UDC 577.2:631[633.111:664.64.016.8]

MICROSATELLITE MARKERS ASSOCIATED WITH GRAIN COLOUR OF UKRAINIAN BREAD WHEAT VARIETIES

O. O. Kolesnyk, S. V. Chebotar, O. M. Khokhlov

The Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigations

Analysis of microsatellite markers associated with grain colour was performed in order to identify the important regions involving in this trait. As a result, 37 marker trait associations (MTAs) were found to be significant in one – three growing seasons, of which 20 MTAs were significantly associated with the lighter grain colour (CI_g) while 17 MTAs showed association with the darker Cl_g. Our study showed that the significant MTAs were located on wheat chromosomes 1B, 3A, 5A, 6D and 7B.

Keywords: *Triticum aestivum L., microsatellite analysis, marker-trait association, grain colour*

The red colour of grain occurs in most common European wheat varieties [18]. It is controlled by one to three dominant alleles *R-A1* (on chromosome 3AL) [13], *R-B1* (3BL) [20] and *R-D1* (3DL) [17]. Contrary, white grain colour is determined by the recessive alleles, i.e. *r-A1*, *r-B1* and *r-D1* [18]. The major genes described for the coloration of glumes are known to be (*Rg1, Rg2, Rg3, Bg*), for anthocyanin pigmentation of coleoptiles (*Rc-A1*, *Rc-B1, Rc-D1*), anthers (*Pan1*, *Pan2*), auricles (*Ra1, Ra2, Ra3*), straw (*Pc1, Pc2*) and grains (*Pp1, Pp2*) [19]. The pigment of grain has been suggested to be a derivative of catechin-tannin generated in the process of biosynthesis of flavonoids and has been associated with economically important characteristics: brightness of wheat flour and level of grain dormancy [8, 9]. From the structural point of view anthocyanins are glycosides composed of hydroxyled or methoxyled 2-phenylbenzopyrilium skeleton with hydroxyl and methoxy groups in the B-ring [7]. The red colour of the grain is associated with a higher content of bitter phenolic components, lower activity of hydrolytic enzymes, and better resistance to

sprouting [18]. The degree of red color is additive, the intensity of the red color depends on the number of *R* alleles, and only those homozygous recessive at all three genes being white (*R-A1a*, *R-B1a* and *R-D1a*). Li et al. [17] have mapped the genes controlling the character of red grain color in Chuanmai 42 derived from synthetic hexaploid wheat by SSR markers, and provided useful markers for breeding white grain colour variety by using synthetic hexaploid wheats and Chuanmai 42 as genetic resources.

In the present study we have applied microsatellite markers [21, 22] for the association with major QTLs controlling wheat grain colour in a core collection from modern Ukrainian bread wheat varieties [12, unpublished data]. Furthermore we report the distribution of alleles at microsatellite loci associated with lighter and darker colours of wheat grains obtained with the help of digital image analysis (DIA) [25]. DIA is the process of converting digital images of individual objects, such as plant organs, into quantitative measurements. DIA methods that convert photographs of plant organs into quantitative data based on measures of axes or pixel counts have been used by numerous research groups [3, 4, 6, 14]. A major objective of this scientific work was to find microsatellite markers associated with the grain colour intensity (CIg) which can be applied to breeding programs.

Materials and research methods. Ukrainian bread wheat core collection consisted of 47 bread winter wheat varieties (*Triticum aestivum* L.) originated in Plant breeding and genetics institute (PBGI) and registered in State Register of plant varieties suitable for dissemination in Ukraine in 2003-2013 years. The genotypes of taken varieties outlined in Kolesnyk et al. [12] were characterized using 17 SSR markers abundantly described in the literature [5, 15, 21, 22]. Varieties Albatros odes'kyi (1990) and two collection samples of variety Bezosta 1 (1955) were added into research as standard (etalon) samples. The plants have been grown by 2-rows mini-plots in field experiment conditions in 2010/11, 2011/12, 2012/13 seasons by the laboratory of Variety Investigation and Breeding Process Modelling of PBGI, located in Odesa, Ukraine (46° 27' 3", 30° 39' 18"). In 2010 from each variety in a randomized way 5 seedlings were taken for microsatellite analysis (MS analysis), of

