area (Syria, Iraq, Jordan and Iran) and a few from Central Asia. The remaining durum wheat germplasm was constituted by breeding material (indicated by ID or – in Table 1) developed by ICARDA breeder.

DNA extraction

Genomic DNA from these genotypes was isolated as described in Chabane et al. (2005). A set of eight wheat genotypes (indicated by * in Table 1) was preliminarily used as a reference set assuming them as a diverse set of genotypes for selecting polymorphic markers to assay the complete germplasm collection.

Sources of markers

Barley EST-SSR markers

A set of 48 barley EST-SSR markers (B-eSSR), distributed throughout all linkage groups of barley, were tested on the reference set of eight diverse genotypes. Subsequently, a set of 13 B-eSSR markers

Table 1 Origin of wheat genotypes from ICARDA genebank

IG[a]	Origin/ID	IG	Origin/ID	IG	Origin/ID	IG	Origin/ID
44830	SYR	95823	SYR	114293	–	139264	Syrian-4
45491	SYR	95842	SYR	114300	Stojori-6	139265	Terbol-97-2
45663	SYR	95843	SYR	114322	–	139266	Fadda-98
46479	SYR	95844	SYR	114326	Omtel-6		
46516	SYR	95846	SYR	114347	Korifla		
46518	SYR	95913	SYR	114353	Bicre		
81974	TUN	95929	SYR	114355	Chahba-88		
82104	DZA	96149	SYR	114360	Tensift-1		
82107	DZA	96154	SYR	114372	Lahn		
82210	KAZ	96158	SYR	114375	Daki		
82281	EGY	96249	JOR	114385	Gedifla		
82458	IRQ	96338*	MAR	114388	Kabir-3		
82581	AFG	96359	MAR	115810	–		
83033	TUR	96365	MAR	115812	–		
83367	EGY	96628	GRC	118178	–		
83481	EGY	97214	JOR	118179*	–		
83582	MAR	97219	JOR	118182	–		
83671	UZB	97223	JOR	118184	–		
84040	SAU	97224	JOR	118726*	–		
84818	IRQ	97228	JOR	118739	–		
84843	PAK	97360	DZA	118742	–		
84857	SYR	97361	DZA	119375	–		
84858	SYR	98192	AZE	126229	–		
85018	ESP	98320	UZB	129076	Zeina-4		
85508	AFG	98530	UZB	129080	Cham-1		
85615	IRN	98691	KAZ	132492	–		
86317	AZE	99049	JOR	139240*	Zeroud-3		
87193	BGR	99051	JOR	139241	Ain arous-2		
89458	AFG	99066	–	139242	Khabur-1		
89461	AFG	99099	MAR	139243	Belikh-1		
89463	AFG	99124	TUN	139244	Awali-3		
90246	AFG	99149*	TUN	139245	Oronte-4		
92399	SYR	99154	TUN	139246	Omrabi-17		
92755	DZA	99216	YEM	139247	Sabil-3		
92888	DZA	109091	SYR	139248	Amst-1		
92966	DZA	113069	SAU	139249	Heider		
93264	DZA	113075	SAU	139250	Syrica-3		
94625	TUN	114207	–	139251	Loukos-4		
94676	TUN	114214	MAR	139252	Akrache-2		
94687	TUN	114215*	–	139253	Guerou-2		
94874	TUN	114229	Valdamez-6	139254	Ouassel-1		
94898	TUN	114233	Genil-3	139255	Omlahn-3		
94925	TUN	114236*	Genil-4	139256	Moulsabil-1		
95499	–	114239	Lagost-2	139257	Brachdi		
95721	TUN	114241	Omruf-2	139258	Wabrach-2		

Table 1 continued

IG[a]	Origin/ID	IG	Origin/ID	IG	Origin/ID	IG	Origin/ID
95777	PAK	114251	Balloran	139259	Outrob-4		
95788	SYR	114256	Omguer-2	139260	Telset-3		
95789	SYR	114262	–	139261	Bicrecham-1		
95798	SYR	114291*	Awalbit-2	139263	Syrian-3		

The genotypes with (*) were used to screen the polymorphism of the identified W-eSSR and B-eSSRs

[a] IG number in ICARDA genebank

* Genotypes used as a reference set

was selected based on yielding a good quality of amplicons as well as polymorphism data in diverse genotypes. Details of the selected markers are given in Table 2 (Thiel et al. 2003; Varshney et al. 2006).

