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ORIGINAL PAPER

Genetic and physical mapping of new EST-derived SSRs on the A and B genome chromosomes of wheat

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Abstract The availability of genetic maps and phenotypic data of segregating populations allows to localize and map agronomically important genes, and to identify closely associated molecular markers to be used in marker-assisted selection and positional cloning. The objective of the present work was to develop a durum wheat intervarietal genetic and physical map based on genomic microsatellite or genomic simple sequence repeats (gSSR) markers and expressed sequence tag (EST)-derived microsatellite (EST-SSR) markers. A set of 122 new EST-SSR loci amplified by 100 primer pairs was genetically mapped on the wheat A and B genome chromosomes. The whole map also comprises 149 gSSR markers amplified by 120 primer pairs used as anchor chromosome loci, two morphological markers (Black colour, *Bla1*, and spike glaucousness, *Ws*) and two seed storage protein loci (*Gli-A2* and *Gli-B2*). The majority of SSR markers tested (182) was chromosome-specific. Out of 275 loci 241 loci assembled in 25 linkage groups assigned to the chromosomes of the A and B genome and 34 remained unlinked. A higher percentage of markers (54.4%), localized on the B genome chromosomes, in comparison to 45.6% distributed on the A genome. The whole map covered 1,605 cM. The B genome accounted for 852.2 cM of genetic distance; the A genome basic map spanned 753.1 cM with a minimum length of 46.6 cM for chromosome 5A and a maximum of 156.2 cM for chromosome 3A and an average value of 114.5 cM. The primer sets that amplified two or more loci mapped to homoeologous as well as to non-homoeologous sites. Out of 241 genetically mapped loci 213 (88.4%) were physically mapped by using the nulli-tetrasomic, ditelosomic and a stock of 58 deletion lines dividing the A and B genome chromosomes in 94 bins. No discrepancies concerning marker order were observed but the cytogenetic maps revealed in some cases small genetic distance covered large physical regions. Putative function for mapped SSRs were assigned by searching against GenBank nonredundant database using TBLASTX algorithms.

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Introduction

The use of molecular markers in genome analysis, the systematic mapping of agricultural important traits, and marker-assisted selection (MAS) have been greatly advanced by the development of reliable PCR-based markers, such as the amplified fragment length polymorphisms and microsatellite or simple sequence repeats (SSR). Microsatellites represent a valid alternative marker system, because of their abundance in plant genomes and easily detection on polyacrylamide or high resolution agarose gel. The advantages of SSR markers include high information content, codominant inheritance, locus specificity and reproducibility. Recently a new source of SSRs has been identified in expressed sequence tags (ESTs). mRNA transcripts contain repeated motifs and the abundance of microsatellites in the expressed sequences of many species makes these markers very interesting due to a possible role in gene expression or function. The overall frequency of microsatellites among species was found inversely related to genome size and to the proportion of repetitive DNA but

remained constant in the transcribed portion of the genomes (Morgante et al. 2002).

The availability of new ESTs in cereals has provided a valuable resource of non-anonymous DNA-markers (EST-SSRs). The great potential of these markers is due to their physical association with coding regions of the genome, which can enhance the role of genetic markers in mapping agronomically important loci. Although EST-SSRs were shown to be less polymorphic markers than genomic SSR (gSSR) markers (Becker and Heun 1996; Eujayl et al. 2001), the use of markers from genic regions may be functionally more informative than SSRs from unexpressed chromosome regions. EST-SSR mapping in wheat is difficult due to a low level of polymorphism, as a result of their conserved nature, the genetic mapping (Gao et al. 2004; Nicot et al. 2004; Yu et al. 2004; Xue et al. 2008) and physical mapping of EST-SSRs (Yu et al. 2004; Qi et al. 2004; Goyal et al. 2005; Peng and Lapitan 2005; Mohan et al. 2007) have been recently reported.

Microsatellites have been developed and widely used for the construction of genetic linkage maps, MAS and for estimating the genetic diversity in major crops (Gupta et al. 1999). In common and durum wheats molecular markers have been developed and mapped mainly in segregating populations derived from cultivated and wild parental lines (Röder et al. 1998; Blanco et al. 1998; Gupta et al. 2002) because of higher level of polymorphism. However, the genome size of wheat is much larger than that of rice, maize and barley and genetic length corresponds to about 4,000 cM (Sourdille et al. 2003), therefore the identification of markers tightly linked to important agronomic traits could be carried out in intervarietal populations. Moreover, wheat genomic research is increasing the use of genetic maps, particularly in map-based gene cloning efforts. Map-based cloning requires an accurate and fine genetic map to correctly place a gene of interest between close flanking markers.

The aim of this work was to develop an intervarietal durum wheat genetic and physical map using gSSR markers as anchor chromosome loci and new EST-derived SSRs as functional markers.

Materials and methods

Plant materials

The mapping population is represented by a set of 120 recombinant inbred lines developed from a cross between the durum wheat commercial cvs. Ciccio and Svevo by advancing random individual F_2 plants to the F_7 generation by single seed descent. The two parents were chosen for differences for important qualitative and quantitative traits, like grain yield components, grain protein content, yellow pigment and adaptive traits.

