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Development of New SSR Markers for Homoeologous *WFZP* Gene Loci Based on the Study of the Structure and Location of Microsatellites in Gene-Rich Regions of Chromosomes 2AS, 2BS, and 2DS in Bread Wheat

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Abstract—Microsatellites, or simple sequence repeats, are widely distributed in eukaryotic genomes, including plant genomes. The peculiarities of the structure and location of the microsatellite loci determine their potential as molecular genetic markers and can influence the assumed function of microsatellites in important biological processes. The identification and study of the distribution of microsatellite loci in gene-rich genome regions of the bread wheat and the development (based on them) of new microsatellite markers are of practical interest and are important for the study of the organization of the bread wheat genome. The sequences of BAC clones that contain the homoeologous *WFZP* genes of the bread wheat (*Triticum aestivum* L.) controlling the development of the ear were the basis for the identification and localization of microsatellite loci in gene-rich regions of the 2AS, 2BS, and 2DS chromosomes. Di- and trinucleotide microsatellite repeats are the most widespread in the studied sequences. The AG and GA/TC motifs prevail among the dinucleotide motifs; the dinucleotide repeats are found in noncoding gene regions, mobile elements, and nonannotated DNA sequences. Most of the trinucleotide repeats are associated with mobile genetic elements. It was found that homoeologous microsatellite loci are located either in the genes or in the nonannotated DNA sequences. The comparison of the structure of homoeologous loci demonstrated that the divergence in them is associated both with a change in the number of repeats and with nucleotide substitutions. The new microsatellite markers, which are colocalized in the genetic maps with the *WFZP-A-B-D* genes and can be used for marking these genes in molecular genetic studies and in breeding controlled by markers, were developed.

Keywords: microsatellite loci, SSR markers, BAC clone, bread wheat, *WFZP*

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INTRODUCTION

Microsatellites, or simple sequence repeats (SSRs) are DNA regions consisting of tandemly repeated short (1–6 bp) elements (motifs). Microsatellite repeats are classified depending on the structure: (1) perfect microsatellite repeats are an uninterrupted sequence consisting of the same motifs; (2) imperfect microsatellite repeats consist of blocks of similar motifs divided by several nucleotides differing from the ones of the repetition; (3) complex microsatellites (or compounds) are blocks of motifs of one or different types divided into not more than 100 bp. Depending on the length, there are two classes of microsatellites, including class I (≥20 bp) and class II (≤19 bp) (Temnykh et al., 2001). The frequency of mutations in microsatellite loci significantly exceeds the expected frequency of spontaneous mutagenesis (Wierdl et al., 1997; Thuillet et al., 2002). The variability of the SSR loci is first of all associated with the change in the number of simple repeats and arises as a result of DNA replication errors due to DNA polymerase slippage or an uneven crossingover (Sia et al., 1997). It was found that the variability of the microsatellite loci is correlated to their length. Thus, class I microsatellites are more polymorphic than class II microsatellites (Temnykh et al., 2001; Webster et al., 2002). The transformation of complete microsatellites into incomplete microsatellites or compounds stabilizes them, as a result of which they become less variable (Thuillet et al., 2002).

Microsatellites are widely distributed in eukaryotic genomes (Tautz et al., 1984); it was demonstrated that they constitute approximately 0.69% of the rice genome (Grover et al., 2007). The microsatellite loci are located both in coding and noncoding genome regions, and the density and distribution of the different types of microsatellites are not the same in different genome fractions (Li et al., 2002; Morgante et al., 2002; Grover et al., 2007).

