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EVALUATION OF THE GENETIC VARIABILITY OF HOMOEOLOGOUS GROUP 3 SSRs IN BREAD WHEAT

Thorough characterization of the genetic variability in bread wheat (Triticum aestivum L.) is important for a better improvement of this key crop and to increase cereal yield in the context of sustainable agriculture to face human needs in the next decades. To study the genetic variability of SSRs on wheat homoeologous group 3 chromosomes, we characterized 38 hexaploid and two tetraploid wheat lines using a set of 165 microsatellites that we cytogenetically assigned to the 17 deletion bins for chromosomes group 3. A comparative analysis of the genetic variability through the PIC value study, allele numbers and SSR lengths indicated that there were no statistically significant differences ($p > 0.05$) between the three chromosomes of this homoeologous group despite the fact that SSRs from chromosome 3B exhibited slightly more alleles per locus compared to chromosomes 3A and 3D as well as slightly higher PIC values compared to chromosome 3D. However, there was a stronger correlation between SSR length and allele number on the short arms compared to the long arms and the correlation increased from the centromeres toward the telomeres. We did not find statistically significant differences in allele numbers and PIC values for SSRs located in more distal bins on 3A and 3B chromosomes. On the contrary, for chromosome 3D, we observed significant differences ($p < 0.05$) between the PIC values determined for SSRs assigned to deletion bin 3DL3–0.81–1.00 bin that is located distal compare to the more proximal region (C-3DL3–0.81). These results suggest that recombination which is higher in the telomeric regions does not contribute to increase a lot the variability of the SSRs.

Introduction. Genetic variability in cereals and especially in hexaploid wheat is extremely extended but remains largely underexploited. Characterization of genetic variation within natural populations and among breeding lines is crucial for effective conservation and exploitation of genetic resources for crop improvement programs. Thus, their evaluation should be a major activity of germplasm centres to identify useful genetic variation and make it available to breeders. A range of methods are now available to depict and evaluate genetic resources diversity. Especially Simple Sequence Repeats (SSRs), also called microsatellites (MS), have proven to be useful for assessment of genetic variation in wheat germplasm collections [1, 2]. SSRs are a widespread and highly abundant class of repeated DNA sequences within genomes of wheat [3–9]. They have been found to be dispersed throughout the genomes with additional clusters at heterochromatic regions [10, 11]. Both mutation factors and indirect selective events vary according to the local recombination rate and therefore jointly influence the level of polymorphism at microsatellite loci in wheat [12]. However, we have no idea whether their genetic variation is uniform overall the genome or located in specific regions (coding vs non-coding regions), and if it also varies along the chromosomes. One way to answer this question would be to study the genetic variability of SSRs distributed all over a chromosome and compare it according to their cytogenetical and/or physical position.

Recent progress in wheat genomics has made possible the elaboration of wheat chromosome-specific BAC libraries [13–15]. Moreover, the International Wheat Genome Sequencing Consortium (IWGSC, http://www.wheatgenome.org) established in January 2005, aims at the construction of an accurate, sequence-ready physical map of the hexaploid wheat genome (cv. Chinese Spring) which is anchored to the genetic map. In order to have a model to study the structure, function and evolution of the wheat genome, a physical map of wheat chromosome 3B is currently under construction [16]. Chromosome 3B is 995 Mb in length [17], which represents about 5.86 % of the nuclear genome of bread wheat (16.974 Mb/1C) [18]. It also shows a wide range of C-banding patterns [19]. Moreover, gene density is higher at its ends [20–22], and comparisons with chromosomes from other related species indicate that it is the most conserved in gene content and order [23].

Table 1

Name and country of origin of the wheat cultivars tested. (Durum wheats are indicated with *)

Country of origin	Cultivars
Afghanistan	A4 (748)
Australia	Aurore (1110)
Belgium	Zandra (8058)
Brazil	Cotipora (2353)
Canada	Glenlea (3358)
China	Chinese Spring
Germany	Opal (5486)
Spain	Xeres* (7657)
Finland	Hopea (9048)
France	Apache (13481), Arche (964), Courtot (2358), Eureka (2830), Magnif 27 (4503), OxC37, Ornicar (13471), Pernel (5681), Récital (6027), Renan (6086), Soisson (6607), Thésée (6932), V23, Oligoculm
Hungary	Gobolloi 15 (3366)
India	NP120 (5308)
Israel	M708 (4482)
Italy	Frumento Cupo* (3173)
Japan	Nyu Bay (5399)
Korea	Seu Seun 27 (6529)
Libanon	Miskaagani (4874)
Mexico	W7984, Bob White, Opata (13811), Pitic 62 (5748)
Nepal	Chyamtang (2171)
Portugal	Mocho de Espiga Branca (4901)
Russia	Bezostaya 1 (1341), Chortandinka (2153)
Turkey	Coppadra (2330)
Yugoslavia	Balkan (1192)

In addition, chromosome 3B also carries many genes of agronomical interest such as resistance genes [24–26], pre-harvest sprouting tolerance genes [27] as well as numerous enzyme-coding genes (for a review, see [28]). Chromosome 3B also exhibits internal as well as external duplications according to content, distribution, and comparative-genome relationship analyzes of expressed sequence tags (ESTs) [23].

The purposes of the present study were: 1) to evaluate the variability of wheat SSRs distributed throughout the chromosomes from homoeologous group 3 (3A, 3B and 3D) using a set of lines covering a large part of the existing genetic variability; 2) to compare the variability at the distal part of chromosome arms vs pericentromeric regions for 3A, 3B and 3D chromosomes.