Nulli-tetrasomic (NT) lines of Chinese Spring (Sears 1954, 1966) and the set of durum wheat cv. Langdon D genome disomic-substitutions lines (Joppa and Williams 1988) were used to assign molecular markers to each chromosome. Chinese Spring ditelosomic lines (Sears and Sears 1978) were used for chromosomal arm assignment of markers. Physical location on chromosome bins of each genomic and EST-SSR marker was obtained using a set of 58 common wheat deletions lines dividing the A and B genome chromosomes in 94 bins (kindly provided by B.S. Gill, USDA-ARS, Kansas State University) (Endo and Gill 1996). The lines were chosen according to their ease in multiplying and to the presence of heterozygous and interstitial deletions (Qi et al. 2003). The length of each deletion bin was measured as a percentage of the chromosome arm missing. Each bin is defined as the name of the proximal line and the percentage of arm present in the next line (Qi et al. 2003).

Marker analysis

A total of 500 gSSR primer pairs (GWM, WMC, BARC, CFD, CFA) were analysed between the two parents in order to find two to four polymorphic markers for each chromosome arm to be used as anchor loci (one marker every 20–30 cM). The development of gSSR markers, primer sequences, chromosome location and annealing temperature were reported by Röder et al. (1998) for GWM, Gupta et al. (2002) for WMC, Song et al. (2005) for BARC, Guyomarc'h et al. (2002) and http://wheat.pw.usda.gov for CFA and CFD.

A set of 1,189 new EST-SSR primer pairs developed by La Rota et al. (2005) and available in the public database http://wheat.pw.usda.gov was synthesized by Sigma-Genosys. Primer pairs were chosen in order to represent each microsatellite class and to produce PCR products ranging from 100 to 250 bp in length. A total of 380 dinucleotide, 657 trinucleotide, 64 tetranucleotide and 88 pentanucleotide (EST-SSR markers) were examined. Primer sequences, annealing temperature and expected PCR product size are reported in the web site http://wheat.pw.usda.gov. In the present work EST-SSR markers are indicated by GenBank accession number.

Polymorphisms between parental lines were examined using conventional PCR. DNA amplifications were carried out in 25-µl reaction mixtures, each containing 100-ng template DNA, 2 µM of SSR primer, 200 µM of dNTP, 2.5 mM $MgCl_2$, 1× PCR buffer (10 mM Tris–HCl, pH 8.3, 10 mM KCl) and 1 U of *Taq* DNA-polymerase. The following PCR profile in a Perkin Elmer DNA Thermal Cycler was used: initial denaturation at 95°C for 3 min, followed

by 35 cycles of 95°C for 1 min, 50°C/65°C for 1 min, 72°C for 2 min with a final extension at 72°C for 10 min. The amplification products were resolved on 6% polyacrylamide gels and stained with ethidium bromide.

The majority of polymorphic SSRs was assayed in the RIL population by capillary electrophoresis.

DNA amplifications were carried out in 12.5 µl reaction mixtures, each containing 25 ng template DNA, 2 µM of each microsatellite primer, 200 µM of each dNTP, 2.5 mM $MgCl_2$, 1× PCR buffer (10 mM Tris–HCl, pH 8.3, 10 mM KCl) and 0.5 U of *Taq* DNA-polymerase. Forward primers were fluorescently labelled with 6-FAM, 8-HEX or NED. The following PCR profile in a Perkin Elmer DNA Thermal Cycler was used: initial denaturation at 95°C for 2 min, followed by 30 cycles of 95°C for 1 min, 50°C/65°C for 1 min, 72°C for 1 min with a final extension at 72°C for 5 min.

PCR product were detected by capillary electrophoresis using an ABI PRISM 3100 Avant Genetic Analyzer and analysed using GeneMapper v 3.5 genotyping software. The internal molecular weight standard was 500-ROX.

Morphological and biochemical markers

In addition to SSR markers the two parents differed for two morphological markers (black awn, *Bla1*, and spike glaucousness, *Ws*), and two seed storage protein markers (*Gli-A2*, *Gli-B2*). Gliadin components were analysed by SDS-PAGE of endosperm extracts. Total proteins were extracted from single grains with 10 µl/mg of 63 mM Tris–HCl buffer, pH 6.8, containing 2% (v/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol and 0.002% (v/v) bromophenol blue and separated by SDS-PAGE. Approximately equal amounts of protein, as estimated by visual examination of preliminary gels of the same extracts, were loaded in each lane.

Segregation analysis and map construction

The observed segregation ratio for each marker was tested by Chi-square analysis for deviation from the expected 1:1 ratio. The linkage analysis was performed by JoinMap v. 3.0 (Van Ooijen and Voorrips 2001) and the Kosambi mapping function was used to calculate map distances (Kosambi 1944). Linkage groups were established using a minimum LOD score of 4.0 after preliminary analysis using LOD score ranging from 2 to 10. Previously mapped gSSR markers (Röder et al. 1998; Blanco et al. 2004; Somers et al. 2004; Sourdille et al. 2004) were used as anchor loci and for assigning linkage groups to a particular chromosome. When necessary markers were removed and the order recalculated until a stable and consistent order with the physical position of the markers on chromosome bins was reached. The "fixed order" of gSSR selected loci from previously publications was used only for the 5B chromosome map. Final mapping was done by combining two or more linkage groups that belong to the same chromosome if the distance was inferior to 40 cM.

