



Physical Mapping of Wheat and Rye Expressed Sequence Tag–Simple Sequence Repeats on Wheat Chromosomes

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Abstract

Six hundred and seventy two loci belonging to 275 expressed sequence tag–simple sequence repeats [EST–SSRs, including 93 wheat (*Triticum aestivum* L.) and 182 rye (*Secale cereale* L.) EST–SSRs] were physically mapped on 21 wheat chromosomes. The mapping involved two approaches, the wet-lab approach involving use of deletion stocks and the in silico approach involving matching with ESTs that were previously mapped. The number of loci per EST–SSR mapped using the in silico approach was almost double the number of loci mapped using the wet-lab approach (using deletion stocks). The distribution of loci on the three subgenomes, on the seven homoeologous groups and on the 21 individual chromosomes was nonrandom ($P < 0.01$). Long arms had disproportionately (relative to the difference in DNA content) higher number of loci, with more loci mapped to the distal regions of chromosome arms. A fairly high proportion of EST–SSRs had multiple loci, which were largely (81%) homoeoloci. Rye EST–SSRs showed a high level of transferability ($\approx 77\%$) to the wheat genome. Putative functions were assigned to 216 SSR-containing ESTs through homology searches against the protein database. As many as 104 SSR-containing ESTs (a subset of the above ESTs) were also mapped to the 12 rice chromosomes, which corresponded with the known homology between wheat and rice chromosomes. These physical maps of EST–SSRs should prove useful for comparative genomics, gene tagging, fine mapping, and cloning of genes and QTLs.

Abbreviations: DT, ditelosomic; EST, expressed sequence tag; NT, nullisomic–tetrasomic; SSR, simple sequence repeat.

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DNA-BASED MOLECULAR MARKERS, particularly SSRs, have been developed and mapped on chromosomes in a variety of crop plants. In bread wheat, genetic and physical mapping of SSRs has been an ongoing exercise, and, to date, ≈ 2450 SSRs ($1 \text{ SSR } 1.63 \text{ cM}^{-1}$) have been genetically mapped (for details see Torada et al., 2006) and ≈ 1320 SSRs ($62 \text{ SSRs chromosome}^{-1}$) have been physically mapped (for details see Goyal et al., 2005). With a genome size of $\approx 16\,000$ Mbp, it is evident that despite concerted efforts, the density of mapped SSRs in bread wheat remains relatively low and continued efforts are needed to increase the density of these SSRs on available genetic and physical maps.

In recent years, emphasis has also shifted from genomic SSRs to EST–SSRs due to the availability of very large databases of ESTs from all of the cereals including bread wheat. Consequently, the number of EST–SSRs in cereals now includes 43 598 from bread wheat (Peng and Lapitan, 2005), 16 917 from rice and 184 from rye (La Rota et al., 2005; Hackauf and Wehling, 2002). The genetic mapping of these EST–SSRs is difficult due to a low level of polymorphism, as a result of their conserved nature. Physical mapping of these EST–SSRs in wheat is equally difficult due to the occurrence of homoeoloci exhibiting no polymorphism. This

has discouraged wheat researchers from undertaking a large-scale project to genetically or physically map wheat EST-SSRs although genetic mapping of 325 EST-SSRs (Gao et al., 2004; Nicot et al., 2004; Yu et al., 2004) and physical mapping of 305 EST-SSRs was recently undertaken (Yu et al., 2004; Zhang et al., 2005; Peng and Lapitan, 2005). We previously reported genetic mapping of 58 and physical mapping of 270 genomic SSRs (Gupta et al., 2002; Goyal et al., 2005). The present study is an extension of our earlier studies on physical mapping of SSRs and involved

The *in silico* approach allowed mapping of twice the number of loci (per EST-SSR) mapped using wet-lab analysis.

both wet-lab and *in silico* approaches, leading to the successful mapping of as many as 672 loci.

Materials and Methods

Cytogenetic Stocks

For chromosome bin mapping of EST-SSRs, the following cytogenetic stocks of common wheat 'Chinese Spring' were used for sequential assignment as follows: (i) 42 compensating nullisomic-tetrasomic (NT) lines were used for assigning EST-SSRs to individual chromosomes (Sears, 1954, 1966); (ii) 24 ditelosomic (DT) lines were used for assigning EST-SSRs to short or long arms of individual chromosomes (Sears and Sears, 1978), and (iii) 101 deletion lines (including 74 single deletion lines, 21 double deletion lines, and 6 triple deletion lines) were used for subarm mapping (Endo and Gill, 1996; Qi et al., 2003). The seed material for NT and DT lines was procured from Dr. B. S. Gill of Kansas State University, Kansas, USA, and that of deletion lines was procured from Dr. T. R. Endo of Kyoto University, Japan. These deletion lines provide complete coverage of the wheat genome, subdividing it into 159 chromosome bins. The details of the above cytogenetic stocks used are listed in Supplemental Table 1.

DNA Isolation and PCR Amplification

The genomic DNA for all the cytogenetic stocks was isolated from week-old seedlings following the CTAB method described elsewhere (Prasad et al., 2000). The PCR was performed using the genomic DNA of each wheat cytogenetic stock in a final volume of 20 μ L in an Eppendorf mastercycler, as described elsewhere (Gupta et al., 2003). After elec-

trophoresis, polyacrylamide gels were silver-stained following Tegelström (1992).