Materials and methods. ***Plant material.*** A total of 40 wheat varieties (38 bread and two durum wheats, Table 1) were used to analyze the genetic variability of SSRs. These lines were selected according to their geographical origins, growth habits (winter or spring type), date of release (landraces, old or recent cultivars) and data on neutral polymorphism based on a set of 42 microsatellite loci, one per chromosome arm [29]. The lines covered 92 % of the world genetic variability based on the evaluation of a core collection [30]. This panel also included the parental lines of several genetic mapping populations. Seeds were obtained from the Centre of Biological Resources on Cereal Crops, INRA-Clermont-Ferrand. Moreover, the set of the three nulli-tetrasomic (NT) and six ditelosomic (DT) wheat lines from homoeologous group 3 ([31, 32]; kindly provided by Dr Steve Reader, John Innes Centre, United Kingdom) was used for chromosomal assignment of markers. A more accurate location of the SSRs on the chromosomes was obtained using a set of respectively four, five and three deletion lines from chromosomes 3A (3AS-4, 3AS-2, 3AL-3, 3AL-5), 3B (3BS-8, 3BS-9, 3BS-1, 3BL-10, 3BL-7) and 3D (3DS-6, 3DS-3, 3DL-3) [33]. Between five and 10 seeds from self-pollinated ears of each lines were sown for further DNA extraction.

DNA extraction, PCR amplification and SSR detection. DNA was extracted from fresh leaves ground in liquid nitrogen using a CTAB protocol [34]. A set of 41, 76 and 48 SSRs was selected from Graingenes data [35] and Somers et al. [9] for chromosomes 3A, 3B and 3D, respectively. SSR order along the chromosomes that is presented in the tables and diagrams was derived from the maps that indicated in Graingenes and in [9] and according to our assignment using deletion bin and ditelosomic lines. PCR reactions using the M13 protocol were carried out as described in [36] with an annealing temperature of 60 °C for 30 cycles (30 sec 94 °C, 30 sec 60 °C, 30 sec 72 °C) and 56 °C for 8 cycles. Amplification products were visualized using an ABI PRISM®3100 Genetic Analyzer (Applied Biosystems). Finally, fragment sizes were calculated using Genescan and Genotyper softwares (Applied Biosystems), where different alleles are represented by different amplification sizes for tandem repeats. Two alleles were considered identical when they showed the same fragment size.

Estimation of PIC values and statistical analyses. Allelic polymorphism information content (PIC) values were calculated in each case using the formula PIC = $1-\sum (Pi)^2$, where P*i* is the proportion of the population carrying the i^{th} allele, calculated for each SSR locus [37]. Comparative analyses of SSR genetic variability overall the chromosomes were computed as follows. For better revealing variability tendencies for microsatellites distributed along chromosomes a simple standard procedure of 5 points smoothing with equal weights for each point involved was applied, according to the usefulness of sequences smoothing procedure recommended by Tukey [38]. Linear regressions between the length and allele number of microsatellites were also calculated. For narrowness of dependencies, coefficients of determination were computed as R^2. Finally, to build up profiles of local correlation between length (define as the number of nucleotides forming the microsatellite X the number of repeats according values given in Graingenes database [35] and allele number, a procedure of point-by-point calculation was used («moving correlation»). Every consequent coefficient was based on 11 points with centrum in point 6. Using these conditions (df = 9), every single coefficient with level equal or greater than 0.6 is significant at p = 0.05. At shorter bases (<11 points), problems with significance may occur while using too long bases (>11) leads to over-losses of information in the two distal parts of chromosomes.

Significance of differences between allele numbers, microsatellite lengths and the PIC values for microsatellites located on homoeologous group 3 chromosomes, on the short and long arms, and those assigned to deletion bins was estimated for this Mann-Whitney U-test [39] the method of nonparametric statistics was applied.

Results. ***Genetic variability of the SSRs.*** Respectively 41, 76 and 48 SSRs were evaluated for chromosomes 3A, 3B and 3D. Results are given in detail in Tables 2–4. This represented a density of one marker every 2.97, 1.95 and 1.65 cM corresponding to one marker every 20.2, 13.1 and 16.1 Mb respectively, which was not statistically different between the different genomes. The number of alleles varied from one (several loci not polymorphic in the plant material tested, but assigned to homoeologous group 3 chromosomes using aneuploid lines) to 21 alleles for loci *Xgpw4431-3B* and *Xcfa2134-3A*. Respectively 276, 524 and 290 alleles were scored for chromosomes 3A, 3B and 3D, which corresponded to means of 6.73, 6.89 and 6.04 alleles/locus. The PIC values for the SSRs were similar and not statistically different between the three chromosomes from homoeologous group 3. They ranged from 0 to 0.91 for chromosome 3A, from 0 to 0.93 for chromosome 3B and from 0 to 0.93 for chromosome 3D with respective means of 0.61, 0.58 and 0.54.

Concerning the type of repeats, we found that dinucleotide repeats were the most abundant class of microsatellite markers mapped on chromosomes from homoeologous group 3 of *T. aestivum*. About 73.2 % of the tested SSRs located on chromosome 3A have dinucleotide motif (25 simple and 5 compound) while this proportion was about 71 % (40 simple and 14 compound) and 75 % (33 simple and 3 compound) for microsatellites from chromosomes 3B and 3D respectively (Table 2–4). This was not unexpected since most of the microsatellites that have been genetically mapped until now are mainly issued from enriched or screened libraries using dinucleotide motifs for enrichment [3, 8, 40]. Only one microsatellite motif $(AT)_n$ and no microsatellite motifs $(GC)_n$ were observed, probably because these motifs were rarely used for library enrichment or screening and also because they are difficult to isolate due to the formation of hairpin loops during the enrichment steps. Here, for dinucleotide motifs, the average number of alleles ranged from 6.25 for $(GT/CA)_n$ to 8.65 for $(GA/CT)_n$. For trinucleotide and tetranucleotide motifs, the average numbers of alleles were respectively 3.1 and 4.6 which was significantly lower than for dinucleotide motifs.