Microsatellites are widely used for the analysis of plant genomes; DNA markers developed based on them are one of the most relevant markers in plant molecular genetics. Molecular genetic mapping is the most important area of their use; in addition, they are irreplaceable in the study of genetic diversity and phylogeny of closely related taxa and are of interest for use in the programs on marker-assisted selection (Ganal and Röder, 2007). This is due to the following properties, including their wide distribution in the genome, multiallelism, codominant nature of inheritance, high reproducibility of the results, and the possibility to automate genotyping. The microsatellite markers obtained based on genomic libraries are called genomic SSR (gSSR). Röder et al (1998) obtained the first large pool of wheat microsatellite markers based on the genomic DNA library of the *Triticum aestivum* L. bread wheat. The search in silico for microsatellite loci in the expressed sequence tags (ESTs) and the marker development based on them is another way of development of microsatellite markers. Such markers are called EST microsatellites. Both types of markers are widely used for mapping the genes and genomes of the bread wheat and its congeners (Salina et al., 2006; Ganal and Röder, 2007; Leonova et al., 2008; Dobrovolskaya et al., 2009; Dobrovolskaya et al., 2011). At present, use of the accumulated data massifs obtained as a result of the performance of projects on plant genome sequencing has provided new possibilities for identifying microsatellite sequences and developing new microsatellite markers. BACend sequences (BES)-SSR is a new type of microsatellite markers obtained as a result of the terminal sequencing of bacterial artificial chromosome (BAC) clones. It is widely used for integration of physical and genetic plant maps (including the bread wheat) (Paux et al., 2008). Determination of the reference sequence of chromosome 3B (Choulet et al., 2014) and obtaining the results of the draft sequencing of isolated bread wheat chromosomes (IWGSC, 2014) allowed to make important conclusions about the structural and functional genome organization in the bread wheat. More than 5000 known microsatellite markers were physically mapped in individual chromosomes (IWGSC, 2014) and new SSR markers of chromosome 3B were developed (Paux et al., 2008). The study of the structure and distribution of microsatellite repeats in DNA sequences of the bread wheat will provide the possibility of detecting regularities of their localization in different fractions of genomic sequences and establishing the functional role of these repeats in biological processes, and will be used for the creation of new SSR markers.

The aim of the present work is to study the structure and location of microsatellite loci in gene-rich regions of chromosomes 2A, 2B, and 2D on the example of the sequences of the homoeologous *WFZP-A-B-D* gene loci and the development of new SSR markers marking these regions.

MATERIALS AND METHODS

The sequences of DNA regions of chromosomes 2AS, 2BS, and 2DS obtained as a result of 454Roch-sequencing of BAC clones containing homoeologous *WFZP-A, -B*, and *-D* bread wheat genes were the object of the study in this work. The CS248B13, CS184F24, and CS305H5 BAC clones were selected during the screening of the genomic BAC library obtained based on the bread wheat variety Chinese Spring (http://cnrgv.toulouse.inra.fr/). The information about the screening, sequencing, and annotation of the DNA sequences of these BAC clones was previously published (Dobrovolskaya et al., 2015). In order to identify SSR repeats, the *SSR locator* program was used (Maia et al., 2008) at the following search parameters, including at least 6 repeats for dinucleotide microsatellites, 5 repeats for trinucleotide microsatellites, and 4 repeats for tetra-/penta-/ hexanucleotide microsatellites for the perfect repeats; up to 3 nonrecurring bases separating microsatellites for the imperfect repeats; and up to 100 bp between microsatellite blocks for the compounds. The primers to the microsatellite loci were developed using the Primer3 program (http://bioinfo.ut.ee/primer3-0.4.0/). The structure of the developed primers is given in Table 1. The polymerase chain reaction (PCR) was conducted using the samples of total DNA previously isolated from the plants of the bread wheat lines and varieties listed in Table 1, as well as from the individual plants of the F_2 mapping population obtained from the cross between the Chinese Spring and Renan bread wheat varieties (Dobrovolskaya et al., 2009; 2015) according to the protocol of Nicot et al. (2004). The PCR fragments were separated on an automatic ABI PRISM 3100 Genetic Analyser sequencer (Applied Biosystems, Foster City, CA, United States). The fragment size was calculated by the ABI GeneScan computer program (version 2.1) developed by the Applied Biosystems company. New microsatellite markers were integrated in previously constructed genetic maps of the bread wheat chromosomes 2A, 2B, and 2D (Dobrovolskaya et al., 2015) by the computer program MAPMAKER/EXP ver. 3.0b (Lander et al., 1987) using the Kosambi mapping function (Kosambi, 1943) at LOD value ≥ 3.00.