In Silico Mining of Rye EST-SSRs

A total of 9196 rye ESTs (details available at www.ncbi.nlm.nih.gov; verified 10 Oct. 2006) were searched *in silico* for the presence of SSRs using a Macro program that was kindly provided by Dr. N. D. Young (University of Minnesota, St. Paul, Minnesota, USA). Nonredundant SSR-containing ESTs were identified using the CAP3 sequence assembly program (<http://pbil.univ-lyon1.fr/cap3.php>; verified 11 Oct. 2006), and were used for physical mapping.

Primers for Wet-Lab Mapping

Primers for 160 wheat EST-SSRs were synthesized by Illumina (www.illumina.com; verified 10 Oct. 2006), using sequences available in the published literature (Gupta et al., 2003; La Rota et al., 2005). Aliquots for 157 rye EST-SSR primers were a generous gift by B. Hackauf of The Institute of Crop Plants, Gross Luesewitz, Germany (Hackauf and Wehling, 2002).

EST Sequences for *In Silico* Mapping

A nonredundant set of 399 SSR-containing rye EST sequences (mined from the rye EST database as above; details given in Supplemental Table 2) were used for *in silico* physical mapping on the wheat genome. Additionally, sequences of SSR-containing wheat ESTs, which could not be physically mapped through a wet-lab approach, were also used for *in silico* physical mapping, using mapped ESTs (<http://wheat.pw.usda.gov/cgi-bin/westsq/locus.cgi>; verified 10 Oct. 2006).

Physical Mapping of EST-SSRs

An individual EST-SSR locus was assigned to a chromosome, an arm, or a bin according to whether or not the expected PCR amplified product was obtained when genomic DNA from a particular cytogenetic stock was used as a template. If the expected amplified product was generated in all deletion lines but absent in NT and DT lines, the fragment was assigned to a centromeric bin of the corresponding arm; similarly, if the expected amplified product was absent in NT, DT, and also in all corresponding deletion lines, it was assigned to the terminal bin, and if the product was obtained in only some of the deletion lines, it was logically assigned to a specific interstitial bin (Fig. 1). Wherever ditelosomics for a chromosome were not available, deletions for both arms were used for bin mapping. For *in silico* physical mapping, sequences of SSR-containing ESTs of wheat and rye were subjected to a

BLASTN search against the GrainGenes database of wheat EST sequences that were bin mapped earlier using deletion stocks of Chinese Spring (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi; verified 10 Oct. 2006). Optimized BLASTN search parameters [cut-off E (expectation) value of $\leq e^{-15}$] and bit score 100 were used. Only the top hit from the BLASTN results were assigned to specific bins of wheat chromosomes.

Alignment of SSR-containing ESTs to the Rice Genome and Mapping onto Rice Chromosomes

The SSR-containing ESTs that were mapped during the present study were also used for BLASTN homology search with the rice whole genome sequence available at Gramene (www.gramene.org; verified 21 Nov. 2006). The significant matches were determined using a cut-off E value of 10^{-15} . Using this approach, SSR-containing ESTs were placed onto the rice chromosomes with the help of KaryoView software available at Gramene (www.gramene.org/Oryza_sativa/karyoview; verified 10 Oct. 2006).

Assignment of Putative Functions to SSR-containing ESTs

The SSR-containing ESTs were subjected to BLASTX analysis against the nonredundant protein database for assigning putative functions at a cut-off E value of 10^{-15} (www.ncbi.nlm.nih.gov/BLAST; verified 10 Oct. 2006).

Statistical Tests for Random Distribution of Mapped Loci

On the basis of physical size (in micrometers) and DNA contents of chromosomes and chromosome arms (as given in Gill et al., 1991; Furuta and Nishikawa, 1991), the expected number of EST-SSR loci was estimated under the assumption of random distribution among chromosomes or chromosome arms, among the three subgenomes, and seven homoeologous groups. The expected number of EST-SSR loci on the rice chromosomes was estimated on the basis of size (Mb) of each rice chromosome (www.Gramene.org/Oryza_sativa; verified 10 Oct. 2006) assuming random distribution of loci. The χ^2 test for goodness-of-fit was used for testing the random distribution of EST-SSR loci among chromosomes, chromosome arms, the three subgenomes, the seven homoeologous groups of wheat and among 12 rice chromosomes. Independence of the distribution of loci in the three subgenomes vs.