Results

Marker evaluation and polymorphism

A total of 1,189 primer pairs were selected among a collection of 5,424 wheat EST-derived SSR markers (available at the web site http://wheat.pw.usda.gov) and tested on genomic DNA of the two parents of the mapping population Svevo × Ciccio in order to assess amplification products and polymorphism. Only 960 (80.7%) of the primer pairs led to at least one PCR product, the other 229 (19.3%) primer pairs produced no PCR product. More than half (500 markers) of the 960 amplifying primer pairs gave one or two PCR products (50.0% dinucleotide and 38.7% trinucleotide EST-SSRs, respectively). The other 460 primer pairs amplified multiple PCR discrete products including bands of expected and unexpected size. Multiple discrete PCR products were observed in both dinucleotide and trinucleotide EST-SSRs (20.2 and 30.5%, respectively). Ten percent of markers (96 markers) amplified bands of higher size than expected, suggesting the presence of introns in the corresponding genomic DNA. Less than 2.0% of primers (18 markers) gave smaller products than expected. The results of all 2,378 amplifications (1,189 EST-SSRs × 2 wheat genotypes) were carefully examined. Out of 1,189 markers, 100 (8.4%) were found polymorphic between the parental lines, of which 39 were dinucleotide, 52 trinucleotide, 5 tetranucleotide and 4 pentanucleotide SSRs. All the 100 polymorphic primer pairs demonstrated reproducible amplification and were used for genetic and physical mapping.

Out of 500 gSSR markers analysed between Svevo and Ciccio 120 were found to be polymorphic markers (24%) with an average of eight to nine markers for each chromosome (Table 1).

Genetic mapping

A set of 122 new EST-SSR loci amplified by 100 primer pairs and 149 gSSR loci amplified by 120 primer pairs were genetically mapped on the wheat A and B genome chromosomes. Moreover two morphological markers (black awn, *Bla1*, and spike glaucousness, *Ws*), and two seed storage protein loci (*Gli-A2*, *Gli-B2*) were included since these loci segregate as Mendelian loci. EST-SSR and gSSR markers were assembled into linkage groups ordered at a minimum LOD score of 4, with a minimum of two loci and a maxi-

Table 1 Total markers screened and polymorphisms detected among the parental, lines Svevo and Ciccio, of the durum wheat mapping population

Marker	Markers screened (*n*)	Polymorphic markers (*n*)	Polymorphism (%)
Genomic SSR	500	120	24.0
EST-SSR	1,189	100	8.4
Morphological markers (*Bla1*, *Ws*)	2	2	–
Biochemical markers	6	2	–
Total	1,697	224	13.2

mum of 22 loci per group. Out of 275 loci, 241 assembled in 25 groups were assigned to chromosomes of the A and B genomes and 34 loci remained unlinked (Fig. 1). The centromeres were positioned using the Chinese Spring ditelosomic lines and data reported in wheat high-density microsatellite consensus map by Somers et al. (2004).

The 241 mapped loci were distributed randomly over all 14 chromosomes ranging from 6 on 5A to 26 on 1B and 7B, with an average of 17.2 markers per chromosome (Table 2). An higher percentage of markers, 54.4%, localized on the B genome chromosomes, in comparison to 45.6% distributed on the A genome. The whole map covered 1,605 cM. The B genome accounted for 852.2 cM of genetic distance; the A genome basic map spanned 753.1 cM with a minimum length of 46.6 cM for chromosome 5A and a maximum of 156.2 cM for chromosome 3A, and an average value of 114.5 cM. The percentage of markers showing significant distortion from the expected 1:1 ratio was 13.7% (12.7% among gSSR and 15.5% among EST-SSR markers). Distorted markers were randomly distributed along chromosomes and five were unlinked. The majority of the microsatellite set tested (182 out of 220) constitutes genome-specific markers. The primer pairs that amplified two or more loci (20 gSSRs and 18 EST-SSRs) mapped to homoeologous as well as to non-homoeologous sites. The highest number of loci was produced by BJ262177 (AC motif) with five loci mapping on non-homoeologous groups of the A genome (4A, 5A, 7A) and by CA594434 (AG motif) with four alleles mapping on homoeologous groups. In 12 cases, SSRs mapped on the two homoeologous chromosomes with a minimum of one marker (*Xwmc382*) mapped on homoeologous group 2 and a maximum of three mapped on homoeologous group 1 (TC69046, TC85294, TC87195), group 3 (TC89014, *Xwmc527*, *Xgwm108*) and group 6 (TC85303, TC84481, *Xgwm132*). A co-linearity between multi-allelic markers located on homoeologous chromosomes was observed for the groups 1, 3 and 7, where markers maintained the same genetic order, while absence of co-linearity was observed for *Xgwm132* localized on 6A and 6B chromosomes.