Table 1. Microsatellite markers

Marker	Motif	Primers	Polymorphism (alleles, bp)
CS248B13-1	(AG)16	F: 5'-CTCCAAGAAGATCGAGGTGAACAT-3' R: 5'-TTGTTACCCTACCGATGATGTGTG-3'	163^{8}, $167^{1, 2, 3, 7}$ $171^{5, 6}$, 175^{9}
CS248B13-2	(AT)11	F: 5'-GTGCACTTTTGACCTCCCTACACT-3' R: 5'-ATTTTGGGTTAAGTGGACGTAGCA-3'	432 NP
CS248B13-4	(AGCC)4	F: 5'-CGCTGACTCTACACCTTACCTCGT-3' R: 5'-ACTTTTAATCGAATCGCACACG-3'	406 NP
CS248B13-3	(GCC)4(GCG)4	F: 5'-CGAGCTACATTTAGTGCATCTGGA-3' R: 5'-TGACCGCTTTAGAGCCTTG-3'	427 NP
CS184F24-1	(TC)15	F: 5'-CCATGGTGATGTGTGAGTAGTTCC-3' R: 5'-GTCGTAGAGTAAGGACACCGCAAT-3'	$367^{2, 6, 7, 9}$ $371^{1, 3, 4, 5, 8}$
CS305H5-1	(TA)20	F: 5'-AACAATGATGCAATGAAGGAACAA-3' R: 5'-CGGGTTTGATTCCTGATGAGTTAG-3'	$301^{1, 2, 3}$, $325^{6, 7, 9}$ 334^{8}, $null^{4, 5}$
CS305H5-2	(GAG)8	F: 5'-ACTACACCGACACCAACGTCTTC-3' R: 5'-GAAGACTAAGGCATGACTTGGAGG-3'	351 NP

NP is the nonpolymorphic fragment of the indicated size; superscript numbers designate the bread wheat lines and varieties in which these alleles were found: $^{1, 2, 3, 4, 5}$, the Ruc163, Ruc167, So149, Ruc204, and Skle128 lines (Dobrovolskaya et al., 2009, 2015); $^{6, 7, 8, 9}$, the Saratovskaya 29, Skala, Chinese Spring, and Renan varieties, respectively.

RESULTS AND DISCUSSION

During the work on determining the primary structure of homoeolog *WFZP-A-B-D* genes regulating the development of the wheat ear and determining the fate of the spikelet meristem, the sequencing of three BAC clones (CS248B13, CS184F24, CS305H5) carrying the target genes was conducted and the data on the structural organization of the regions of homoeologous chromosomes 2AS, 2BS, and 2DS were obtained; the genes and mobile elements within these sequences were annotated, and the order of their mutual position was determined (Dobrovolskaya et al., 2015).

The *mrs1/WFZP-D* gene was localized in the 2S0.8 gene-rich region of the homoeologous group-2 chromosomes (Dobrovolskaya et al., 2009). The gene-rich regions of the chromosomes were determined during the study of the localization of the expressed DNA sequence fragments or expressed sequence tag (EST) sequences in the deletion bins of the bread wheat chromosomes (Erayman et al., 2004). The uneven gene distribution in the chromosomes was confirmed by the results of sequencing the extended DNA regions of the wheat chromosome 3B; however, no clear separation on the large blocks of gene-rich and gene-poor regions in chromosome 3B was found. It was demonstrated that most of the genes (75%) formed small gene islands consisting of on average three genes separated by blocks of mobile elements, while the extended non-coding DNA regions (longer than 800000 bp long) are found very rarely (Choulet et al., 2010, 2014).

In the present study, the sequences of BAC clones containing homoeologous *WFZP* genes were the basis for the identification and localization of microsatellite loci in the gene-rich regions of chromosomes 2AS, 2BS, and 2DS. It was found that AG, GA/TC (61.5%), and TA/AT (27%) are prevalent among the dinucleotide repeats (Table 2). Previously, it was demonstrated that the same classes of dinucleotide repeats (AG/CT and AT/TA) are prevalent dinucleotide repeats in the rice genome (Grover et al., 2007), the genomes of nine species of cereals, including the *Triticum urartu* (the wheat A genome donor) and *Aegilops taushii* (the wheat D genome donor) species (Wang et al., 2015), and in general are typical for the plant genomes (Lagercrantz et al., 1993). Dinucleotide microsatellites (that we identified) are localized both in the gene loci and the loci of transposable elements (TE), as well as in nonannotated DNA sequences. Trinucleotide microsatellite repeats were found less frequently than dinucleotide microsatellite repeats; no prevalent motif/motifs were found. Most of the trinucleotide microsatellites were presented by short class II (≤19 bp) SSR repeats associated with TE (Table 2).