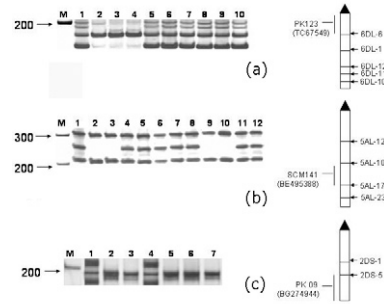


Figure 1. PCR profiles showing strategy followed for deletion bin mapping: (a) Physical mapping of wEST-SSR PK123 (TC67549) on centromeric bin of 6DL (notice amplification in all the deletion stocks), Lanes M 100-bp ladder, 1 CS, 2 N6DT6A, 3 N6DT6B, 4 Dt6DS, 5 Dt6DL, 6 6DL-6, 7 6DL-1, 8 6DL-12, 9 6DL-11, 10 6DL-10. (b) Physical mapping of rEST-SSR SCM141 (BE495388) on interstitial bin defined by deletion breakpoints 5AL-10 and 5AL-17 (notice amplification in only some deletion stocks); Lanes M 100-bp ladder, 1 CS, 2 N5AT5B, 3 N5AT5D, 4 Dt5AL, 5 5AS-1, 6 5AS-3, 7 5AS-7, 8 5AS-10, 9 5AL-12, 10 5AL-10, 11 5AL-17, 12 5AL-23. (c) Physical mapping of wEST-SSR PK9 (BG274944) in distal bin of 2DS (notice absence of amplification in all the deletion stocks); Lanes M 100-bp ladder, 1 CS, 2 N2DT2A, 3 N2DT2B, 4 Dt2DS, 5 Dt2DL, 6 2DS-1, 7 2DS-5.

the seven homoeologous groups was tested using 3×7 contingency table for χ^2 test of independence.

Results and Discussion

Physical Mapping of EST-SSRs

In the present study, 672 loci belonging to 275 EST-SSRs were mapped, with an average of 2.44 loci per EST-SSR (Table 1; Fig. 2). As many as 716 EST-SSRs (including 160 wEST-SSRs and 556 rEST-SSRs; w stands for wheat and r stands for rye) were tried for this purpose, giving a successful mapping effort of 38.40%. Of these 672 mapped loci, 110 loci belonging to 74 EST-SSRs (43 wEST-SSRs + 31 rEST-SSR) were mapped using a wet-lab approach (1.48 loci/EST-SSR), and 562 loci belonging to 201 EST-SSRs (50 wEST-SSRs + 151 rEST-SSRs) were mapped using an in silico approach (2.8 loci/EST-SSR). The results

Table 1. Details of the EST-SSR loci physically mapped using wet-lab analysis and in silico analysis in wheat (in each case, number of EST-SSRs used for mapping are given in parenthesis).

	Number of EST-SSRs loci mapped		Total
	Wet-lab analysis	In silico analysis	
Wheat	67 (43)	143 (50)	210 (93)
Rye	43 (31)	419 (151)	462 (182)
Total	110 (74)	562 (201)	672 (275)