Wheat EST-SSR markers

About 517,319 *Triticum aestivum* EST sequences available in the public domain (3 September 2004) were downloaded. SSRs search, cluster analysis and primer designing for developing non-redundant SSR markers for wheat was carried out as described in Varshney et al. (2002) and Thiel et al. (2003). Although primer pairs were developed for non-redundant wheat SSRs, out of 28, 12 markers (Table 3) were selected randomly for analysing the durum wheat populations.

To locate the newly developed W-eSSR markers in the wheat genome, these markers were analyzed on 115 recombinant inbred lines (RIL) of the International Triticeae Mapping Initiative (ITMI) population derived from Opata 85 × Synthetic 7984. Genetic mapping of polymorphic markers was carried out as described in Röder et al. (1998). Mapped wheat microsatellite loci were designated as ICARDA Wheat Microsatellite (IWM).

Marker analysis

PCR was done in 10 µl reactions containing 20 ng genomic DNA, 0.25 U Taq DNA polymerase (Qiagen, Hilden, Germany) for all microsatellite markers including B-eSSR and W-eSSR and I. The following touch down PCR profile was used: 3 min at 94°C; 10 cycles of 30 s at 94°C, 30 s at 60°C minus 0.5°C per cycle, 30 s at 72°C; 25 cycles of 30 s at 94°C, 30 s at 55°C, 30 s at 72°C; and 5 min at 72°C

Table 2 Characteristics of B-eSSR loci derived from *H. vulgare* database, including their repeat motif, the total of alleles per locus (A_0), PIC value and number of alleles by wheat landraces

Marker	Locus	PIC value	Location	Total number of alleles	Number of alleles by accession
GBM 1008	(AAC)10	0.853222	6H	16	3
GBM 1029	(AG)10	0.787963	1H	23	3
GBM 1031	(AG)15	0.936064	3H	30	3
GBM 1033	(AT)9	0.79614	7H	19	2
GBM 1035	(CT)8	0.817892	2H	31	4
GBM 1043	(AAC)5	0.792467	3H	15	3
GBM 1054	(CCG)5	0.895244	5H	22	3
GBM 1059	(GGT)5	0.919689	3H	27	4
GBM 1064	(AGGG)5	0.78325	5H	15	3
GBM 1405	(CGCA)5	0.59061	3H	9	4
GBM 1419	(CTCAT)5	0.785226	7H	15	4
GBM 1459	(AC)7	0.904873	2H	25	3
GBM 1464	(CAG)8n(CAG)5	0.8724	7H	22	3
Average		0.825772		21	3

Table 3 Description of W-eSSR derived microsatellites used on the 153 genotypes

Locus name	Repeat	Forward primer1 (5′-3′)	Reverse primer1 (5′-3′)	Blast match	E-value
IWM0001	(AG)49	attcggcacgaggagagag	gggccatggctgtactatgt	*Ae. tauschii* Coss. mRNA for ribulose…	0.27
IWM0002	(AT)31	tgccgagctaaagaagaagg	atacatcttaacgcgcctgc	nd*	–
IWM0003	(CT)25	ccccctctccttatggctac	ggagggaatactaggcgagg	*Zea mays* PCO074099 mRNA sequence	0.27
IWM0004	(GA)40	gaatccagccgaacaatttc	agtactccgacaccacgtcc	*Triticum aestivum* L. em. Thell. clone wip1c.pk00…	0.072
IWM0005	(GA)59	ctgtttgggttttcaggctc	caaggtagggaaatggctagg	*Zea mays* L. CL12353_1 mRNA sequence	0.30
IWM0006	(ACA)31	tcatgctattattctgcatcaaca	cctggcctgatggatattgt	*Triticum aestivum* L. em. Thell. alpha-gliadin GLi2	0.001
IWM0007	(ATA)27	gttgaggttggactttgcgt	atgcaaagatttaatgcgcc	*Saccharomyces. pombe* crk1 gene	1.1
IWM0008	(TTC)19	gccaccaaaggtactgctact	acccgcttagacgttttcct	*Oryza sativa* L. alpha-expansin OsEXPA4	0.019
IWM0009	(TATG)34	tgcgctcgggataaattaaa	tacacaagccgacgtgtcat	nd	–
IWM0010	(TATAGA)16	cgagtcgaagctggtttagg	tttcatgacgatttgtgtatgtagt	*Medicago truncatula* clone mth2-135i1	0.35
IWM0011	(CA)43	tgagttactgtacgcacacacg	cacaaccctgtggatctcct	*Mus musculus* BAC clone RP23-58N15…	0.31
IWM0012	(AAG)17	ctttcgaagtagctgaggcg	ggatccatccatattgtaaatgtc	*Oryza sativa* L. (*japonica* cultivar-g…)	1.3