In general, most of the microsatellite loci that we found were associated with the class I TE (retrotransposons) and were located directly in the internal TE regions. We did not find any series of homoeologous microsatellite loci associated with TE (the microsatellite loci of a single type with homologous flanking sequences localized in homoeologous chromosomes of different wheat subgenomes are homoeologous microsatellite loci). This is apparently associated with the fact that TE is a rapidly evolving fraction of genomes, which mainly contributes to the interspecific divergence. A high percentage of microsatellite

Table 2. Type and localization of microsatellite loci

Motif	SRR locus	Localization	SRR marker
2AS (CS248B13)			
AG/GA/CT	(GA)6	na	
	(GA)20[a]	G (5'-region)	*
	(GA)4(GA)6[b]	na	
	(AG)6	TE (I)	
	(AG)16[c]	na	CS248B13-1
	(CT)6	G (3'-region)	
AT/TA	**(AT)11**	na	CS248B13-2
	(AT)7	na	
	(TA)8	TE (II)	
	(TA)19	TE (I)	
	(TA)6[1]	G (intron)	
TG/GT	(TG)6	TE (I)	
AGG/GGA	(AGG)6	TE (I)	
	(GGA)5	TE (II)	
TGA	**(TGA)7**	TE (I)	
	(TGA)2(TGA)4	TE (I)	
CTT	(CTT)5	TE (I)	
ACA	(ACA)5	TE (I)	
GCC, GCG	(GCC)4(GCG)4[d]	G (CDS)	CS248B13-3
AGCC	(AGCC)4[1]	na	
AAGAA	**(AAGAA)4**	na	
2BS (CS184F24)			
AG/GA/TC	(AG)7	na	
	(AG)7	TE (I)	
	(GA)7	TE (I)	
	(GA)10	TE (I)	
	(GA)34	na	*
	(TC)6[a]	G (5'-region)	*
	(TC)5-CA-(TC)10[b]	na	CS184F24-1
GGC/CGG/GCG	(GGC)5	TE (I)	
	(GGC)19 imp	TE (I)	
	(CGG)5	TE (I)	
	(CGG)5(CGG)4	TE (I)	
	(GCG)4(GCG)4	TE (I)	
GTG	(GTG)5	TE (I)	
TTTA	(TTTA)4(TTTA)2		

Table 2. (Contd.)

Motif	SRR locus	Localization	SRR marker
2DS (CS305H5)			
AG/GA/TC	(GA)9[c]	na	
	(GA)7(GA)3[b]	na	
	(AG)8-G-(GA)12	TE (I)	
GC	(GC)6	G (5'-region)	
TCC	(TCC)5	TE (II)	
GAG	**(GAG)8**	na	CS305H5-2
GCC, GCG	(GCC)5, (GCG)4[d]	G (CDS)	
TTAT	(TTAT)4	na	
	(TC)12[a]	G (5'-region)	*
AT/TA	**(TA)20**	G (5'-region)	CS305H5-1
	(TA)7	TE (II)	

The class I (length ≥20 bp) microsatellite loci are highlighted. The superscript letter ([a, b, c] or [d]) designates homoeologous microsatellite loci. [1] is the homoeologous locus with the number of repeats smaller than in the specified search criteria identified for this locus; G is a gene; TE is the class I or II transposable element; na is nonannotated DNA sequences; 5'- and 3'-regions are noncoding DNA regions with a length of up to 1800 bp (in this study) adjacent to the start or terminal codons of the gene, respectively; CDS is the gene coding region; imp is an imperfect repeat; * means the locus is located at the border of the contig.

repeats associated with retrotransposons was previously found during the development of the barley genomic library saturated with microsatellite repeats (Ramsay et al., 1999).