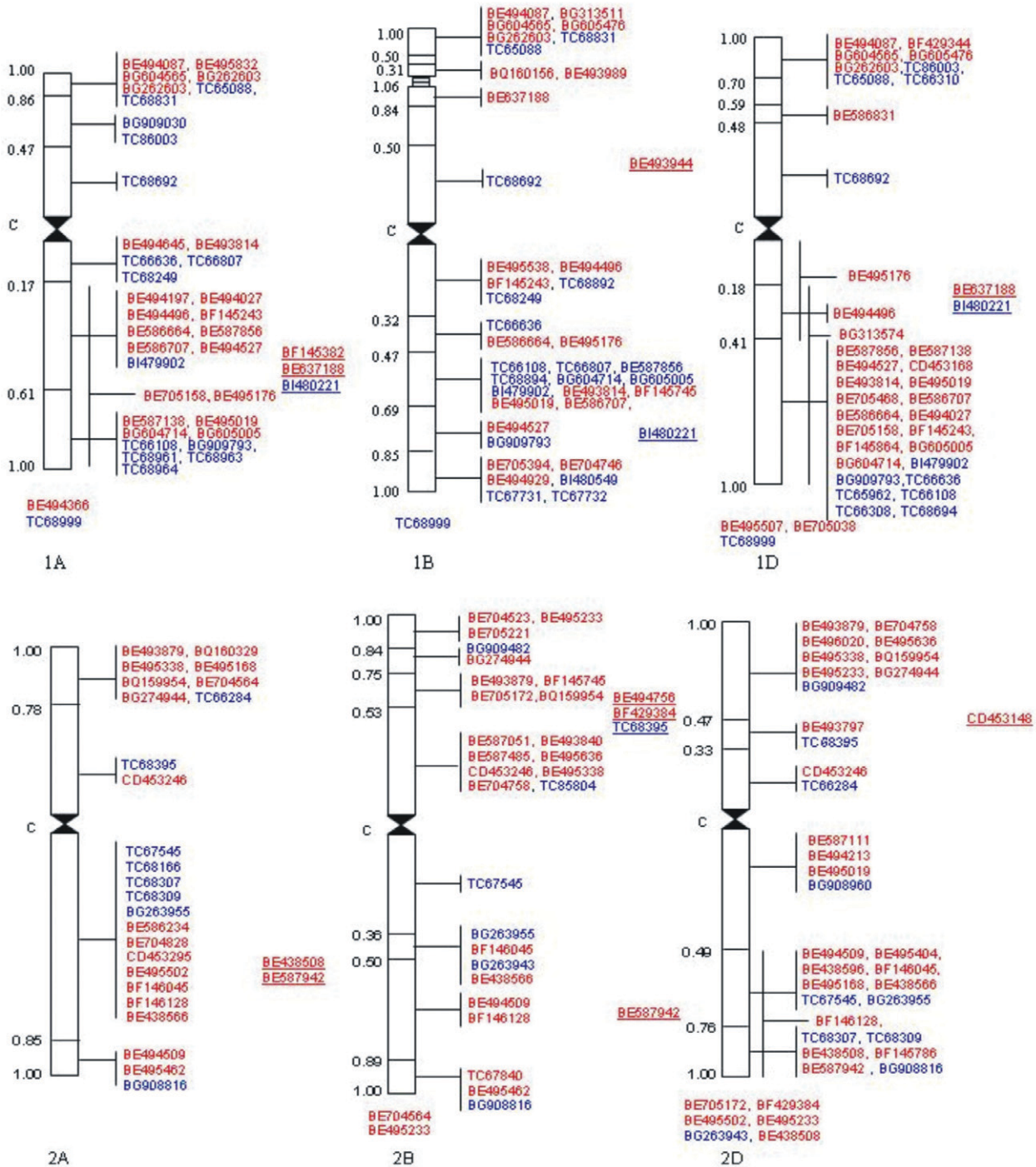


Figure 2. Physical mapping of wheat and rye EST-SSRs on 21 wheat chromosomes. The accession numbers of ESTs, which correspond mapped EST-SSRs are shown on the right and the fraction lengths are given on the left. Vertical lines on the right side span the bin length. The accession numbers in blue belong to wheat EST-SSRs, and those in red belong to rye EST-SSRs. The EST-SSRs, which could not be assigned to bins and were assigned to chromosome arms only are underlined and those assigned to chromosomes (with no information about arm) are given under each individual chromosome.

of the present study are in agreement with those of earlier reports, where an average number of mapped loci per EST-SSR in wheat ranged from 1.3 to 2.8 (Yu et al., 2004; Zhang et al., 2005; Qi et al., 2004). As

can be seen, the in silico approach allowed mapping of twice the number of loci (per EST-SSR) mapped using the wet-lab analysis. Since the cytogenetic stocks covered the whole genome, this difference

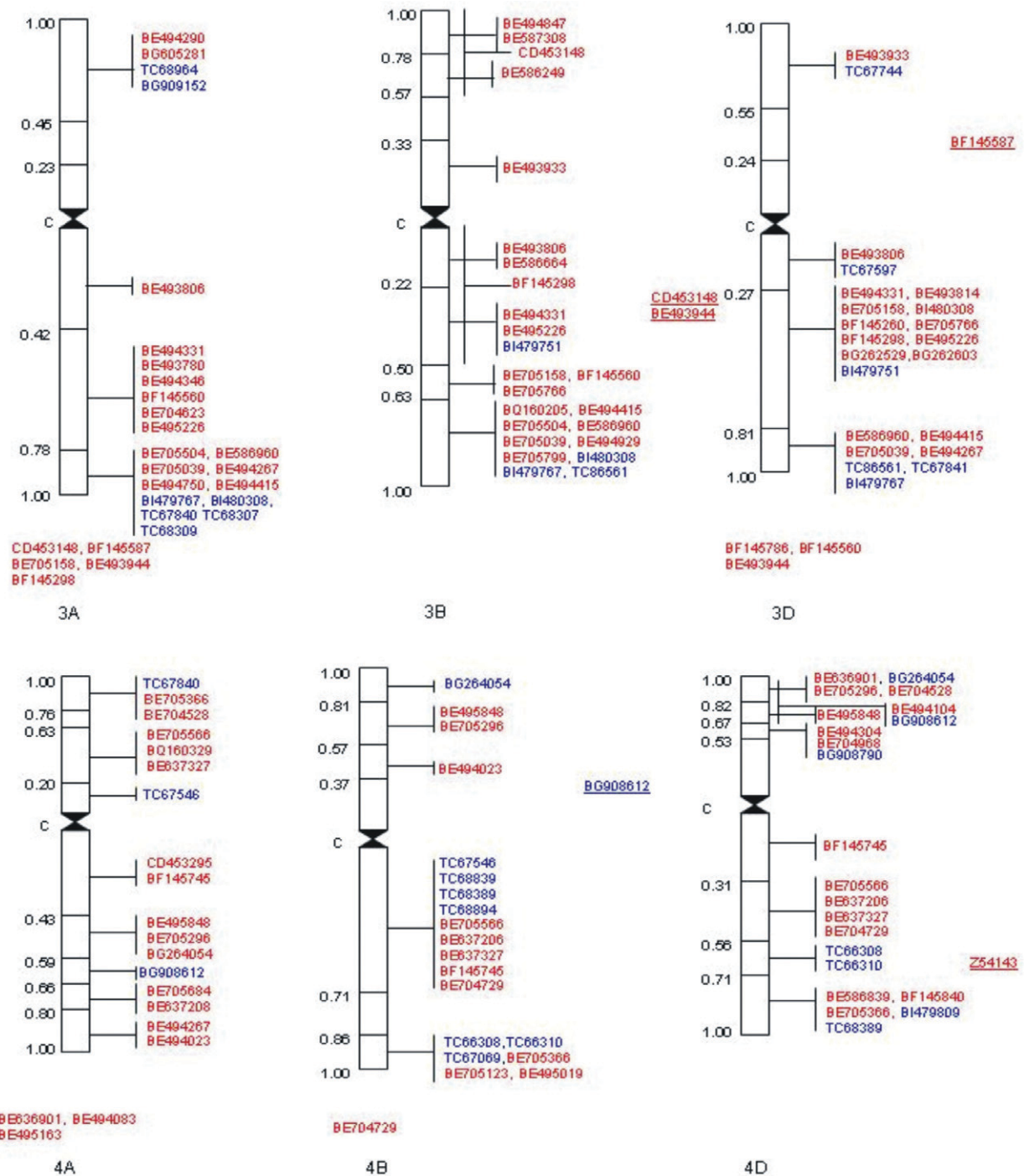


Figure 2. Continued.