* not determined

for final extension. Post-amplification, 3–5 µl of multiplexed PCR were mixed with sterile distilled water to reach a final volume of 15 µl. Then 4 µl of the combined PCR products were mixed with 9 µl of Hi-Di formamide and 0.45 µl of the GeneScan-350 ROX size standard (Applied Biosystems). All samples were denatured for 2 min at 95°C, and then cooled on ice for 2 min before testing. The fragments were separated on an ABI377 sequencer and analyzed using GenoTyper 3.7 (Applied Biosystems).

Statistical analysis

Polymorphism information content (PIC)

The PIC-value for markers was calculated as follows (Anderson et al. 1993):

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

where k is the total number of alleles detected for a locus of a marker and P_i the frequency of the ith allele in the set of the investigated wheat accessions.

Diversity analysis

The profiles produced by EST-SSR were scored: each allele was scored as present (1) or absent (0) for the SSR loci. The 0/1 matrix for the examined genotypes was used to calculate genetic dissimilarity according to Nei's method (1978), SAHN clustering and the construction of UPGMA (Unweighted Pair Group Method Arithmetic Average) phenogram using NTSYS program (version 2.1).

Results and discussion

Development of wheat EST-SSR markers

About 517,319 *Triticum aestivum* ESTs, corresponding to 249 MB were employed for searching of microsatellites as a source for marker development. Using *MISA* software tool, 38,121 microsatellites containing sequences were identified (SSR sequences available on request). The total number of detected SSR was 45,456 (9%), and 4846 sequences (10%) contained more than one SSR. However, 5306 SSRs (1%) were present in compound formation. As

expected, trimeric SSRs constituted the major portion (56% of the total SSRs identified). As reported earlier (Metzgar et al. 2000; Varshney et al. 2005a; Swarup et al. 2006), this could be due to the suppression of non-trimeric SSRs in coding regions due to risk of frame shift mutations. Pentameric and hexameric microsatellites were present at less than 1% of total SSRs searched (0.9% versus 0.3%). These results are in accordance with earlier studies on database mining of SSRs in ESTs in cereal genomes (Kantety et al. 2002; Varshney et al. 2002; Thiel et al. 2003).

Out of 12 wheat markers, only three markers (IWM5004, IWM5007 and IWM5008) detected polymorphism between parental genotypes (Opata 85 × Synthetic 7984) of the ITMI mapping population. As a result four SSR loci including two loci IWM5004a and IWM5004b detected by IWM5004 marker and two other loci IWM5007 and IWM5008 were successfully integrated in the reference map of ITMI population (Fig. 1a, b). These four EST wheat SSR loci were integrated into four linkage groups (2A, 2B, 6A and 1D). Two independent loci