Tetra- (three loci) and pentanucleotide (single locus) microsatellites were found only in nonannotated DNA sequences (Table 2). Of the nine microsatellites found in the gene loci, five were in 5'-noncoding gene regions; two, in the coding regions; and one, in the 3'-region and intron; at the same time, the trinucleotide repeat was localized in the coding region, while the dinucleotide microsatellites were localized in the others. The results of many studies indicate that different types of microsatellite loci are unevenly distributed in the plant genome. Thus, it was found that tri- and hexanucleotide repeats are most frequently found in the coding sequences of Arabidopsis, rice, maize, and wheat, while the noncoding fraction contains other types of microsatellites (Morgante et al., 2002). A high density of microsatellites in the sequences adjacent to the start codon (including 5'-untranslate region (5'-UTR)) was found in rice; at the same time, dinucleotide microsatellites (AG)n and (CT)n were the most well represented, while (AT)n was the prevalent dinucleotide in the rice genome (Grover et al., 2007). The high frequency of microsatellites in the regions adjacent to the start codon can assume its functional role. The microsatellite markers obtained based on such loci are of interest for the development of functional genetic markers in order to use them in functional studies and breeding.

We note that the homoeologous microsatellite loci that we found were located either in the genes (including the 5'-regions adjacent to the start codon) or in nonannotated sequences (Table 2) reflecting the conserved nature of these regions. The comparison of the structure of these loci demonstrated that the divergence in them is associated both with the change in the number of repeats and with the nucleotide substitutions (Fig. 1); the presence of the insertion–deletion polymorphism and single nucleotide substitutions in the regions flanking the microsatellite repeats allows us to develop locus-specific SSR markers (Tables 1, 2).