between numbers of mapped loci could not be due to incomplete coverage of the genome by the cytogenetic stocks. Instead, this may be due to the failure to map, through the wet-lab approach, the homoeoloci that lacked polymorphism since the expected product will be visible in all NT, DT, and deletion stocks. Instead, the in silico approach will allow mapping of such homoeoloci, since the EST sequence will match with more than one mapped sequence.

Distribution of EST-SSR Loci among Subgenomes and Homoeologous Groups

The distribution of loci in the three subgenomes (Table 2), when tested for randomness using their lengths in micrometers (Gill et al., 1991), were statistically significant ($P < 0.01$), suggesting a nonrandom distribution. Similar results were obtained when DNA contents (instead of length in

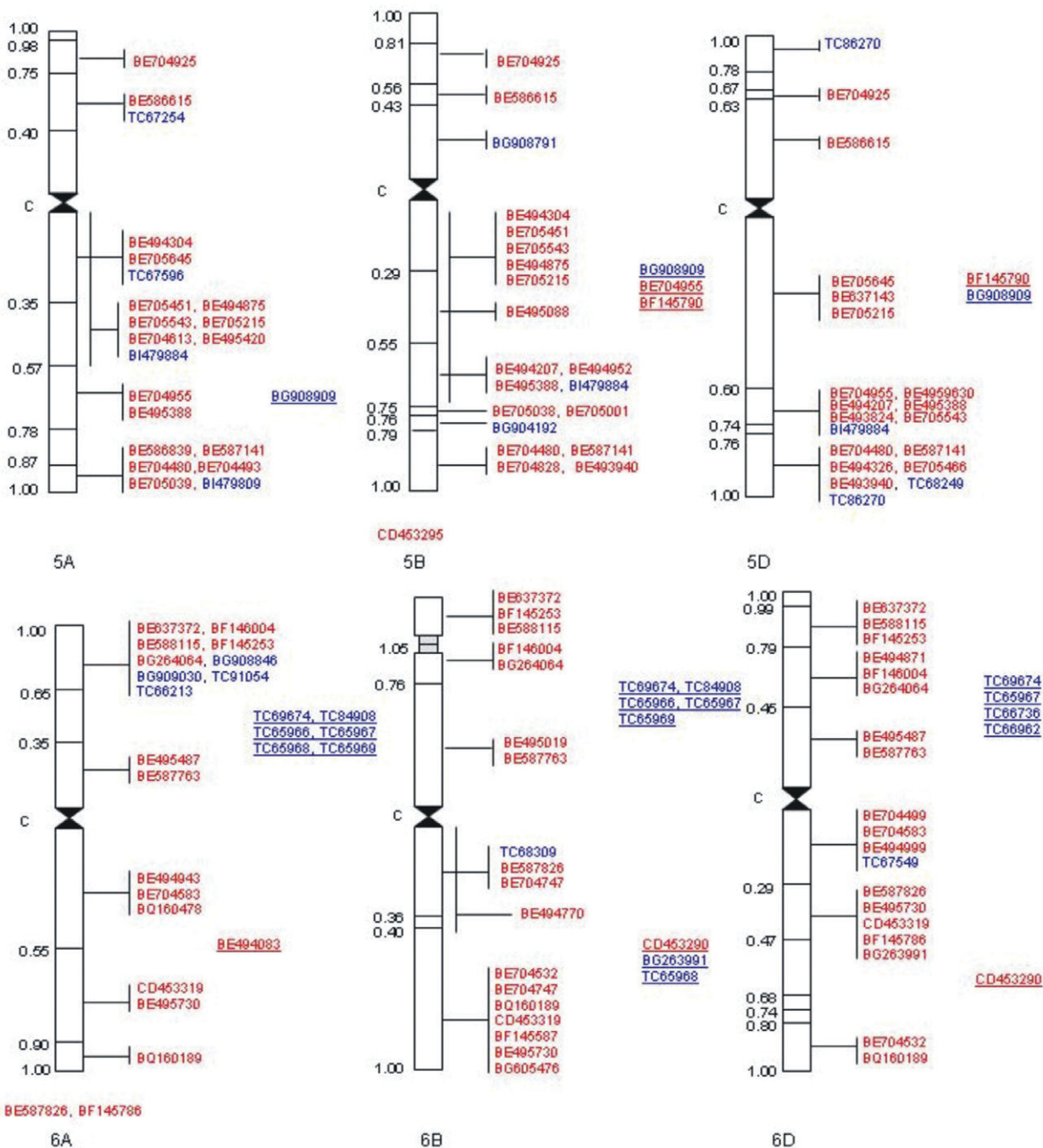


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micrometers) of the three subgenomes were used for these tests. In Table 2 it can be seen that the A genome, which is larger than the D genome, had the minimum number of mapped loci. This is similar to earlier studies that reported many more EST loci mapped to the B subgenome than either on the A or on the D subgenome (Qi et al., 2004; Akhunov et al., 2003; Peng and Lapitan, 2005).