Variability of microsatellite loci along chromosome 3A. Between one and 14 SSR loci have been assigned to the six deletion bins from chromosome 3A (Table 2). Especially, four SSRs loci were assigned to deletion bin 3AS2 – 0.23–0.45 while none were assigned to this bin previously [41, 42]. The allele number for the 41 microsatellites which were located on chromosome 3A ranged from one for the three loci *Xgpw7031-3A*, *Xgpw7553-3A* and *Xgpw7467-3A* to 21 for the locus *Xcfa2134-3A*. The PIC values ranged from 0 to 0.91 (mean 0.61) while SSR length varied from 14 to 156 bp. We analyzed the variation of allele numbers, microsatellite lengths and PIC values along this chromosome

Table 2

Microsatellite markers from chromosome 3A evaluated for genetic variability and PIC values

Part of chromosome and loci	Motif	Length	Numbers of alleles	PIC	Fragment size in 'CS' (in bp)
3AS4 – 0.45–1.0					
Xgpw7031	$(AGC)_6$	18	1	0,00	192
Xwmc532	$(GA)_5$ / $(GA)_{11}$	32	7	0,80	195
Xbarc45	$(TAA)_{10}$	30	7	0,54	202
Xgpw4074	$(CT)_{15}$	30	4	0,63	261
Xgpw2142	$(GT)_{40}$	80	5	0,36	266
Xgwm2	$(CA)_{18}$	36	9	0,72	143
Xwmc505	$(CA)_{31}$	62	5	0,69	127
Xgpw4221	$(TC)_{12}$	24	4	0,43	262
3AS2 – 0.23–0.45					
Xgwm674	$(CT)_{16}$ / CCC / $(GT)_4$	43	5	0,51	184
Xcfa2234	(CA)17	34	3	0,47	161
Xgpw5216	$(GA)_{21.5}$ / $(CATG)_7$	71	3	0,53	355
Xgpw3223	$(AG)_{27}$	54	11	0,88	298
C-3AS3 – 0.23					
Xwmc664	$(GA)_{44}$	88	15	0,89	179
C-3AL3 – 0.42					
Xgpw8038	$(TC)_{19}$	38	5	0,69	170
Xgpw7553	$(CTG)_6$	18	1	0,00	150
Xgpw7467	$(CAG)_{16}$ / $(CAA)_{30}$ / $(CAG)_6$	156	1	0,00	297
Xgpw5007	$(CT)_{23}$	46	8	0,84	199
3AL3 – 0.42–0.78					
Xwmc428	$(CA)_{25}$ / $(CA)_4$	58	8	0,67	276
Xwmc153	$(GT)_{20}$ / $(GT)_{18}$ / $(GT)_{11}$	98	10	0,77	201
Xgpw2169	$(TG)_{21}$	42	7	0,73	330
Xgpw5042	$(CA)_9$	18	6	0,67	191
Xbarc67	$(GATA)_9$ / $(GATA)_3$	48	5	0,44	122
Xwmc627	$(GA)_9$	18	8	0,75	134
Xcfa2134	$(CT)_{23}$	46	21	0,91	258
Xgpw8072	$(CG)_7$	14	5	0,72	172
Xgpw2266	$(CA)_{16}$	32	16	0,84	280
Xgpw4352	$(GT)_{15}$	30	5	0,59	275
3AL5 – 0.78–1.00					
Xgwm480	$(CT)_{16}(CA)_{13}$	58	10	0,60	189
Xwmc264	$(GA)_{16}$	32	9	0,78	151
Xgpw7219	$(AGG)_7$	21	3	0,52	150
Xwmc594	$(GA)_{43}$	86	14	0,91	193
Xwmc559	$(GT)_{16}$ / $(GT)_{14}$ / $(GT)_{10}$	80	8	0,82	277
Xcfa2193	$(GT)_{12}$	24	8	0,83	228
Xgpw7643	$(CAG)_{9.7}$ / $(CAG)_5$ / $(GCA)_{24}$	116,1	3	0,52	374
Xgpw7663	$(GGC)_5$	15	2	0,23	314
Xcfa2170	$(GA)_{31}$	62	6	0,65	219
Xgpw307	$(CA)_{21}$	42	5	0,71	148
Xgpw5271	$(AG)_{12}$	24	5	0,64	255
Xcfa2183	$(CA)_{26}$	52	7	0,68	178
Xcfa2037	$(CA)_{23}$	46	4	0,38	196
Xwmc169	$(CA)_{25}$	50	7	0,62	127

Table 3

Microsatellite markers from chromosome 3B evaluated for genetic variability and PIC values

Part of chromosome and loci	Motif	Length	Numbers of alleles	PIC	Fragment size in 'CS' (in bp)
3BS8 – 0.78–1.00					
Xgpw8020	$(CT)_8$	16	2	0,50	null
Xgpw7757	$(GCC)_6$	18	3	0,53	243
Xbarc75	$(TAG)_2$ / $(TAGA)_5$	26	4	0,58	125
Xbarc180	$(ATT)_{21}$	63	8	0,77	212
Xbarc147	$(CA)_{14}$	28	13	0,65	177
Xgwm493	$(CA)_{43}$ imperfect	86	12	0,82	212
Xgpw3156	$(C)_{17}$	17	5	0,67	130
Xgwm389	$(CT)_{14}$ / $(GT)_{16}$	60	11	0,87	131
Xwmc623	$(GA)_{31}$	62	15	0,86	208
Xgpw7031	$(AGC)_6$	18	1	0,00	198
Xgpw3092	$(AG)_{15}$	30	11	0,78	200
3BS9 – 0.57–0.78					
Xcfa2191	$(TCCC)_4$	16	2	0,10	179
Xgwm533	$(CT)_{18}$ / $(CA)_{20}$	76	11	0,76	172
Xbarc092	$(TTA)_{11}$	33	3	0,38	169
Xcfa2226	$(GCT_6$	18	4	0,49	192
Xgpw7774	$(AGC)_8$	24	3	0,49	130
Xgpw7452	$(CA)_{23}$	46	7	0,70	136
Xwmc231	$(GA)_{10}$ / $(GT)_8$	36	7	0,75	247
Xgpw4146	$(TG)_{15}$	30	7	0,62	null
Xwmc43	$(GT)_{14}$	28	4	0,27	342
Xgwm284	$(GA)_{17}$	34	6	0,80	138
Xgwm566	$(CA)_{21}$ / $(GA)_2$ / $(TA)_8$	62	8	0,79	148
barc068	$(TC)_3$ / $(TATC)_6$ / $(TATC)_7$	30	6	0,76	151
Xwmc777	$(GA)_{36}$	72	7	0,53	157
3BS1 – 0.33–0.57					
Xgwm77	$(CA)_{10}$ / $(GA)_{40}$	100	10	0,53	168
Xwmc78	$(CA)_{18}$	36	9	0,77	279
Xwmc540	$(GT)_9$	18	2	0,22	239
Xwmc533	$(CA)_{20}$	40	1	0,00	150
Xwmc762	$(GA)_{34}$	68	15	0,87	140
Xgpw1120	$(CA)_{24}$	48	8	0,85	265
C-3BS1 – 0.33					
Xgpw4207	$(TG)_{12}$	24	6	0,60	368
Xwmc612	$(GA)_{40}$	80	15	0,88	302
Xwmc274	$(GAAAA)_2$	45	1	0,00	153
Xwmc625	$(CT)_{10}$ / $(CT)_8$	36	11	0,59	141
Xgpw3134	$(GA)_{19}$	38	6	0,77	130
Xwmc544	$(CA)_{14}$ / $(CA)_{10}$	48	7	0,59	398
Xgpw4225	$(TC)_{26}$	52	20	0,93	202
Xgwm285	$(GA)_{27}$	54	17	0,88	241
C-3BL10 – 0.50–0.63					
Xgpw1145	$(CA)_{19}$	38	4	0,38	187
Xwmc1	$(CT)(CA)$ / $(CA)_{14}$	32	6	0,84	173
Xgpw3254	$(AG)_{13}$	26	7	0,75	250
Xgwm376	$(CA)_{16}$ / $(GA)_{22}$	76	8	0,80	158
Xgpw2266	$(CA)_{16}$	32	1	0,00	280