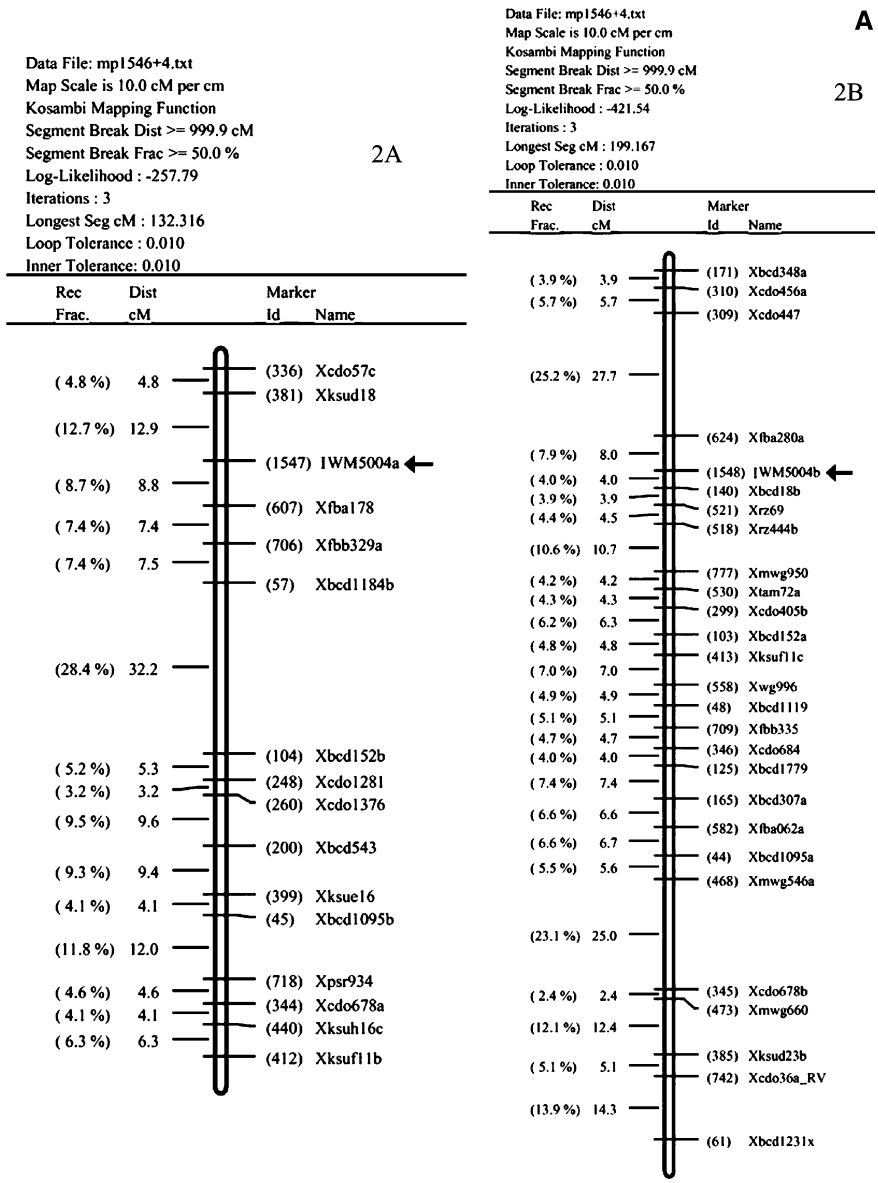
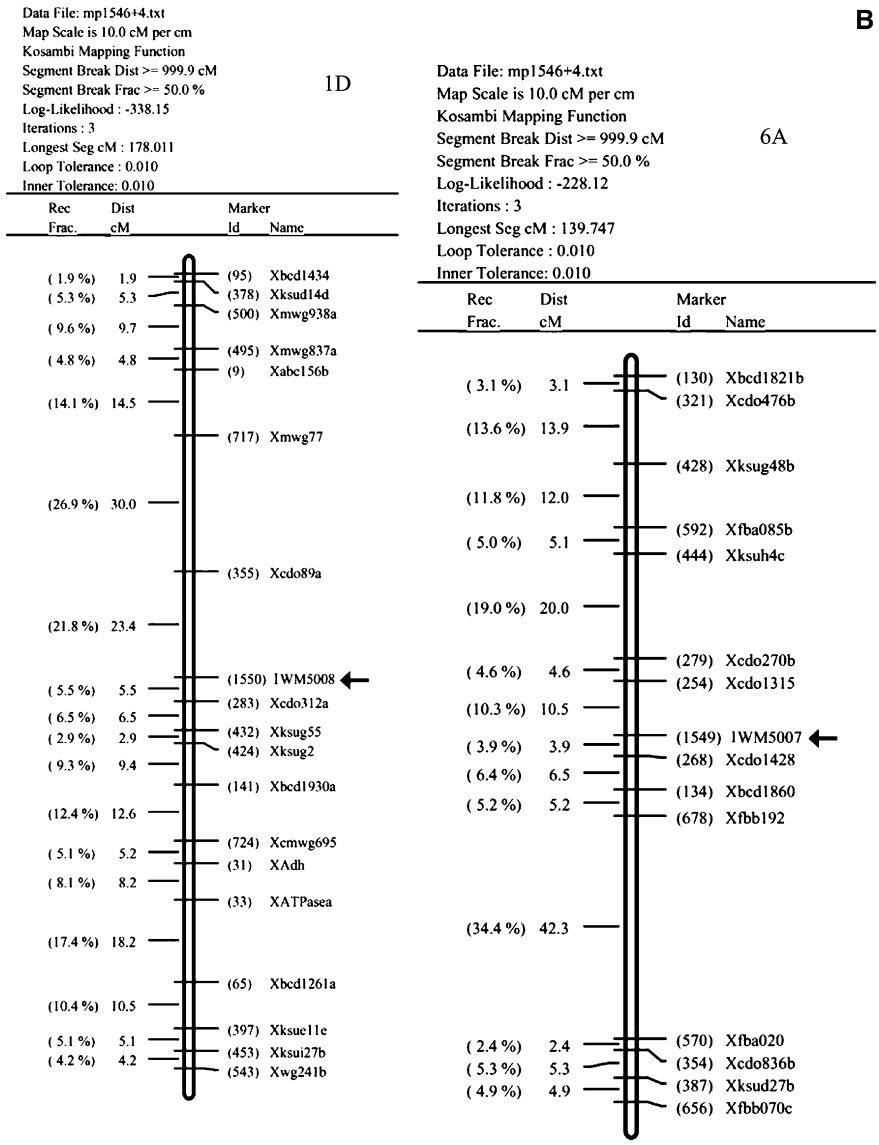