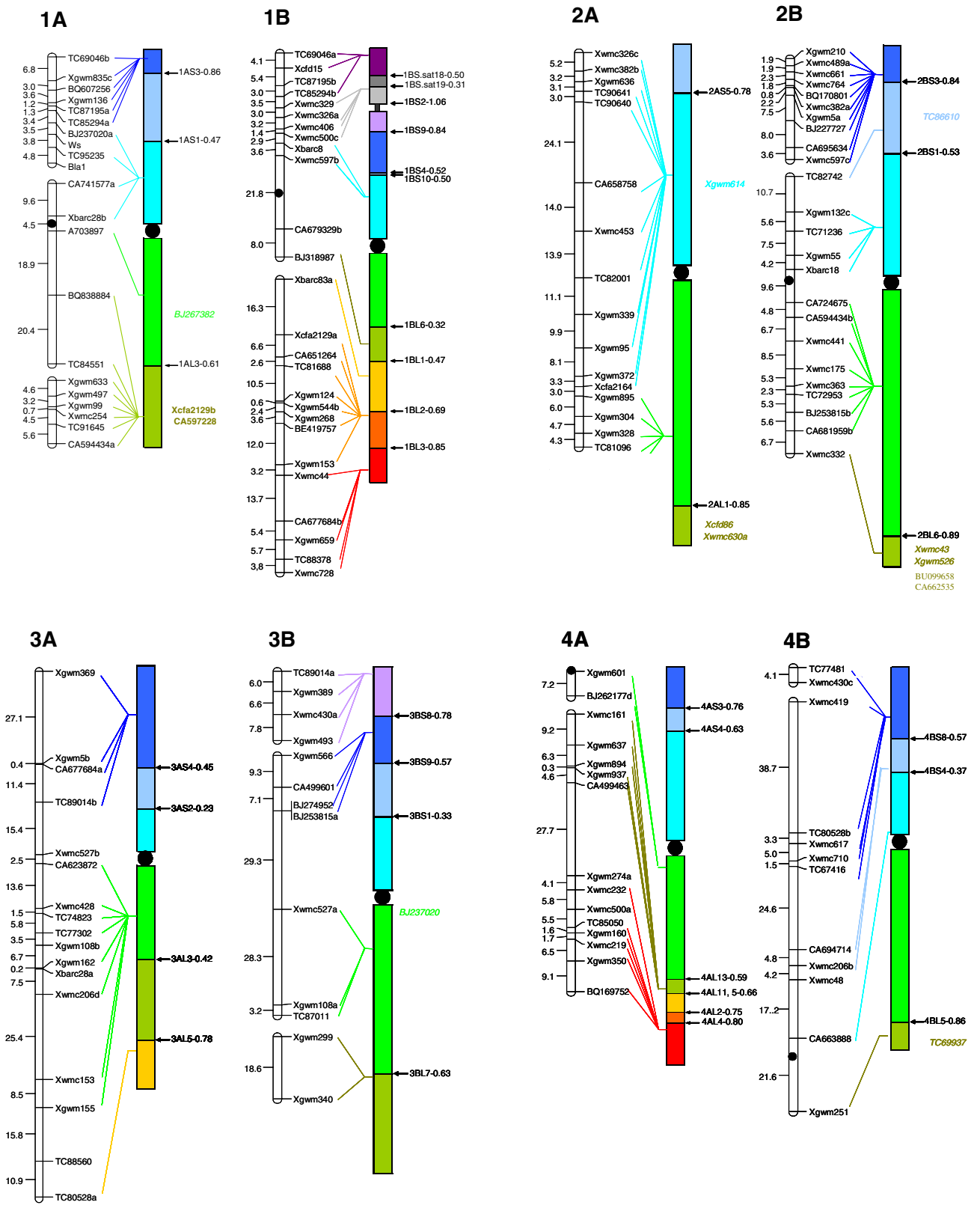
Fig. 1 Genetic and physical maps of the A and B genome of durum wheat developed using a RIL population derived by crossing the cultivars Ciccio and Svevo. The genomic loci are indicated with *Xgwm*, *Xwmc*, *Xbarc*, *Xcfa* and *Xcfd*. The EST-SSR loci are named with the international assigned accession number. *Numbers* on the *left side* of each map show the distances in centimorgans between markers. Microsatellites amplifying two or more loci mapped on the same or different chromosomes have the suffix "a", "b", etc. For each chromosome the partitioning into bins based on deletion breakpoints is shown on the *right side*. *Black arrows* indicate the name of deletion lines and the fraction length value of breakpoints according to Endo and Gill (1996). *The coloured lines* show the genetic/physical relationships for each marker. Molecular markers mapped physically and not genetically are reported on the *right* of each deletion map with the same colour of the bin. The centromeres, indicated with a *black circle*, were positioned using Chinese Spring ditelosomic lines and data reported in the high-density microsatellite consensus map by Somers et al. (2004) ▶

Physical mapping

Genomic SSR and EST-SSR markers were physically mapped using the NT and ditelosomic lines of Chinese Spring, and a stock of 58 deletion lines dividing the A and B genome chromosomes in 94 bins. Out of 241 mapped loci, 213 (88.4%) were assigned to the bins. Up to 57% of the bins (54 of the 94) where characterized by at least one microsatellite marker (range from 1 to 13 SSRs, mean 4.2 SSR\bin). The bins with best coverage were located on chromosome 2B (2BS3-84-100) and 7B (7BL10-78-100). Moreover, we assigned a physical location to 15 of 34 SSRs lacking a genetic map position (Fig. 1). For 25 loci genetically mapped it was not possible to assign a physical location because the alleles mapped in the segregant population were lacking in the Chinese Spring aneuploid lines.