The distribution of EST-SSR loci among the seven homoeologous groups of wheat chromosomes was also nonrandom ($P < 0.01$), ranging from

74 loci on homoeologous Group 4 to 128 loci on homoeologous Group 1. These results are in agreement with earlier reports, where the maximum number of SSR loci were mapped to the homoeologous Group 1 (Gao et al., 2004), and the homoeologous Group 4 was found to carry the minimum number of mapped loci, which was attributed to the lack of polymorphism in this group (Nelson et al., 1995; Cadalen et al., 1997; Paillard et al., 2003; Zhang et al., 2005).

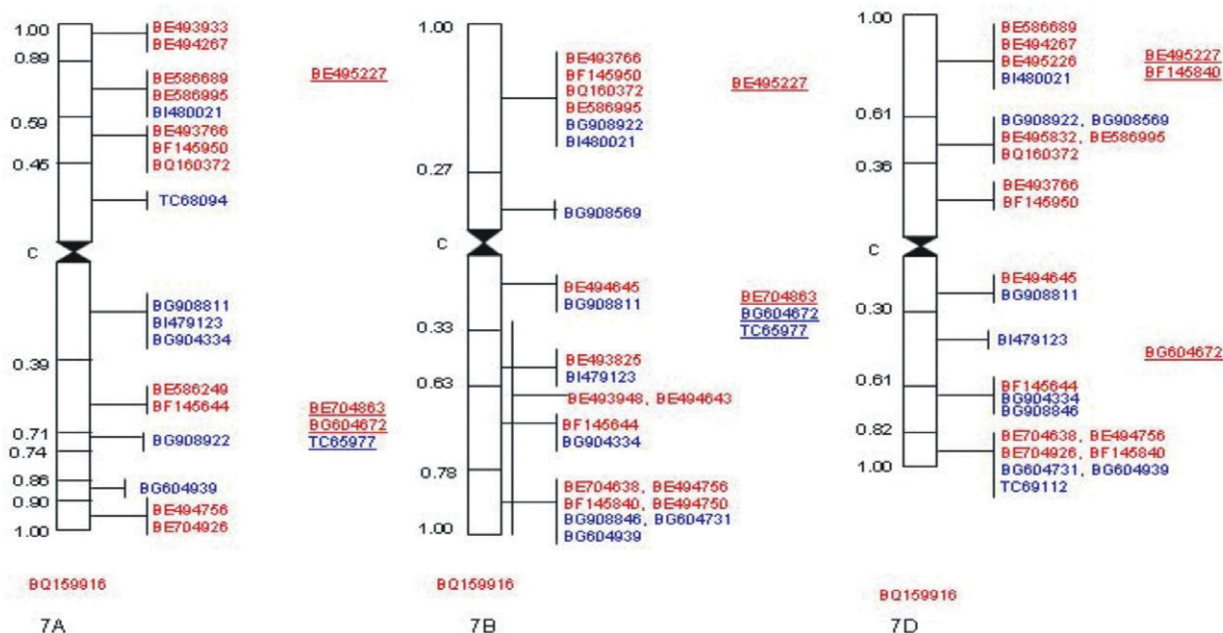


Figure 2. Continued.

However, the distribution of EST-SSR loci among the three subgenomes and among the seven homoeologous groups was independent ($P = 0.62$), although one would expect a lack of independence, since each subgenome has seven chromosomes, one from each of the seven homoeologous groups. This unexpected independent distribution may be due to the fact that many EST-SSRs with homoeoloci could not be mapped through the wet-lab approach.

Distribution of EST-SSR Loci on Individual Chromosomes and Their Arms

The distribution of EST-SSR loci among the 21 wheat chromosomes was also nonrandom ($P \lll 0.01$) with a minimum number of 20 EST-SSR loci on Chromosome 4B and a maximum number of 46 EST-SSR loci mapped on Chromosome 1B. These results are also in agreement with earlier reports, where Chromosome 4B was found to carry the minimum number of SSR markers with the lowest gene density (Qi et al., 2004; Song et al., 2005), and 1B (along with 2A) was found to carry the maximum number of EST-SSR loci (Torada et al., 2006). The distribution of loci on long and short arms (excluding 18 loci, which could not be assigned to arms) was also not in proportion to their relative lengths or DNA contents ($P \lll 0.01$). Long arms carried almost double the number of loci (434 loci) carried by the short arms (220 loci), although the long arms are known to contain only 38% excess DNA (computed from data from Furuta and Nishikawa, 1991), suggesting that long arms contain many more

expressed genes than the short arms, as also inferred in earlier studies (Sorrells et al., 2003; Qi et al., 2004).