Fig. 1 (**a**, **b**) Linkage map of wheat. The W-eSSR loci mapped in this study are indicated by narrows. The scale to the left of the chromosome shows map distances in centiMorgans (cM)

Fig. 1 continued



developed by using one EST (derived marker IWM5004) were mapped in homologous positions on chromosome 2A and 2B (Röder et al. 1998).

Genomic regions having group 2 homoeoloci were suggested by Nelson et al. (1995) and Nachit et al. (2001) for *Triticum* adaptation (photoperiod and vernalization responses). This group is also important for disease resistance as numerous resistance genes are located on chromosomes 2A and 2B (rust, *Septoria nodorum* blotch and bunt). IWM5007 was mapped on chromosome 6A, which is the location for resistance genes to biotic stresses, including leaf, yellow and stem rust.

Interspecific transferability

A set of 48 B-eSSR markers was first screened on a set of eight wheat diverse genotypes to confirm the transferability of B-eSSR markers and to select polymorphic B-eSSR markers. These 48 B-eSSR markers are distributed throughout all the barley genome (Thiel et al. 2003; Varshney et al. 2006). After analyzing B-eSSR markers on wheat genotypes, 13 markers were selected which could be transferred to wheat, single peak (strong amplification) and polymorphic among accessions tested. B-eSSR markers showed 1 to 3 amplicons in the examined germplasm collection (Table 3).

These observations are in good accordance with the existence of up to three homoeologous loci within the A, B and D genomes of wheats. These results suggest the utility of B-eSSR markers for analyzing wheat genetic diversity. Indeed, transferability of B-eSSR markers to wheat species and vice versa has been demonstrated earlier by Holton et al. (2002). The high level of transferability of EST-SSR markers in related species has been demonstrated for eSSR markers derived from grape (Scott et al. 2000), rice (Cho et al. 2000), sugarcane (Cordeiro et al. 2001), barley (Thiel et al. 2003; Varshney et al. 2005b). The transferability of eSSR markers from one species to another provides the opportunity to compare maps between species and to follow gene introgression from wild species.

Polymorphic information content (PIC)

Generally, eSSR markers display a low level of polymorphism, but in the present study, they showed a high level of polymorphism in the examined germplasm: PIC values for the markers ranged from 0.590 to 0.936 with an average of 0.831. The B-eSSR markers showed a PIC value ranging from 0.590 (GBM1405, 3H) to 0.936 (GBM1031, 3H), average 0.825 (Table 3). The W-eSSR markers on the other hand had PIC values 0.773 (IWM0007) to 0.894 (IWM0010), average 0.842 (Table 4). Markers derived from wheat showed a higher polymorphism than markers derived from barley. That difference could be explained by the conserved nature of the genome from which these markers are derived.

Genetic relationships

Finally, a total of 159 and 263 bands (alleles) on 153 genotypes were obtained by eSSR markers from barley and wheat respectively. The EST-SSR bands were used to determine genetic distances between different genotypes. In our study, genetic distance matrices (for any two markers) did not show a high correlation using the Mantel test. Comparative studies on different marker systems (especially AFLP and SSR) for diversity and population structure in several plant species also shows that diversity estimates from different types of markers are often incongruent (Nybom 2004; Woodhead et al. 2004). To estimate genetic distance more accurately, combined analysis was carried out using all the EST-SSR bands together. A clustering phenogram was drawn using NTSYS software (Fig. 2), to show the relationships between different genotypes.