Temnykh et al. (2001) suggested dividing the microsatellite loci into two classes depending on the length (class I (≥20 bp) and class II (≤19 bp)). Such separation into classes reflects the potential of microsatellites as molecular markers, since class I is highly polymorphic, while class II does not differ by the mutation frequency from the unique DNA sequences (Temnykh et al., 2001). Among the microsatellites that we identified, 14 loci belonged to class I (Table 2), while five of them were associated with TE (Table 2) and were not suitable for the development of markers, since nonconserved flanking sequences do not allow us to develop locus-specific SRR markers. It was not possible to develop one of the primers for several class I loci located on the border of the contigs (Table 2). The primer pairs were developed to seven microsatellite loci and tested on nine bread wheat lines and varieties. The information about the developed markers is presented in Table 1.

```
                10        20        30        40        50        60        70
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....
2A      CCATTGGGGAGAAAATGTTCATCGATCTTGCTGCCTCCATG---ATGTGTGAGTAGT-CCTTCAGGACCTACGAG
2B      CCAGTGGGGAGACAATCTTCATCGAGCTTGCTGTCTCCATGGTGATGTGTGAGTAGTTCCTTCAGGACCTACGGG
2D      CCATTGGGGAGACAATGTTCATCGACCTTGCTGCCTCCATG---ATGTGTGAGTAGTTCCTTCAGGACCTACGAG

            80        90        100       110       120       130       140
        |....|....|....|....|....|....|....|....|....|....|....|....|....|....
2A      TCCATAGCAGTAGCTAGACGTCTCTCTCTCTCTCCTCCTCTCTCTC--------CAATACAATGATCTCCTCT
2B      TCCATAGCAGTAGCTAGATCTCTCTCTCCATCTCTCTCTCTCTCTCTCGATCTTCAACACAATGATCTCCGCT
2D      TCCATAGCAGTAGCTAGACGTTTCTCTCTCCTC------TCTCTCTCTCTC----CAATACAATGATCTCCTCT
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Fig. 1. Structure of homoeologous microsatellite loci.
The regions of the microsatellite CS184F24-1 marker primers are underlined. Complementary sequences of the contigs of the CS248B13 (2A) and CS305H5 (2D) BAC-clones containing the microsatellite loci were used for the alignment.

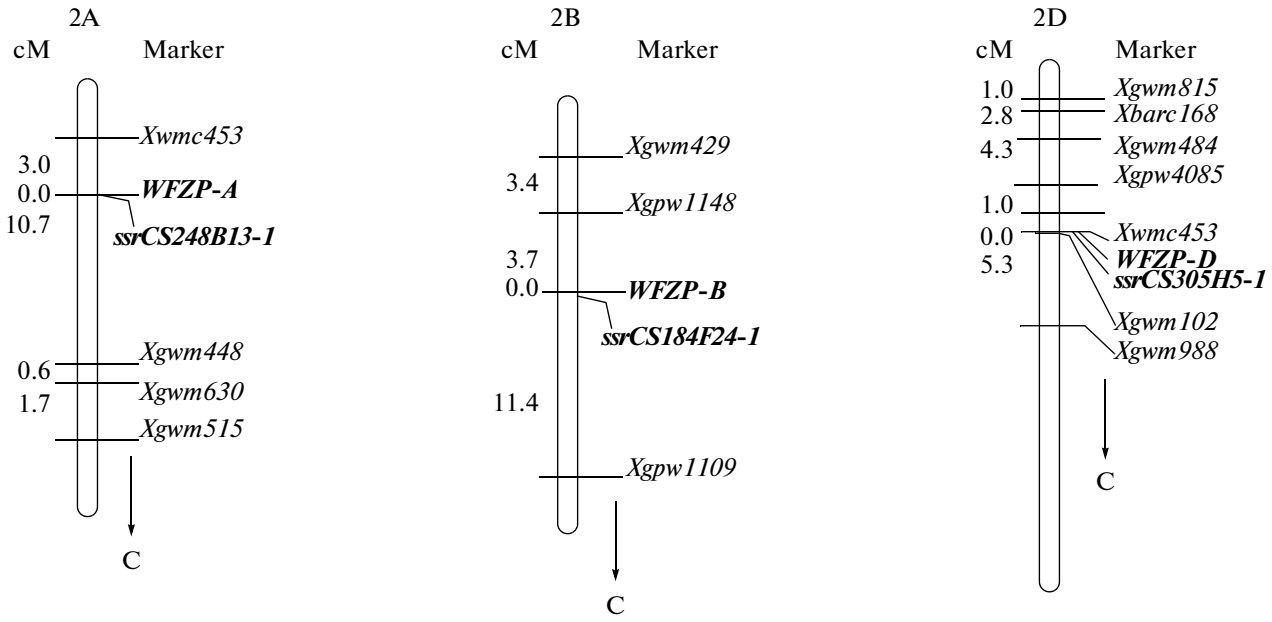

Fig. 2. Microsatellite maps of the chromosomes 2AS, 2BS, and 2DS, including the *WFZP-A-B-D* genes.
C is centromere; genetics distances in cM are indicated to the left of each map; the names of the microsatellite markers and genes, to the right.

Polymorphic markers were used for the genotyping of individual plants from the F_2 population obtained from the crossing between the Chinese Spring and Renan bread wheat varieties and were integrated in the previously constructed genetic maps of chromosomes 2AS, 2BS, and 2DS (Dobrovolskaya et al., 2015). It was found that they are colocalized with the *WFZP-A*, *WFZP-B*, and *WFZP-D* genes (Fig. 2). The microsatellite CS248B13-1 and CS305H5-1 markers can be subsequently used for marking the *WFZP-A* and *WFZP-D* gene loci in the genetic background of different wheat varieties, including during the marker-assisted transfer of these genes, along with the previously developed allele-specific markers (Dobrovolskaya et al., 2015). In addition, new microsatellite markers are of interest for the BAC clone marking and genetic mapping of the genes localized in the gene-rich 2S0.8 region of the homoeologous group-2 chromosomes.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Choulet, F., Wicker, T., Rustenholz, C., Paux, E., Salse, J., Leroy, P., Pingault, L., Sourdille, P., Couloux, A., Paux, E., Leroy, P., Mangenot, S., Guilhot, N., Le Gousis, J., Alaux, M., et al., Megabase level sequencing reveals contrasted organization and evolution patterns of the wheat gene and transposable element spaces, *Plant Cell*, 2010, vol. 22, pp. 1686–1701. doi 10.1105/tpc.110.074187