Continued Table 3

Part of chromosome and loci	Motif	Length	Numbers of alleles	PIC	Fragment size in 'CS' (in bp)
Xgpw7220	$(AG)_7$	14	2	0,43	160
Xgpw7816	$(CAC)_{8.7}$	26,1	4	0,34	275
Xgpw4078	$(TG)_{16.5}$	33	7	0,71	251
Xwmc307	$(GT)_8$ / $(GA)_{13}$	42	6	0,76	166
Xgpw4034	$(GT)_{16}$	32	7	0,73	246
Xgpw1107	$(GA)_{19}$	38	5	0,70	148
Xgpw7805	$(CGG)_{7.3}$ / $(TCC)_4$	33,9	1	0,00	184
Xwmc527	$(CA)_{15}$ / $(GA)_{20}$	70	9	0,72	397
Xgpw3053	$(TG)_{10}$	20	5	0,70	313
Xwmc291	$(GT)_{26}$	52	7	0,82	245
Xgpw7467	$(CAG)_{16}$ / $(CAA)_{30}$ / $(CAG)_6$	156	1	0,00	300
Xgpw4451	$(CT)_{20}$	40	2	0,15	152
Xwmc326	$(CT)_{16}$	32	13	0,88	204
Xwmc632	$(GA)_{38}$	76	15	0,90	198
Xgpw7335	$(GCC)_4$	12	1	0,00	384
3BL10 – 0.50–0.63					
Xgpw4497	$(TC)_{37}$ / $(CA)_{24}$	122	13	0,91	193
Xgpw4431	$(TC)_{24}$	48	21	0,93	280
Xbarc164	$(ATT)_{18}$	54	11	0,83	222
Xwmc418	$(GT)_{13}$	26	1	0,00	266
Xgpw4143	$(TC)_{12}$	24	5	0,68	245
3BL7 – 0.63–1.0					
Xcfd283	$(CCCA)_3$	12	2	0,38	252
Xgpw4505	$(TC)_{17}$	34	7	0,78	176
Xgpw8064	$(AT)_8$	16	2	0,44	149
Xgpw1025	$(CA)_{48}$	96	9	0,75	181
Xcfa2170	$(GA)_{31}$	62	4	0,53	176
Xbarc77	$(ATCT)_6$ / $(ATCT)_{18}$	96	12	0,88	226
Xgpw7586	$(GTG)_4$	12	1	0,00	348
Xgpw7213	$(CGCCG)_3$	15	2	0,40	330
Xgpw5007	$(CT)_{23}$	46	4	0,38	165
Xgpw5271	$(GA)_{10}$ / $(GA)_{12}$	44	1	0,00	222
Xgpw7088	$(CATG)_7$ / $(GCATG)_5$	53	2	0,49	207
Xgwm247	$(GA)_{24}$	48	13	0,90	180
Xgwm340	$(GA)_{26}$	52	15	0,89	128

using the standard method of moving means (see M&Ms; Fig. 1, *a*). The three curves followed similar profiles on the short arm of the chromosome as well as on the distal part of the long arm (deletion bin 3AL5 – 0.78–1.00). On the contrary, the SSR length curve showed an opposite profile compared to the other two along the deletion bins C-3AL3 – 0.42 and 3AL3 – 0.42–0.78 (slanting arrows on Fig. 1, *a*). Moreover, these two bins showed different profiles: in the former, SSR length was high compared to the level of polymorphism they revealed while in the latter, SSRs were short but revealed a high level of polymorphism. In order to further study the effect of SSR length on allele number, the diagrams of moving correlation between these two parameters at microsatellite loci along the chromosome 3A were built (Fig. 2, *a*). We observed that the correlation was high ($r > 0.4$) in the middle of each arm while it was lower near the telomere. The correlation between SSR length and allele number was negative in bin C-3AL3 – 0.42 contrary to the distal part of bin 3AL3 – 0.42–0.78 where correlation was highly positive. This suggests that the correlation between length

Table 4

Microsatellite markers from chromosome 3D evaluated for genetic variability and PIC values