All the genotypes could be classified in three main groups (A, B and C). The first group consists mainly of ICARDA durum wheat breeding germplasm. However, the five sub-species were associated with ICARDA's breeding genotypes (Group A), suggesting that *T. turgidum* subsp. *dicoccoides* (AABB) is the ancestor of all the tetraploid wheat species. *T. turgidum* ssp. *dicoccoides* was domesticated to form *T. turgidum* ssp. *dicoccum* (Schrank) Thell. and successive domestication steps generated durum wheat (*T. turgidum* ssp. *durum*), the most cultivated tetraploid wheat (Salamini et al. 2002).

The closest relationships of *T. turgidum* ssp. *dicoccoides* (originating from southern Syria) with breeding genotypes supports the conclusions of

Table 4 Characteristics of W-eSSR loci derived from *T. aestivum* database, including their repeat motif, the total of alleles per locus (A_0), PIC value and number of alleles by wheat genotypes

Marker ID	SSR motif	PIC value	Chr	A_0	Number of alleles by accession
IWM0002	AG(49)	0.834166	na	24	2
IWM0004	AT(31)	0.891351	2A/2B	25	4
IWM0006	ACA(31)	0.769476	na	12	4
IWM0007	ATA(27)	0.773641	6A	23	4
IWM0008	TTC(29)	0.857612	1D	24	3
IWM0010	TATAGA(16)	0.894169	na	21	4
IWM0011	CA(43)	0.87571	na	27	3
Average		0.842304		22	3