The microsatellite loci were found to be relatively evenly distributed along the chromosome length. We detected 48.2% of the total SSR loci in the distal bins covering 20% of the physical length of the genome, and 31.1% of the loci in the proximal bins covering 41% of the physical length. The distribution of EST-SSR markers along the bins showed that 51 out of 99 (51.5%) physically mapped were located into distal regions as compared to 32 (32.3%) localized into peri-centromeric regions. The remaining EST-SSRs (16.2%) were distributed randomly along the chromosomes. Recombination hot spots were observed close to the telomeres, especially on chromosome arms 1AS (1AS3-0.86-1.00), 1AL (1AL3-0.61-1.00), 1BS (1BS.sat18-0.50-1.00), 2BS (2BS3-0.84-1.00), 4AL (4AL4-0.80-1.00), 6AS (6AS1-0.35-1.00), 6BS (6BS5-0.76-1.00), 7BL (7BL10-0.78-1.00).

A co-linearity of multi-allelic markers located on homoeologous groups was confirmed with the physical maps for the groups 1 and 3. No discrepancies were observed in the order of markers between genetic and physically maps, but in some cases markers mapping closely genetically were physically mapped in distant bins. For instance, TC91851



and *Xwmc705* distant only 7.2 cM on chromosome 5A were physically mapped on the bins 5AS3-0.75-0.98 and 5AL12-0.35-0.78, respectively.

A comparison between physical location of gSSR anchor loci was made using as references two recently published maps (Goyal et al. 2005; Sourdille et al. 2004).