Choulet, F., Alberti, A., Theil, S., Glover, N., Barbe, V., Daron, J., Pingault, L., Sourdille, P., Couloux, A., Paux, E., Leroy, P., Guilhot, N., Le Gouis, J., Balfourier, F., Alaux, et al., Structural and functional partitioning of bread wheat chromosome 3B, *Science*, 2014, vol. 345, pp. 1249721–1. doi 10.1126/science.1249721

Dobrovolskaya, O.B., Sourdille, P., Bernard, M., and Salina, E.A., Chromosome synteny of the genome of two evolutionary wheat lines, *Russ. J. Genet.*, 2009, vol. 45, pp. 1368–1375.

Dobrovolskaya, P., Boeuf, C., Salse, J., Pont, C., Sourdille, P., Bernard, M., and Salina, E., Microsatellite mapping of *Ae. speltoides* and map-based comparative analysis of the S, G, and B genomes of Triticeae species, *Theor. Appl. Genet.*, 2011, vol. 123, pp. 1145–1157. doi 10.1007/s00122-011- 1655-z

Dobrovolskaya, O., Pont, C., Sibout, R., Martinek, P., Badaeva, E., Chosson, A., Watanabe, N., Prat, E., Gautier, N., Gautier, V., Oncet, C., Orlov, Y.L., Krasnikov, A.A., Berges, H., Salina, et al., *FRIZZY PANICLE* drives supernume (*T. aestivum* L.), *Plant Physiol.*, 2015, vol. 167, pp. 189–199. doi 10.1104/pp.114.250043

Dobrovoskaya, O., Martinek, P., Voylokov, A.V., Korzun, V., Röder, M.S., and Börner, A., Microsatellite mapping of genes that determine supernumerary spikelets in wheat (*T. aestivum*) and rye (*S. cereale*), *Theor. Appl. Genet.*, 2009, vol. 119, pp. 867–874. doi 10.1007/ s00122-009-1095-1

Erayman, M., Sandhu, D., Sidhu, D., Dilbirligi, M., Baenziger, P.S., and Gill, K.S., Demarcating gene-rich regions of the wheat genome, *Nucleic Acids Res.*, 2004, vol. 32, pp. 3546–3565. doi 10.1093/nar/gkh639

Ganal, M.W. and Röder, M.S., Microsatellite and SNP markers in wheat breeding, in *Genomics Assisted Crop Improvement*, Varshney, R.K. and Tuberosa, R., Dordrecht, 2007, vol. 2.

Grover, A., Aishwarya, V., and Sharma, P.C., Biased distribution of microsatellite motifs in the rice genome, *Mol. Gen. Genom.*, 2007, vol. 277, pp. 469–480. doi 10.1007/s00438-006-0204-y

IWGSC (International Wheat Genome Sequencing Consortium). A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome, *Science*, 2014, vol. 345, no. 6194. doi 10.1136/science.1251788

Kosambi, D.D., The estimation of map distances from recombination values, *Ann. Eugen.*, 1943, vol. 12, pp. 172–175.

Lagercrantz, U., Ellegren, H., and Andersson, L., The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates, *Nucleic Acids Res.*, 1993, vol. 21, pp. 1111–1115.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., and Newburg, L., MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations, *Genomics*, 1987, vol. 1, pp. 174–181.

Leonova, I.N., Röder, M.S., Kalinina, N.P., and Budashkina, E.B., Genetic analysis and localization of loci controlling leaf rust resistance of *Triticum aestivum* × *Triticum timopheevii* introgression lines, *Russ. J. Genet.*, 2008, vol. 44, no. 12, 1431–1437.

Leonova, I.N., Roder, M.S., Kalinina, N.P., and Budashkina, E.B., Geneticheskii analiz i lokalizatsiya lokusov, kontroliruyushchikh ustoichivost' introgressivnykh linii Triticum aestivum Triticum timopheevii k listovoi rzhavchine, *Genetika*, 2008, vol. 44, pp. 1652–1659.