Seven polymorphic loci of twelve W-eEST derived microsatellites are presented

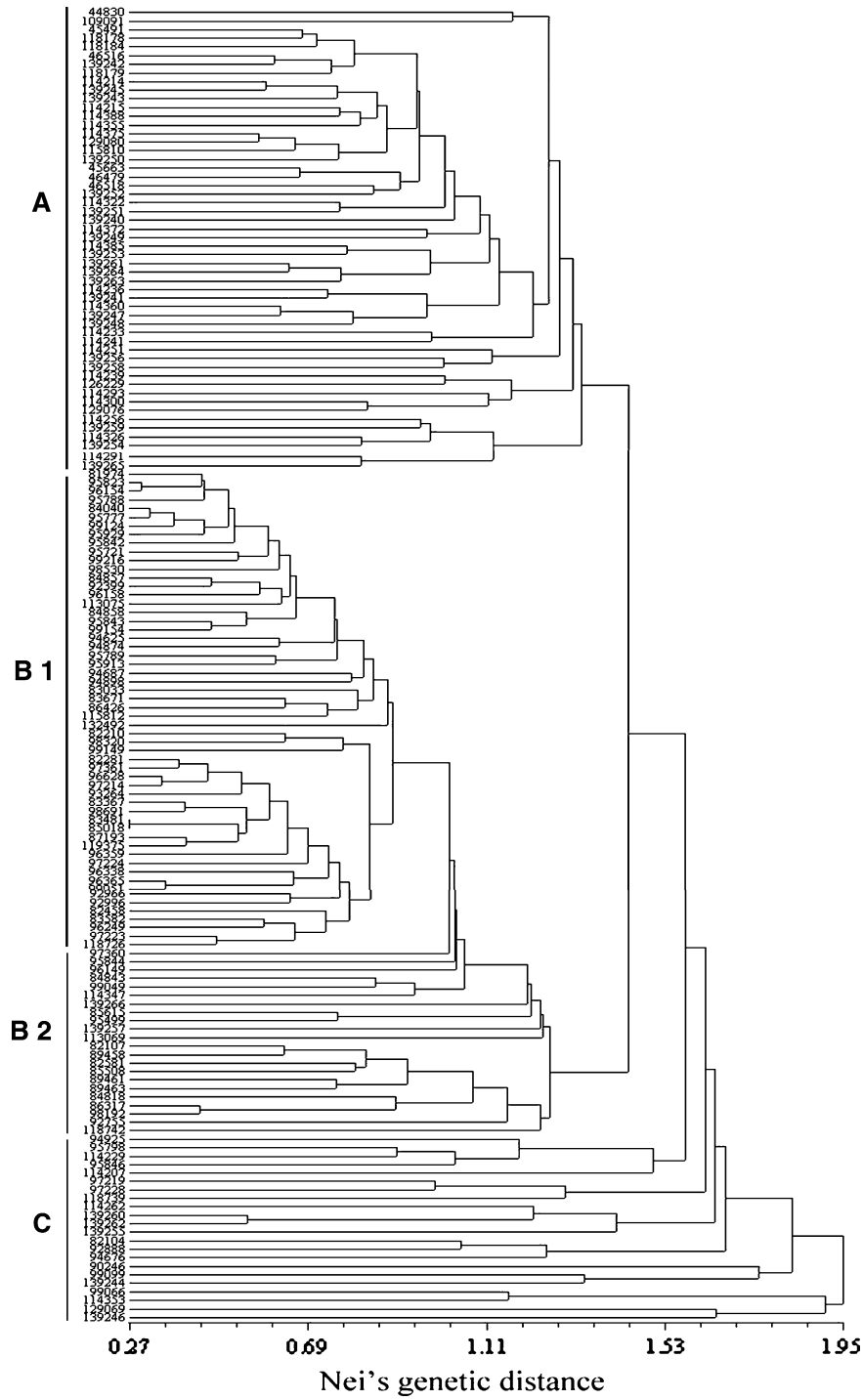


Fig. 2 Genetic relationship between 153 wheat accessions, landraces and breeding germplasm, based on Nei's coefficient and UPGMA cluster analysis using combined W-eSSR and B-eSSRs data

archeologists and molecular studies (Heun et al. 1997; Blumler 1998; Chabane and Valkoun 2001) that the origin of agriculture could be the southern and central Levant where three Pre-Pottery Neolithic A (PPNA) sites yielded cereal remains interpreted as domestic. More recently, Dubcovsky and Dvorak (2007) suggested that the region west of Dyarbakir in southeastern Turkey is the most likely site of their

domestication. It also suggested that from this area, the expansion of agriculture lead to the dissemination of domesticated einkorn (*T. monococcum* L. genomes A^mA^m) and domesticated emmer (*T. turgidum* subspecies, genomes BBAA) across Asia, Europe and Africa. Two sub clusters (B1 and B2) constitute the second group. The first sub cluster B1 is constituted by populations from Syria and North Africa countries (Tunisia, Morocco and Algeria) while B2 comprises durum wheat populations from Afghanistan. Our results are in accordance with those obtained by Autrique et al. (1996) using RFLP and morphological traits where differences have been observed between landraces and improved cultivars for different areas of origin and adaptation. Maccaferi et al. (2006) using SSR and AFLP markers, found similar results such as suggested by our study where different subgroups were identified. These results are indicating the presence of a complex pattern of familial relationships among the genotypes. There was no clear clustering in the last group, C, which comprised durum wheat landraces from Syria, Jordan, and Algeria, associated with a few ICARDA breeding durum wheat genotypes. The difficulty in separating accurately this group could be explained by the proportion of common bands between the species, which reflect the high conservation of the coding sequences between Triticeae species as suggested by Zhang et al. (2006). These observations could be explained as suggested by Dubcovsky and Dvorak (2007) by gene exchanges between the northern domesticated emmer and the southern wild emmer populations or emmer domesticated in the southern region resulting in the formation of a center of domesticated emmer diversity in southern Levant.

Classification of the examined germplasm, in more or less separated clusters, showed a clear geographical repartition (growth habitat) of the different populations of durum. Three pools were clearly identified as Syria (Middle East), North Africa and Central Asia. The classification of the examined germplasm in more or less separate clusters according to their growth habitat underscores the utility of molecular marker systems for fingerprinting and diversity analyses.

The utility of EST-gene derived microsatellites markers for fingerprinting and diversity analyses has been already demonstrated in different studies (Eujayl et al. 2001; Thiel et al. 2003; Leigh et al. 2003; Perry 2004; Chabane et al. 2005).