Part of chromosome and loci	Motif	Length	Numbers of alleles	PIC	Fragment size in 'CS' (in bp)
3DS6 – 0.55–1.00					
Xcfd35	$(GT)_{13}$ / $(CT)_{20}$	66	7	0,78	207
Xgwm161	$(CT)_{15}$	30	7	0,64	172
Xcfd141	$(TCAA)_4$	16	2	0,11	172
Xcfd55	$(GT)_{23}$	46	8	0,80	295
Xgpw7553	$(CTG)_6$	18	2	0,05	152
Xgpw5248	$(CA)_{41.5}$	83	5	0,58	234
Xgpw5213	$(AG)_{23}$	46	15	0,91	294
Xwmc43	$(GT)_{14}$	28	4	0,17	350
Xcfd79	$(GA)_{26}$	52	8	0,80	280
Xgpw4074	$(CT)_{15}$	30	4	0,62	259
Xcfd70	$(CA)_{19}$	38	8	0,74	190
Xcfd34	$(GGA)_5$ / $(G)_{13}$	28	3	0,58	null
Xgpw5216	$(GA)_{2.5}$ / $(CATG)_7$	32	6	0,72	390
Xwmc533	$(CA)_{20}$	40	7	0,79	167
3DS3 – 0.24–0.55					
Xgpw5067	$(GA)_{17}$	34	3	0,55	279
Xgpw4163	$(AGA)_4$ / $(TC)_{21}$	54	9	0,85	409
Xgpw333	$(GT)_{13}$	26	4	0,37	287
Xgpw5104	$(CT)_{24}$	48	9	0,84	188
Xgwm341	$(CT)_{26}$	52	14	0,89	170
C-3DL3 – 0.81					
Xgpw1168	$(GGC)_5$	15	2	0,05	205
Xgpw5166	$(GA)_{19.5}$	39	5	0,59	155
Xgpw7467	$(CAG)_{16}$ / $(CAA)_{30}$ / $(CAG)_6$	156	2	0,10	323
Xgpw3109	$(AG)_{19}$	38	9	0,84	231
Xgpw2266	$(CA)_{16}$	32	1	0,00	236
Xgpw5042	$(CA)_9$	18	3	0,27	173
Xgwm52	$(GT)_4$ / AT / $(GT)_{20}$	50	7	0,75	170
Xgpw1062	$(CA)_{16}$	32	1	0,00	203
Xgpw4451	$(CT)_{20}$	40	5	0,48	166
Xgpw1149	$(CA)_{48}$	96	4	0,50	130
Xgpw8116	$(ATATA)_8$	40	1	0,00	189
Xcfd152	$(TC)_{24}$	48	10	0,80	308
Xgpw7586	$(GTG)_4$	12	2	0,06	393
Xgpw7663	$(GGC)_5$	15	2	0,05	327
Xgpw5123	$(CT)_{18.5}$	37	7	0,79	257
Xgpw7643	$(CAG)_{9.7}$ / $(CAG)_5$ / $(GCA)_{24}$	116,1	4	0,14	391
Xcfd127	$(GCC)_6$	18	2	0,06	170
Xgwm645	$(CT)_{23}$ imperfect	46	11	0,88	148
Xgwm383	$(GT)_{27}$	54	6	0,76	215
3DL3 – 0.81–1.00					
Xcfd211	($GA)_{17}$	34	3	0,61	295
Xcfd223	$(TG)_{18}$	36	7	0,73	188
Xgpw4136	$(CT)_{52}$	104	11	0,85	262
Xgpw5064	$(GA)_{17}$	34	14	0,90	364
Xgpw5067	$(GA)_{17}$	34	5	0,73	274
Xgpw294	$(CA)_{31}$	62	8	0,76	116

Continued Table 4

Part of chromosome and loci	Motif	Length	Numbers of alleles	PIC	Fragment size in 'CS' (in bp)
Xgpw5001	(GA)18	36	6	0,72	212
Xgpw5263	(TC)36	72	4	0,46	306
Xgpw5235	(GA)36	72	3	0,46	305
Xcfd9	(TC)29	58	20	0,93	259

(linked to the number of repeats) and polymorphism which is generally admitted is not systematic.

Variability of microsatellite loci along chromosome 3B. Between five and 20 SSR loci were assigned to the six deletion bins of chromosome 3B (Table 3). The allele number for the 76 microsatellite loci assigned to chromosome 3B ranged between one for ten loci [*Xgpw7031-3B, Xwmc533-3B, Xwmc274-3B, Xgpw2266-3B, Xgpw7805-3B, Xwmc418–3B, Xgpw7467-3B, Xgpw7586-3B, Xgpw7335-3B, Xgpw5271-3B]* to 21 for locus *Xgpw4431-3B*. The PIC values ranged from 0 to 0.93 (mean 0.58) while SSR lengths varied from 12 to 156 bp. Analysis of the variation of allele numbers, PIC values and lengths of microsatellites showed that these traits varied along the chromosome 3B (Fig. 1, *b*). For allele number, it was high at the distal part of the short arm and then decreased slightly in the middle of the arm to increase again in the pericentromeric region. On the long arm, the allele number was low close to the pericentromeric region and in deletion bin 3BL7 – 0.63–1.00 and increased close to and in deletion bin 3BL10 – 0.50–0.63. PIC values and SSR length followed similar patterns except in bins 3BS9 – 0.57–0.78 and C-3BL10 – 0.50 (white arrows on Fig. 1, *b*), where the PIC values were higher and in bin C-3BS1 – 0.33 where the length was lower. Similarly to chromosome 3A, the diagram of moving correlation between the SSR lengths and allele numbers was built (Fig. 2, *b*). The correlation between these two traits was very high (>0.5) on the short arm and in the pericentromeric region of the long arm. Then it decreased abruptly in the middle of the long arm and reached a minimum (–0.374). This was due to the data from locus *Xgpw7467-3B* and removing of this locus from the set of data changed the reduction of correlation from 0.9 to only 0.2 (data not shown). There was a general trend of correlation to increase from the centromere toward the telomere on both chromosome arms.