Li, Y.-C., Korol, A.B., Beiles, A., and Nevo, E., Microsatellites: Genomic distribution, putative functions and mutational mechanisms: A review, *Mol. Ecol.*, 2002, vol. 11, pp. 2453–2465. doi 10.1046/j.1365-294X.2002.01643.x

Maia, L.C.D., Palmieri, D.A., Souza, V.Q.D., and Kopp, M.M., and Costa de Oliveira, A., SSR locator: Tool for simple sequence repeat discovery integrated with primer design and PCR simulation, *Int. J. Plant Genomics*, 2008. doi 10.1155/2008/412696

Morgante, M., Hanafey, M., and Powell, W., Microsatellites are preferentially associated with nonrepetitive dna in plant genomes, *Nat. Genet.*, 2002, vol. 30, pp. 194–200. doi 10.1038/ng822

Nicot, N., Chiquet, V., Gandon, B., Amilhat, L., Legeai, F., Leroy, F., Bernard, M., and Sourdille, P., Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags (ESTs), *Theor. Appl. Genet.*, 2004, vol. 109, pp. 800–805. doi 10.1007/s00122-004-1685-x

Paux, E., Sourdille, P., Salse, J., Saintenac, C., Choulet, F., Leroy, P., Korol, A., Michalak, M., Kianian, S., Spielmeyer, W., Lagudah, E., Somers, D., Kilian, A., Alaux, M., and Vautrin, S., A physical map of the 1-gigabase bread wheat chromosome 3B, *Science*, 2008, vol. 322, pp. 101–104. doi 10.1126/science.1161847

Ramsay, L., Macaulay, M., Cradle, L., Morgante, M., Ivanissevich, S.D., Maestri, E., Powell, W., and Waugh, R., Intimate association of microsatellite repeats with retrotransposons and other dispersed repetitive elements in barley, *Plant J.*, 1999, vol. 17, pp. 415–425. doi 10.1046/j.1365- 313X.1999.00392.x

Röder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leroy, P., and Ganbal, M.W., A microsatellite map of wheat, *Genetics*, 1998, vol. 149, pp. 2007–2023.

Salina, E.A., Leonova, I.N., Efremova, T.T., and Röder, M.S., Wheat genome structure: Translocations during the course of polyploidization, *Funct. Integr. Genomics*, 2006, vol. 6, pp. 71–80.

Sia, E.A., Jinks-Robertson, S., and Petes, T., Genetic control of microsatellite stability, *Mitat. Res.*, 1997, vol. 383, pp. 61–70.

Tautz, D. and Renz, M., Simple sequences are ubiquitous repetitive components of eukaryotic genomes, *Nucleic Acids Res.*, 1984, vol. 12, pp. 4127–4138.

Temnykh, S., DeClerck, G., Lukashova, A., Lipovich, L., Cartinhour, S., and McCouch, S., Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): Frequency, length variation, transposon association, and genetic marker potential, *Genome Res.*, 2001, vol. 11, pp. 1441–1452. doi 10.1101/gr.184001

Thuillet, A.C., Bru, D., David, J., Roumet, P., Santoni, S., Sourdille, P., and Bataillon, T., Direct estimation of mutation rate for 10 microsatellite loci in durum wheat, *Triticum turgidum* (L.) Thell. ssp durum Desf, *Mol. Biol. Evol.,* 2002, vol. 19, pp. 122–125.

Wang, Y., Yang, C., Jin, Q., Zhou, D., Wang, S., Yu, Y., and Yang, L., Genome-wide distribution comparative and composition analysis of the SSRs in Poaceae, *BMC Genet.*, 2015. doi 10.1186/s12863-015-0178-z

Webster, M.T., Smith, N.G.C., and Ellegren, H., Microsatellite evolution inferred from human-chimpanzee genomic sequence alignments, *Proc. Natl. Acad. Sci. USA*, 2002, vol. 99, pp. 8748–8753.

Wierdl, M., Dominska, M., and Petes, T.D., Microsatellite instability in yeast: Dependence on the length of the microsatellite, *Genetics*, 1997, vol. 140, pp. 769–779.

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