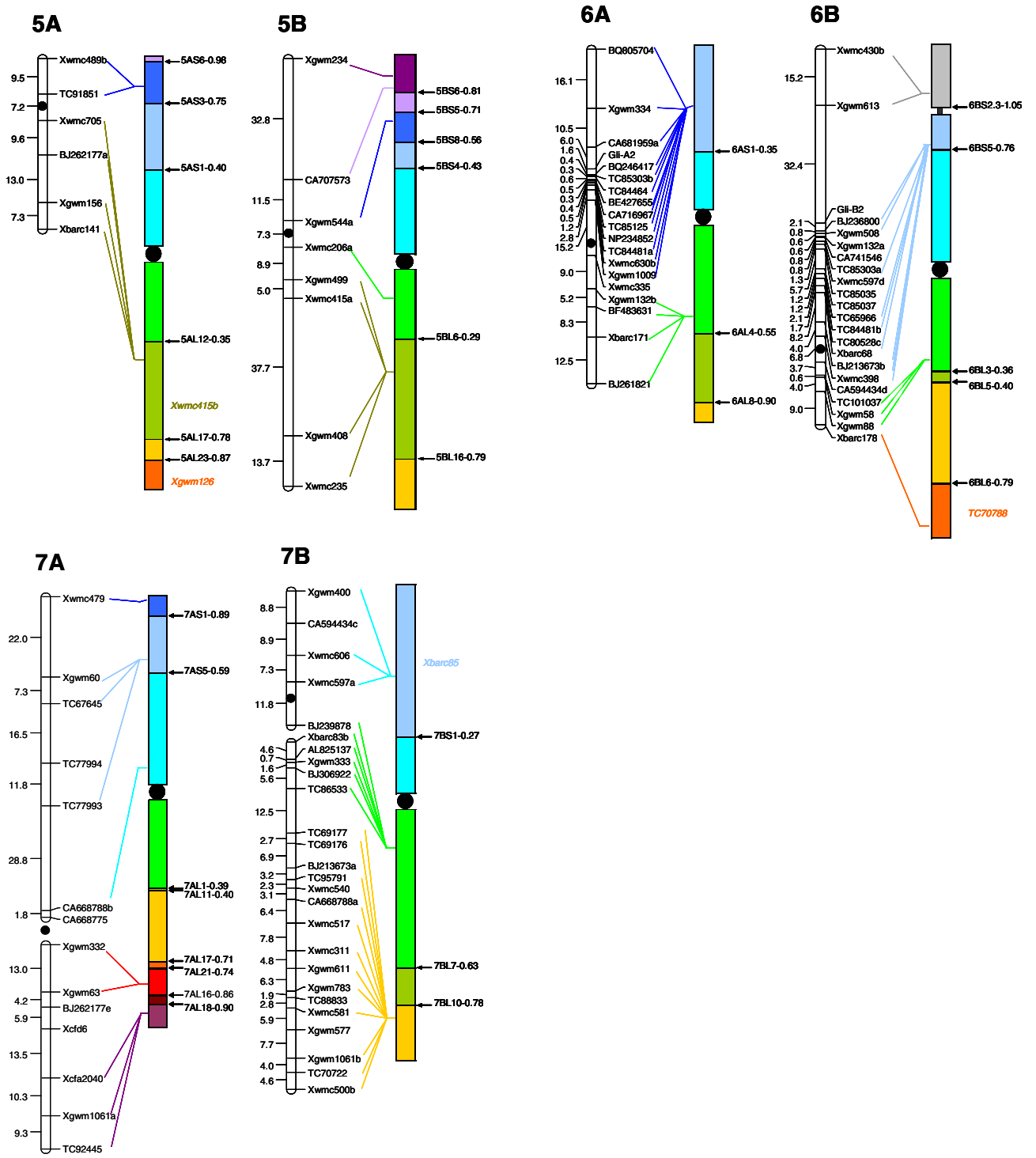


Fig. 1 continued

A different physical bin location was observed for 19 gSSR (Fig. 1), and three gSSR loci (*Xbarc83a-1B*, *Xwmc382b-2A*, *Xwmc415a-5B*) mapped in homoeologous positions with respect to that reported by authors above mentioned.

Putative functions of EST-SSRs

Expressed sequence tag-derived SSR markers were searched against GenBank nonredundant database using TBLASTX algorithms (http://ncbi.nlm.nih.gov/BLAST).

Table 2 Distribution of gSSR and EST-SSR markers across the 14 durum wheat chromosomes in the genetic linkage map Ciccio × Svevo

Chromosome	Genomic SSR markers (*n*)	EST-derived SSR markers (*n*)	Morphological markers (*n*)	Total markers (*n*)
1A	7	12	2	21
1B	16	10	–	26
2A	11	7	–	18
2B	14	10	–	24
3A	10	7	–	17
3B	8	5	–	13
4A	11	4	–	15
4B	7	5	–	12
5A	4	2	–	6
5B	7	1	–	8
6A	6	12	1	19
6B	10	11	1	22
7A	7	7	–	14
7B	14	12	–	26
A genome	56	51	3	110
B genome	76	54	1	131
Total	132	105	4	241

Table 3 lists loci with sequence homology and the respective *E*-value. The data indicated that 43% of the 122 loci had an *E*-value $>10^{-7}$ and corresponded to known function genes. Sequences similarity searches revealed storage proteins, regulatory factors as well as structural genes and genes involved in such diverse processes as DNA synthesis, cell cycle regulation, carbon metabolisms, fatty acid metabolisms, membrane transport and signal transductional. Mostly of seed storage protein (22) were mapped on chromosomes 6A and 6B. Genetically, the α-gliadin genes are located at the *Gli-2* loci on the group 6 homoeologous chromosomes. Three low molecular weight glutenin subunits were localized on chromosome 1A (TC95235, CA741577, TC85294) and two on chromosome 1B (TC87195, TC85294). The EST-SSR TC85294 had two loci mapped in homoeologous position on chromosome arms 1AS and 1BS.

Discussion

Expressed sequence tags are currently the most widely sequenced nucleotide commodity from plant genomes in terms of number of sequences and total nucleotide count. A great emphasis has been recently given to discovering and characterizing protein-coding genes in different species with large genomes. *Arabidopsis thaliana* and *Oryza sativa*, the model plant species with fully sequenced genomes, have the smallest known genomes (125 and 430 Mb, respectively), while hexaploid wheat has genome size of 17 Gb (Rudd 2003). The larger size of wheat genome is due to polyploidy and the presence of regions with repeat motifs that makes difficult the complete genome sequencing. ESTs available in public databases offer the opportunity to identify SSRs and to develop molecular markers useful for the production of functional maps. While gSSRs are anonymous markers, EST-SSRs are physically associated to expressed regions of the genome thus allowing the direct mapping of agronomically important loci (Nicot et al. 2004).

The construction of a genetic and physical map of the A and B genome chromosomes of durum wheat is described in this report. The primary goal was to map new EST-SSR markers and to provide a molecular and functional map suitable for identifying associations between molecular markers, major genes and quantitative traits loci. We developed a durum wheat genetic map including 122 EST-SSR loci never mapped before; besides we physically mapped both gSSR and EST-SSR markers by a set of common wheat deletion lines. The establishment of genetic/physical relationships will open the possibility of molecular cloning of valuable genes that are difficult to study with classical molecular analysis (Sourdille et al. 2004). EST-SSRs are also more transferable across grass species including rice, barley, maize and wheats (Yu et al. 2004) while gSSRs are frequently not transferable to closely related species and thus not suitable in comparative genomics (Sourdille et al. 2001). Out of 1,189 new EST-SSR primer pairs examined, 19.3% did not amplify PCR products, this was lower when compared to the rate (30%) obtained by Nicot et al. (2004) and Holton et al. (2002). A similar value (15%) was observed with gSSRs by Guyomarc'h et al. (2002) and by

Table 3 Chromosomal location and putative function of EST-derived SSR loci assigned by alignment with their GenBank homologue sequences