Variability of microsatellite loci along chromosome 3D. We analyzed the variation of PIC values, microsatellite lengths and allele numbers along the chromosome 3D (Fig. 1, *c*). Between five and 19 SSR loci were assigned to the four deletion bins of chromosome 3D (Table 4). Allele number for the 48 SSR loci assigned to chromosome 3D ranged from one for the three loci *Xgpw1062–3D, Xgpw2266–3D* and *Xgpw8116–3D* to 20 for locus *Xcfd9–3D*. PIC values ranged from 0 to 0.93 (mean 0.54) while SSR length varied from 12 to 156 bp. SSR lengths did not vary much along the short arm but they were a little higher on the long arm. On the contrary, PIC values and allele numbers were similarly higher in short arm and in bin 3DL3 – 0.81–1.00 (white arrows on Fig. 1, *c*). The diagram of moving correlation between SSR lengths and allele numbers was built (Fig. 2, *c*) and showed that the correlation increased considerably from the centromere toward the telomere of the short arm while on the contrary, there was almost no correlation on chromosome arm 3DL. In particular, the correlation was very high for the middle of the short arm (up to 0.8). In contrast, close to the centromere, the correlation equaled zero and remained weak on the rest of the long arm (mean r = 0.2).

Comparative analysis between homoeologous group 3 chromosomes. A comparative analysis of allele number, SSR length and PIC values between the three chromosomes from homoeologous group 3 reveals that each chromosome had unique pattern for the curves and we had not detect clear increasing of the allele numbers or SSR lengths with the distance from the centromere towards the distal part of chromosomes as we initially expected (see Fig. 1). Such a tendency was only slightly observed for the long arm of chromosome 3D. Concerning the correlations between SSR length and allele number, it increased from the pericentromeric region toward the telomeres and coefficients of correlations between SSR length and allele num-

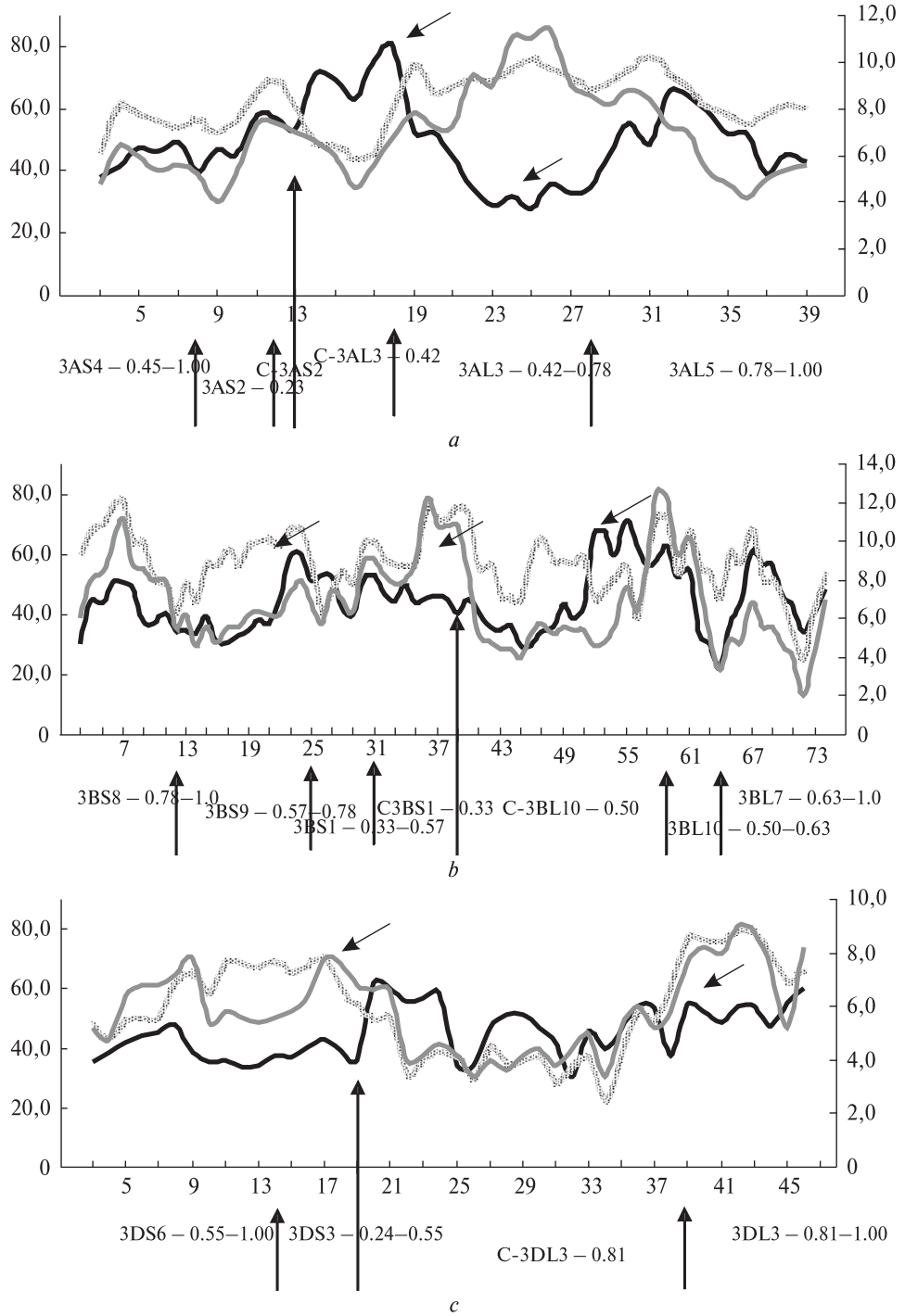

Fig. 1. Variability of allele number (grey curve), length (black curve) and PIC × 100 value (dotted curve) of microsatellites using the method of moving means (5 points) along chromosomes from homoeologous group 3 (*a* – chromosome 3A; *b* – chromosome 3B; *c* – chromosome 3D). Approximate position of the centromere is indicated with the long black arrow. X-axis: microsatellite loci numbers (according to Table 2–4) along the chromosomes. Telomeric regions of the short arms are on the left and the one of the long arms are on the right. Y-axis: left – length of microsatellite in bp and PIC value × 100; right – allele number. Important points as discussed in the text are indicated with white arrows