Conclusions

In the past, a variety of molecular markers such as RFLPs, RAPDs, SSRs and AFLPs have been used for estimating the genetic diversity in different types of wheat's (Leigh et al. 2003; Sasanuma et al. 2004). Zhang et al. (2005) with 73 EST-derived microsatellites developed from *T. aestivum* demonstrated their high level of transferability to closely related Triticeae species (*Triticum turgidum* ssp. *durum*) and wild relatives (*Aegilops speltoides* Tausch, *Ae. tauschii* Coss.). Then, Eujayl et al. (2001) developed 22 DuPw-SSRs showing a high level of discrimination in durum wheat and are still a source of information for assessing genetic relationships. Our modest contribution, by the small number of EST-SSRs tested, will diversify the source of markers to be used for functional diversity in durum wheat. Further EST-SSRs would be developed and tested nearly. Then recent developed SNP markers have also been used for detection of genetic diversity (Rafalski 2002; Vogel et al. 2006; Giancola et al. 2006). The analysis of genetic similarity, with e-SSRs may show homoplasy, in that different SSR alleles correspond to identical underlying sequence allele (Grimaldi et al. 1997; Hayden et al. 2004). This suggests that SNPs are more appropriate in the analysis of phylogenic relationship. On the other hand, e-SSRs may be more indicative of more recent relationship in the germplasm. The use of a particular molecular marker type for estimating the genetic diversity in a germplasm collection, however, depends on many factors including costs of genotyping the large population with a marker assay (Gupta et al. 2002).

In recent years, the SSR and SNP markers derived from ESTs, due to their low inexpensive developmental costs (Kota et al. 2001; Varshney et al. 2005a; Chabane et al. 2006) are being used for genotyping both natural and breeding populations.

Assessment of genetic diversity by using molecular markers is important not only for crop improvement efforts but also for efficient management and conservation of plant genetic resources in the genebanks (Graner et al. 2004). Based on earlier studies, either the SSR or e-SSR markers have been

recommended for diversity studies (Powell et al. 1996; Russell et al. 1997a; Sourdille et al. 2001; Gupta et al. 2002; Nybom 2005; Zhang et al. 2005; Chabane et al. 2005).

In our study, all the primers pairs designated for W-eSSR markers successfully amplified EST-SSR products, and produced strong and clear profiles in durum wheat. Up to 50% of the EST-SSRs identified more than one locus, suggesting an amplification of either the homoeologous or homologous copies. EST-SSR markers are more transferable across closely related genera than genomic SSRs because they originated from conserved transcribed regions that are better conserved between the genomes; this will facilitate their use in comparative mapping (Yu et al. 2004b). B-eSSR markers showed good level of transferability in durum wheat populations. Of 15 markers that showed strong amplification in wheat, 86% were polymorphic. These results are in contrast with those observed with genomic SSRs, which are more genome-specific and thus less transferable to related species (Sourdille et al. 2001).

The application the two types of e-SSRs to analyze the relationships between durum wheat landraces and ICARDA breeding germplasm resulted in the differentiation of different groups related to their geographical origin in Syria, North Africa and Central Asia. This result is in accordance with previous studies in barley (Chabane et al. 2005) where three groups (wild, landraces and elite barley) were identified using barley e-SSRs. Genetic diversity has also been assessed in a collection of elite exotic wheat genotypes (Gupta et al. 2003; Chabane et al. 2007), and the results suggest that e-SSRs can be successfully used for a variety of purposes and may be superior to genomic SSRs for diversity estimation. Recently, Zhang et al. (2006) demonstrated that bread wheat e-SRRs could be used to compare the species according their ploidy level (diploid species as well as tetra- and hexaploid species) for phylogenetic studies. Functional e-SSRs exhibiting sequence similarity to genes with a range of functions could be used directly in determining putative agronomical traits. For example EST-sequences reported by Holton et al. (2002), showed a strong homology to wheat storage protein. This kind of result is vital for plant breeding programs to have sufficient diversity available to enable them to develop new varieties with higher productivity and ability to withstand damage from biotic and abiotic factors. That potential will make them a valuable source of new SSR markers. Since they exist within genes, they may be "perfect" genetic markers and may be more transferable between species. Sourdille et al. (2001) suggested that a difference in transferability would also be depending on the mapping position of the locus.

Finally, we conclude that barley e-SSR markers show a relatively high transferability. This transferability makes them a powerful tool to work on wheat such as durum wheat. The wheat e-SSRs showed a relative high level of polymorphism and are therefore useful for assaying molecular genetic diversity. In addition, e-SSR markers are thus excellent molecular markers that can now be applied in marker-assisted-selection (MAS) in cereals and comparative mapping.

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