EST accession no.	Chromosome arm	Repeat motif	*E*-value	Function	Organism
TC69046	1AS, 1BS	AGG	2e-10	Protein csAtPR5, TacsAtPR5	*Triticum aestivum*
BQ607256	1AS	AAC	2e-70	LMW glutenin	*Thinopyrum ponticum* × *Triticum aestivum*
TC87195	1AS, 1BS	AAC	0	LMW glutenin	*T. aestivum*
TC85294	1AS, 1BS	AAC	5e-94	LMW glutenin	*T. aestivum*
BJ237020	1AS	AAC	8e-93	LMW glutenin	*T. monococcum*
TC95235	1AS	AAG	1e-06	Low-affinity cation transporter	*T. aestivum*
CA741577	1AS	AAT	6e-16	AMP-binding protein (ABP-1)	*T. aestivum*
CA703897	1AL	ACG	4e-09	MAR binding protein 1 (AHM1)	*T. aestivum*
TC84551	1AL	AAC	8e-74	HMW glutenin	*T. aestivum*
TC91645	1AL	AC	2e-53	Acyl-coenzyme A synthetases/ AMP-fatty acid ligases (ABG-1)	*T. aestivum*
CA594434	1AL, 2BL, 6B, 7B	AG	4e-04	Golden 2-like protein	*T. aestivum*
BJ267382	1AL	AG	1e-09	Putative cyclin	*T. aestivum*
CA597228	1AL	ATC	2e-53	TaDof1 zinc finger protein	*T. aestivum*
CA679329	1BL	AAC	8e-05	HMW glutenin	*T. aestivum*
CA651264	1BL	ACC	2e-05	Quinone reductase 2	*T. monococcum*
TC88378	1BL	AGC	2e-10	Pseudo-response regulator	*T. aestivum*
TC82001	2AS	AG	3e-72	CTD-phosphatase-like protein (HvCpl1)	*Hordeum vulgare*
BJ227727	2BS	AC	0.42	Barley B recombinant transcriptional regulator	*Hordeum vulgare*
TC82742	2BS	AG	1e-22	Plasma membrane protein 3	*Leymus chinensis*
TC86610	2BS	AAG	0.002	Histone H2B	*T. aestivum*
CA724675	2BL	ACG	1e-07	Cold acclimation induced protein 2-1	*T. aestivum*
BJ253815	2BL, 3BS	ACGAT	5e-46	Metallothionein	*T. aestivum*
TC89014	3AS, 3BS	AT	4e-153	Expansin EXPB11 protein precursor	*T. aestivum*
TC74823	3AL	AAG	5e-08	Ocs-element binding factor 1	*T. aestivum*
TC80528	3AL, 4BS, 6BS	AC	2e-06	Cold acclimation induced protein 2-1, (TaEsi2-1)	*T. aestivum*
BJ274952	3BS	ACGAT	5e-46	Metallothionein	*T. aestivum*
TC87011	3BL	ACC	2e-06	Cold shock domain protein 2	*T. aestivum*
TC85050	4AL	AAC	9e-168	Putative avenin-like b precursor	*T. aestivum*
TC69937	4BL	ACG	2e-08	Ocs-element binding factor 1	*T. aestivum*
TC91851	5AS	ACT	1e-04	Ocs-element binding factor 1	*T. aestivum*
BQ805704	6AS	AG	2e-146	α-Gliadin	*T. aestivum*
BQ246417	6AS	AAC	3e-41	α-Gliadin	*T. aestivum*
TC85303	6AS, 6BS	AAC	5e-171	α-Gliadin	*T. aestivum*
TC84464	6AS	AAC	4e-168	α-Gliadin	*T. aestivum*
BE427655	6AS	AAC	6e-81	α-Gliadin	*T. aestivum*
CA716967	6AS	AAC	6e-36	α-Gliadin	*T. turgidum*
TC85125	6AS	AAC	1e-176	α-Gliadin	*T. aestivum*
NP234852	6AS		5e-171	α-Gliadin	*T. aestivum*
TC84481	6AS, 6BS	AAC	1e-110	α-Gliadin	*T. turgidum*
BJ236800	6BS	AAC	1e-51	α-Gliadin	*T. aestivum*
TC85035	6BS	AAC	0	α-Gliadin	*T. aestivum*
TC85037	6BS	AAC	0	α-Gliadin	*T. aestivum*
TC65966	6BS	AAC	5e-125	α-Gliadin	*T. aestivum*

Table 3 continued

EST accession no.	Chromosome arm	Repeat motif	*E*-value	Function	Organism
BJ213673	6BS, 7B	AG	1e-41	CTD-phosphatase-like protein	*Hordeum vulgare* subsp. *vulgare*
TC70788	6BL	AGC	3e-04	GATA-type zinc finger protein	*T. aestivum*
TC67645	7AS	ATC	3e-08	Histone deacetylase HDAC2	*T. aestivum*
TC92445	7AL	AC	8e-17	Thioredoxin	*T. aestivum*
BJ239878	7BL	AAT	3e-30	Putative lipase	*Hordeum vulgare*
BJ306922	7BL	AGC	9e-05	Dof zinc finger protein 1	*Hordeum vulgare*
TC86533	7BL	AC	5e-95	ABA induced plasma membrane protein	*T. aestivum*
TC85687	–	AGATC	0	Na^+/H^+ antiporter	*T. aestivum*
TC91009	–	AGG	0.003	RING-H2 finger protein	*T. aestivum*