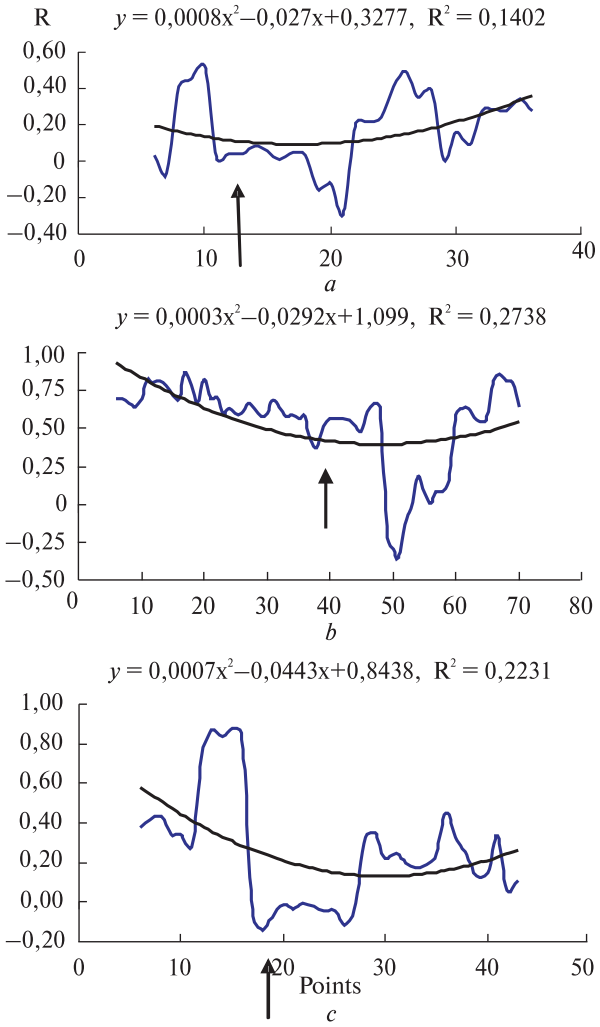

Fig. 2. Moving correlation (11 points) between allele number and microsatellite length along chromosomes from homoeologous group 3 (*a* – chromosome 3A; *b* – chromosome 3B; *c* – chromosome 3D) and general trend of variation tested along. X-axis: microsatellite loci number (according to Table 2–4) along the chromosomes. Approximate position of the centromere is indicated with an arrow. Telomeric short arm regions are on the left and long arms regions are on the right. Y-axis: correlation coefficients between allele number and the length of microsatellites

ber were higher on short arms of 3B and 3D chromosomes than on the long arms.

Discussion. It has been demonstrated that microsatellites are powerful molecular markers in wheat because of the high degree of polymorphism they reveal [4, 43] and also because of their high locus specificity compared to RFLP markers [8, 39, 44]. Thus, they constitute a major tool for establishing genetic variability in wheat collections [1, 2, 45]. Here, we used 165 microsatellites from wheat homoeologous group 3 in order to evaluate the variation of the genetic variability at the chromosome level and to compare the results according to the type and length of motifs as well as between the homoeologous chromosomes. We focused our attention on homoeologous group 3 chromosomes because this group has been retained as a model for the establishment of a physical map of the wheat genome prior to large scale sequencing by the International Wheat Genome Sequencing Consortium (IWGSC; http://www.wheatgenome.org).

Concerning the type and length of the microsatellite motifs, the dinucleotide motifs and especially $(GA)_n$ and $(CA)_n$ repeats appeared to be more abundant in our investigations. No enrichment was observed for microsatellites like $(GC)_n$ or $(AT)_n$, although (AT) microsatellites are very abundant in plants [46–48].

This was expected since these two latter motifs are rarely selected in enrichment or screening techniques [49, 3, 50]. Moreover, because of the complementarities of the motif bases at the level of double strand DNA, these SSRs are making a hairpin, which makes them difficult to isolate [49, 50]. However, from data obtained from random sequencing (11 Mb from BAC end sequences from chromosome 3B [51]), the most likely distribution of microsatellite motifs within the wheat genome was very different from that obtained from enriched library. Particularly, microsatellites with tri- and tetra-nucleotidic motifs were more abundant compared to enriched libraries [51], as are some of them with particular motifs (like AT) which are almost impossible to recover by specific enrichment.

We found that the average number of alleles varied according to the type of motif. The dinucleotide repeats have more alleles than the tri-, tetra- and pentanucleotide repeats which was in accordance to the literature. According to Chakraborty et al. [52], the dinucleotide motifs display a higher mutation rate than the trinucleotide motifs which in turn show a higher rate than the tetranucleotide motifs.

Moreover, Lee et al. [53] confirmed that tetranucleotide repeats are considerably more stable than dinucleotide repeats and in yeast, it was shown that microsatellites with smaller repeat

units are much less stable than those with larger repeat units [54]. In addition, the number of repeats could account for some differences in mutation frequencies. Microsatellites with large numbers of repeats are generally more polymorphic than shorter tracts [55–57].

It has long been known that the level of polymorphism is quite different between the three genomes, the B genome being the most polymorphic while the D genome is generally the less polymorphic [58]. Considering this, we expected variability to be lower on this latter chromosome compared to the other two.

This was actually the case since allele number per locus (6.04) as well as mean PIC values (0.54) for chromosome 3D were smaller compared to chromosome 3A (6.73 allele number / locus and 0.61 PIC values) and 3B (6.89 allele number / locus and 0.58 PIC values) chromosomes, but the differences were not statistically significant (Mann-Whitney U-test, $p > 0.05$). However, the behaviour of the correlations between SSR length and allele number along homoeologous group 3 chromosomes were increased from the centromere toward the telomere, especially for the short arms of 3B and 3D chromosomes.