Distribution of EST-SSR Loci to Distal and Proximal Regions of Chromosome Arms

Chromosome bin mapping was possible for only 568 loci, since the remaining 104 loci could be assigned only to chromosomes or chromosome arms. On the basis of the fraction length of the terminal deletion lines (Endo and Gill, 1996), each chromosome arm was divided into two parts, the proximal region representing the 60% of the arm from the centromere (C-0.60) and the distal region representing the terminal 40% of the arm (0.60–1.00). Out of the above 568 EST-SSR loci, 240 (42%) loci were mapped to the bins which fall in the distal regions, 134 (24%) loci were mapped to bins which fall in the proximal regions, and the remaining 194 (34%) loci were mapped to the long bins which contained parts of both distal and proximal regions. The distribution of loci in distal and proximal bins

Table 2. Distribution of EST-SSR loci according to their assignment to wheat chromosomes arranged in two-way classification.

	Homoeologous Group							Total
	1	2	3	4	5	6	7	
Subgenome A	37	32	32	23	24	31	25	204
Subgenome B	46	38	32	20	26	35	39	236
Subgenome D	45	42	31	31	25	26	32	232
Total	128	112	95	74	75	92	96	672

deviated significantly from the distribution expected on the basis of the relative lengths of these bins ($P \ll 0.01$). The present study, therefore, suggests that more EST-SSR loci tend to map to the distal regions, which are also the regions of recombination hot spots. The preferential localization of the EST-SSRs in the recombination hot spots enhances their value as markers, since these would facilitate fine mapping of genes and QTLs. In earlier studies, EST-density was shown to be positively related with the recombination rate (Akhunov et al., 2003) and was found to increase with increasing relative physical distance from the centromere to the end of a chromosome (Qi et al., 2004).

EST-SSRs Detecting Multiple Loci (Homoeologous and Nonhomoeologous)

Of the 275 mapped EST-SSRs, 178 (64%) detected multiple loci, and each of the remaining 97 detected only a single locus. Of the 178 EST-SSRs with multiple loci, 144 (81%) detected multiple homoeoloci, the remaining 34 (19%) detected multiple loci on nonhomoeologous chromosomes, which might represent duplications. This is in agreement with an estimated 25 to 30% gene duplications reported for the wheat genome (Anderson et al., 1992). In view of the known fact that ESTs generally represent unique single loci (Qi et al., 2004), the occurrence of EST-SSRs with multiple loci is significant and may be attributed to the conserved nature of EST-SSRs, which seem to occur as homoeoloci in the three related subgenomes.

Transferability of Rye EST-SSRs to Wheat

Out of 157 SSR-containing rye ESTs that were used to study transferability between the two species, 121 (77%) gave amplification products in Chinese Spring, indicating a high rate of transferability. The level of transferability between wheat and rye in the present study is comparable to earlier reports of transferability of EST-SSRs in cereals (Gao et al., 2003; Khlestkina et al., 2004; Zhang et al., 2005), but it is higher than the level of transferability (25%) of genomic SSRs (Korzun et al., 2001; Kuleung et al., 2004). This may also be attributed to the conserved nature of EST-SSRs among closely related species.

Distribution and Localization of Mapped EST-SSRs on Rice Chromosomes

In the present study, a total of 104 (38%) mapped EST-SSRs (from wheat and rye) showed significant sequence homology with rice genome sequence (Fig. 3). These results differ from those of La Rota and Sorrells, (2004), who reported 59% of the wheat EST contigs

showing significant homology with rice sequences; the differences may be attributed to the different stringency conditions used in the two studies.

It can be seen in Fig. 3 that the rice genome sequences that are homologous to the 104 EST-SSRs were only nearly random in their distribution on the 12 rice chromosomes ($P = 0.038$; not significant at $P = 0.01$). The distribution ranged from a maximum of 14 EST-SSRs homologous to sequences on rice Chromosome 3 (longest Chromosome 1 had homology with only 12 EST-SSRs) to a minimum of 4 EST-SSRs homologous to sequences on rice Chromosome 8 (smallest Chromosome 10 had homology with 5 EST-SSRs). Also, 66 (63.4%) of the 104 EST-SSRs belonged to the long arms, 32 (30.8%) belonged to the short arms, and the remaining 6 (5.8%) belonged to both arms of wheat chromosomes (Supplemental Table 3). Once again, the wheat EST-SSRs from the long arms sharing homology to the rice genome sequences were more than double the number from the short arms, giving support to the idea that the frequency of expressed genes on the long arms of wheat chromosomes is much higher than what one would expect due to only 38% excess DNA in the long arms (Sorrells et al., 2003).

The present study also confirms some of the known relationships between wheat and rice chromosomes. For instance, EST-SSRs that matched sequences on rice Chromosomes 1, 2, 3, 5, and 6 were generally mapped on related wheat homoeologous Groups 3, 6, 4, 1, and 7, respectively, and EST-SSRs matching with sequences of rice Chromosomes 4 and 7, were mapped to wheat Group 2 chromosomes. The earlier studies also reported that rice Chromosomes 4 and 7 both together contributed to a conserved block of the present day wheat Group 2 (Sorrells et al., 2003; La Rota and Sorrells, 2004).

Putative Functions of Mapped EST-SSRs

The results of the BLASTX analysis revealed that 186 (67.6%) EST-SSRs matched proteins of known functions; 25 (9%) matched proteins of unknown functions, and 5 (1.8%) matched with hypothetical/expressed proteins (Supplemental Table 4). The remaining 59 (21.45%) EST-SSRs did not match with any proteins in the database.