Gadaleta et al. (2007) with EST-SSRs. The lack of amplification could be due to the presence of introns in the genomic DNA. Microsatellites are commonly considered codominant markers and the polymorphism detected among genotypes is the result of variation in the number of tandem repeat units. The polymorphism detected in the present work was only in part due to differences of tandem repeat unit number, as several primers (24%) showed allelic differences as presence/absence of bands due to base deletion and substitution events in the primer sites sequences. The variability within a flanking sequences is sufficient to distinguish even between two duplicate loci in the same genome and this could be particularly useful in wheats for differentiating loci located on homoeologous chromosomes. EST-SSRs generally generated high-quality amplification products as also reported by Eujayl et al. (2002), Nicot et al. (2004) and Gadaleta et al. (2007), suggesting that ESTs are more suited for specific primer pair design than genomic sequences. The polymorphisms observed among the two parents was lower for EST-SSR (8.4%) than gSSR (24%). A relatively low level of polymorphism was reported by using EST-SSR markers in different species including durum wheat (Weising et al. 1996; Smulders et al. 1997; Bandopadhyay et al. 2004; Eujayl et al. 2002). gSSRs are known to evolve faster than coding sequences depending on the type of motif (Ellegren et al. 1995), the structure of the alleles (Brinkmann et al. 1998) or the number of repeats (Wierdl et al. 1997). Conversely coding regions are less prone to mutations as they may frequently lead to a loss of function (Sourdille et al. 2004). The low level of polymorphism revealed among the two parental lines (cvs. Ciccio and Svevo) of our mapping population could be also explained by the genetic similarity of these two elite cultivars.

Out of 275 loci, 241 were genetically mapped on the A and B genome chromosomes while 34 (12.3%) remained unlinked, probably to insufficient coverage of the genome; in fact in some cases we were not able to have a unique linkage group representing one chromosome and no marker were mapped on several bins. Microsatellites tended to be genome-specific markers as the majority of the examined SSRs (182) mapped on individual chromosomes and only 12 SSRs detected multiple loci on homoeologous and non-homoeologous chromosomes. The A genome had a lower number of mapped loci (Table 2), and that confirmed earlier studies reporting more SSR loci on the B genome (Mohan et al. 2007).

We exploited wheat aneuploids to physically map SSR markers to chromosome bins as establishing relationships between genetic and physical maps is a prerequisite to positional cloning of agronomically important genes (Sourdille et al. 2004). We found a good correspondence between physical and genetic map order. However, markers genetically close were often physically mapped in distant bins suggesting that genetic mapping only gives an indication of the relative position of the markers with each other, as already observed by Sourdille et al. (2004). That is probably due to lack of recombination in particular regions or to low polymorphism, such as the regions around the centromeres. Recombination hot spots and gene-rich regions were frequently observed close to the telomeres (for review see Schnable et al. 1998). In the present work 50.5% of EST-SSRs were mapped in the telomeric regions and 32.3% in pericentromeric regions. Sourdille et al. (2004) also reported only few discrepancies concerning marker order on the chromosomes, but their cytogenetic maps revealed small genetic distances covering large physical regions especially around the centromeres and large genetic to physical map ratios close to the telomeres. The comparison of the gSSRs physical position found by Sourdille et al. (2004) and Goyal et al. (2005) revealed a different bin location for 15.8% of the tested markers. This could be explained with an error in the markers assignment to chromosomes, to rearrangements occurred in same bin deletion lines or to the fact that physically mapped bands were different between the parental lines of the mapping populations.

This study can be a starting point for the construction of a durum wheat functional map using EST-SSRs. Most of the mapped EST-SSR markers (43%) represented genes with a stringent sequence similarity threshold. Two gene islands were found on homoeologous chromosomes 6A and 6B with 18 of 22 loci (81.8%) corresponding to known function sequences. Interestingly 13 ESTs were genes coding for storage proteins (gliadins) and all were characterized by the same repeat motif (AAC). Genetically, the α-gliadin genes are located at the *Gli-2* loci on the group 6 homoeologous chromosomes. Like other wheat prolamins, the α-gliadin proteins are encoded by members of a multigene family. Six EST-SSRs matching gliadin proteins were also mapped on the short arm of group 1 chromosomes which are known to occur gliadin genes on the corresponding position (Liu and Shepherd 1995). A Dof protein was mapped on 1AL chromosome arm. Dof proteins are a large family of transcription factors recently discovered and present only in plants and have effects on seed germination in *Arabidopsis* (Papi et al. 2000, 2002). *Dof* gene "DAG1" was recently described and mapped on wheat chromosome 1BS by Gao et al. (2004), while we found an EST-SSR with an homology sequence with *Dof* gene on chromosome 1AL. The map position of this gene family may help further research to determine their functions in wheat.

The availability of functional genetic linkage maps combined with EST homology information can offer attractive approach to comparative genomics and to marker-assisted breeding by direct gene selection for target traits. Moreover, the establishment of genetic/physical relationships will open the possibility to molecular cloning of valuable genes.

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