We did not reveal the increasing of lengths of microsatellites with distance from centromer. For example, the average length of SSRs that located on distal bin 3AL5 – 0.78–1.00 was 50.6 bp and for microsatellites assigned to more proximal bin 3AL3 – 0.42–0.78 average length was 40.4 bp, but difference was not statistically significant. The average lengths of SSRs that have been mapped on bins 3BS8 – 0.78–1.0 and 3BS9 – 0.57–0.78 were similar 38.5 bp and 38.9 bp, respectively. The same situation we found for microsatellites on distal bin 3BL7 – 0.63–1.0 and on more proximal region of long arm 3B chromosome (C-3BL10–0.50), where the average lengths were 45.1 and 44.1 bp, respectively.

Difference between average of lengths of SSRs located on proximal bins C-3DL3 – 0.81 (45.7 bp) and distal bin 3DL3 – 0.81–1.00 (54.2 bp) also was not statistically significant.

We did not find statistically significant differences in allele numbers and PIC values for SSRs located on more distal bins on 3A and 3B chromosomes. On 3D we tested significant differences (Mann-Whitney U-test, $p < 0.05$) between the PIC values determined for SSRs assigned to 3DL3 – 0.81–1.0 bin that is distal compare to the more proximal C-3DL3 – 0.81 bin. Average PIC values on these two comparing regions were 0.71 (3DL3 – 0.81–1.0) and 0.37 (C-3DL3 – 0.81). Analysis of allele numbers of SSRs assigned to the regions, which we have described above (3DL3 – 0.81–1.0 vs C-3DL3 – 0.81) revealed average allele numbers per locus 8.1 and 4.4 respectively, differences were significant ($p < 0.05$).

It is well known that each chromosome of wheat has a unique pattern of recombination rate [59]. In general, recombination is strongly reduced in the pericentromeric regions of chromosomes contrary to the telomeric regions where it is high. In wheat, local recombination rate is positively correlated with physical distance from the centromere [12]. According to these authors, microsatellite loci located in distal regions with presumably high recombination rates show longer allele size and more polymorphism than loci located in proximal regions. Similarly, Huang et al. [60] found a positive correlation between the genetic distance from centromere and the number of alleles per microsatellite locus in hexaploid wheat.

However, Huang et al. [60] used genetic distances as reference, while in some cases, there are huge differences between genetic and physical distances; it is thus possible that the SSRs they tested were located in the same deletion bin in the telomeric region. In our study it was possible to choose microsatellites located in more centromeric regions because SSRs were assigned to deletion bins using deletion lines.

According to our results, we can conclude that the genetic variability of SSR loci on homoeologous group 3 chromosomes varied along the chromosomes. However, the variability at distal regions was not as extended as expected, which suggests that recombination which occurs more frequently in the distal part of the chromosomes does not significantly contribute to the extension of the variability of the microsatellite loci. SSR distributions are associated with sites of recombination, most probably as a consequence of repetitive sequences being involved in recombination rather than being a consequence of it [61, 62]. SSR genetic variability is thus probably due to other phenomena such as the model for SSR mutation based on replication slippage previously formulated by Levinson et

al. [63]. Replication slippage or slipped strand mispairing refers to the out-of-register alignment of the two DNA strands following dissociation at the time when the DNA polymerase traverses the repetitive region. This slippage implies the formation of a single-strand loop during DNA synthesis and addition (or suppression, depending on the strand concerned with the loop) of a motif. Most of these primary mutations are corrected by the mismatch repair system, and only the small fraction that is not repaired ends up as microsatellite mutation events [64]. This model, which can be applied in distal as well as in proximal chromosome parts, is thus probably favoured compared to unequal recombination for evolution of SSR variability in wheat.

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ИССЛЕДОВАНИЕ ГЕНЕТИЧЕСКОЙ ВАРИАБЕЛЬНОСТИ МИКРОСАТЕЛЛИТНЫХ ЛОКУСОВ ТРЕТЬЕЙ ГОМЕОЛОГИЧНОЙ ГРУППЫ ХРОМОСОМ МЯГКОЙ ПШЕНИЦЫ

Изучали вариабельность МС-локусов третьей гомеологичной группы хромосом *T. aestivum* L., осуществили сопоставление изменчивости микросателлитов в дистальных и проксимальных областях хромосом и физическое картирование МС-локусов с помощью делеционных, дителосомных, нуллитетрасомных линий и провели сравнительный анализ вариабельности микросателлитных локусов хромосом 3A, 3B и 3D. Физически картировано 165 микросателлитов на хромосомах третьей гомеологичной группы *T. aestivum* L. Выполненные исследования вносят существенный вклад в насыщение физических карт 3A, 3B и 3D хромосом МС-маркерами. Характер варьирования числа аллелей МС и их длины на всей протяженности исследованных хромосом главным образом нерегулярен. Не выявили статистически значимых достоверных различий по количеству аллелей, значениям PIC или длине МС-локусов для микросателлитов, локализованных на хромосомах 3A, 3B и 3D мягкой пшеницы.

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ДОСЛІДЖЕННЯ ГЕНЕТИЧНОЇ ВАРІАБЕЛЬНОСТІ МІКРОСАТЕЛІТНИХ ЛОКУСІВ ТРЕТЬОЇ ГОМЕОЛОГІЧНОЇ ГРУПИ ХРОМОСОМ М'ЯКОЇ ПШЕНИЦІ

Вивчали варіабельність МС-локусів третьої гомеологічної групи хромосом *T. aestivum* L., здійснили порівняння мінливості мікросателітів у дистальних та проксимальних областях хромосом, а також фізичне картування МС-локусів за допомогою делеційних, дителосомних, нулітетрасомних ліній та провели порівняльний аналіз варіабельності мікросателітних локусів хромосом 3A, 3B і 3D. Фізично картовано 165 мікросателітів на хромосомах третьої гомеологічної групи *T. aestivum* L. Виконані дослідження є істотним внеском у насичення фізичних карт хромосом 3A, 3B і 3D МС-маркерами. Характер варіювання числа алелів МС та їх довжини по всій довжині досліджених хромосом головним чином нерегулярний. Не виявлено статистично значущих достовірних відмінностей за кількістю алелів, значенням PIC та довжині МС-локусів для мікросателітів, локалізованих на хромосомах 3A, 3B і 3D м'якої пшениці.

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