Among the 186 EST-SSRs matching proteins of known functions, seven EST-SSRs that were mapped to the short arms of Group 1 chromosomes matched with genes belonging to the gliadin family (a class of seed storage proteins), which are known to occur on the corresponding positions of Group 1 chromosomes (Payne et al., 1984; Payne, 1987; Sandhu et al., 2001; Erayman et al., 2004). The EST-SSR, PK74 (mapped to terminal short arms of wheat Group

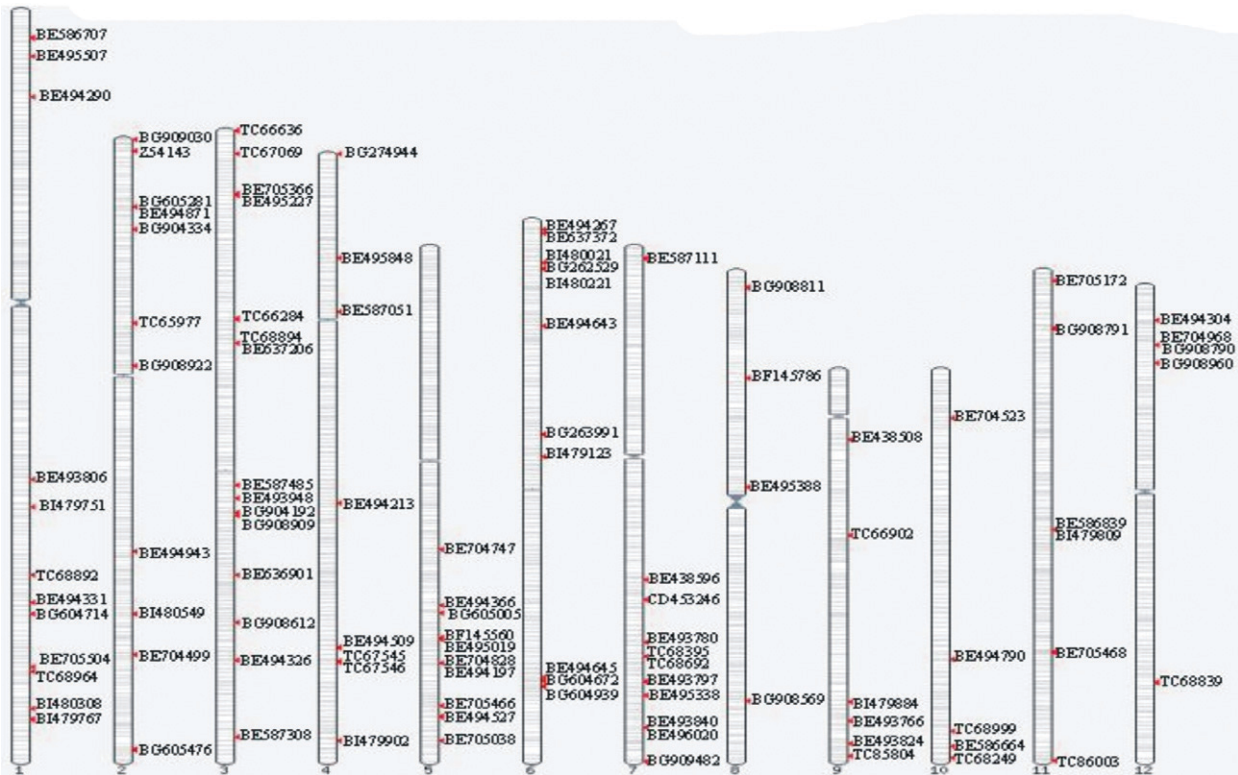


Figure 3. Rice chromosomes showing physical positions of SSR-containing ESTs, mapped through homology search.

1) matched glutenin, confirming the known mapping positions of the corresponding genes (Sandhu et al., 2001; Erayman et al., 2004). This is also in agreement with the report that the genes coding for both gliadins and glutenins occur in the gene-rich region (GRR) 1S 0.8; 48 such GRRs were reported in the wheat genome (Erayman et al., 2004). However, EST-SSR PK82 that was physically mapped to 6AS matched with a Dof DNA binding protein (a multigene family encoding transcription factors), the genes for which were earlier mapped to 1BS (Gao et al., 2004) and 5A/5B (Ravel et al., 2006), so that the present study suggests an additional locus on 6AS for this gene family. Another EST-SSR mapping to Chromosome 4A matched an ice recrystallization inhibition protein, encoded by gene *TaRI-1* with unknown position on the wheat genome (Tremblay et al., 2005). Thus the mapping of the SSR-containing EST (SCM178) belonging to a gene coding for ice recrystallization inhibition protein on wheat Chromosome 4A is reported for the first time in the present study.

EST-SSRs for Cereal Breeding

EST-derived SSR markers are a novel source of markers from the transcribed regions of the genome and increase the repertoire of perfect markers for studies related to germplasm evaluation, fine mapping of genes or QTLs, marker-assisted selection in

plant breeding, and for positional cloning of genes or QTLs (Gupta and Rustgi, 2004). The transferability and physical mapping of rEST-SSRs to wheat will also help in further elucidating the relationships between the wheat and rye genomes and make them a valuable source for comparative genomics research. Because of their higher level of transferability, EST-SSRs may also be used for gene introgression from related wild species, which are an important source of both biotic and abiotic resistance genes.

Acknowledgments

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