which one was selected for the further growing of its seed progenies in consecutive seasons according to spike-row scheme. Each season, the sowing was carried out in 1st decade of October - optimum time for this climatic zone. Plants were harvested in early July, in stage of fully ripened seeds. Genomic DNA was extracted from seedlings using modified CTAB method [23]. Polymerase chain reactions (PCR) was performed on a Tertsyk thermocycler (DNA Technology, Russia) according to Röder et al. [21], with 35 instead of 45 cycles of denaturation for 1 min at 94 ^{0}C, annealing for 1 min at 50 ^{0}C (55 or 60 ^{0}C depending on the primer) and extension for 1 min at 72 ^{0}C followed by a final extension step for 5 min at 72 ^{0}C. PCR was carried out in a final reaction volume of 25 μL containing: 60 ng of DNA; 0.25 μM of each primer; 10x PCR-buffer (40 mM Tris-HCl pH 8,4; 25 mM KC1; 1.5 mM of $MgCl_2$; 0,01 % Tween-20); 0,2 mM of each dNTP; 1 unit of Taq-polymerase. The amplification products (10-μL aliquot of the PCR mixture) were separated in 7% polyacrylamide gel in 1 x TBE using Hoefer scientific instruments device (United States) according to the manufacturer's instruction. Visualization of PCR products of electrophoretic division was performed by the staining of gels in $AgNO_3$ according to "Silver sequence TM DNA Sequencing System Technical Manual" ("Promega", United States). Image Master VDS video system (Amersham Pharmacia Biotech, United States) was used to assess the fragment size of the alleles at each microsatellite locus according to the recommendations of the manufacturer [24]. The pUC19 DNA/MspI and 100 bp DNA Ladder were used as standard ladders.

Each year the obtained grain material was analyzed for colorimetric characteristics extracted from the digital images of kernels using a computer program ImageJ ver.1.49h (National Institute of Health, USA). The program was tested and adapted for objects of varieties investigation [2]. In order to standardize images characteristics and to improve productivity the set of macros was developed making it possible to perform high-speed routine estimations. The grains, 150-200 kernels for each sample, were scanned on the device HP 3570c, against a dark background, with 200 dpi (pixels / inch) resolution, as recommended by Цевма and Хохлов [1]. The hardware/software configuration applied is able to register colour-related information

from all three Red-Green-Blue channels. But since it was known beforehand, that all varieties taken into investigation are graded as "red-grained", the characteristics were restricted by "level of grey" only with full range 0-255 units for 8-bit gray images. All five available characteristics were registered (mean, median, mode, skewness, and kurtosis). But, in view of tight correlations among all these, the only first of them is presented here. Grain color intensity (CI_g) was expressed by grey level values with full scale from 0 (entirely black) to 255 (pure white). The data were used "as is", without transformation, calibration or any other treatment. Other words, raw readings were interpreted as common quantitative trait. Only classes with not less than three samples were taken into statistical consideration. Over this limitation only about half of all alleles presented were suited for association detection. Differences significance between alleles means were the main criterion of associations. They were evaluated by instruments of descriptive statistics of EXCEL and also SIMFIT, ver.7.0.5 package. For preliminary evaluation of variation characteristics within each sample was used dedicated instrument incorporated into ImageJ. It was detected here that the average level of standard error for sample consisted of 150 kernels was around 0,4-0,7 units which allowed fine discriminant ability even while working with samples of one colour grade only.

The research results. Microsatellite markers have a great potential of applying into the research as they are apt to describe polymorphism in the SSR loci and show wheat genetic diversity [5, 15]. In our previous study the allelic composition of 47 bread wheat varieties has showed that it would be quite enough to choose 7-8 most informative microsatellite markers for complete variety identification and differentiation [12]. 35-36 varieties were also chosen to form standard varieties collection, which included carriers of alleles typical for varieties released by PBGI.

CI_g of the Ukrainian bread wheat collection was measured in three growing seasons (2010/11, 2011/12, 2012/13). Mean values of CI_g in three years analyzed showed considerable levels of diversity within each of year (Table 1).

1.Variability of grain colour intensity among studied varieties in three growing seasons

CHARACTERISTICS	2010/2011	2011/2012	2012/2013
CI_g, mean	138,9	132,8	131,2
min	131,2	122,7	121,9
max	149,6	147,7	144,3
SD	4,19	5,13	4,49
Stds	0,60	0,87	0,76
CV	3,02	3,86	3,42
Shapiro-Wilks normality test:			
W-statistic	0,980	0,981	0,970
significance level	0,767	0,597	0,231

*CI_g – grain colour intensity; Mean – average values; SD – standard deviation; Stds – standard error; CV – coefficient of variation, %

Over weather conditions in first season level of CI_g was higher (more light grains). Though all studied varieties are considered as 'red-grained', wide scale of CI_g was registered among them. Genetic nature of these differences was confirmed by significant positive correlations between years (0,35-0,58). LSD_{05} criterion for variety means comparing, determined by ANOVA, was 5,8. The lowest level of CI_g in all three seasons displayed Bezostaya 1, 126,6 in average. This 'milestone' for world breeding variety was famous for its large, dark-red kernels. Podiaka variety showed the same color (127,4). For comparison: the experimental lines with deap purple colour of pericarp have CI_g level about 105-115. The highest rate of CI_g was typical for Zaporuka variety, 145,9 in average. It was close to varieties grown in these very conditions and graded as 'white-grained' (about 150, in general). Light grain (CI_g from 138 to140) was registered also in varieties Albatros odes’kyi, Scarbnytsia, Zmina, Ednist', Istyna, Gospodynia, and Oksana. The last was the only variety with soft endosperm, so opacity was the reason of its increased CI_g level.

Shapiro-Wilks test indicated normality of variety-specific CI_g distribution in all three seasons. It is also obvious in Figure. Normality is one of conditions required for implementation of simple (without transformations etc.) t-test at associations finding.

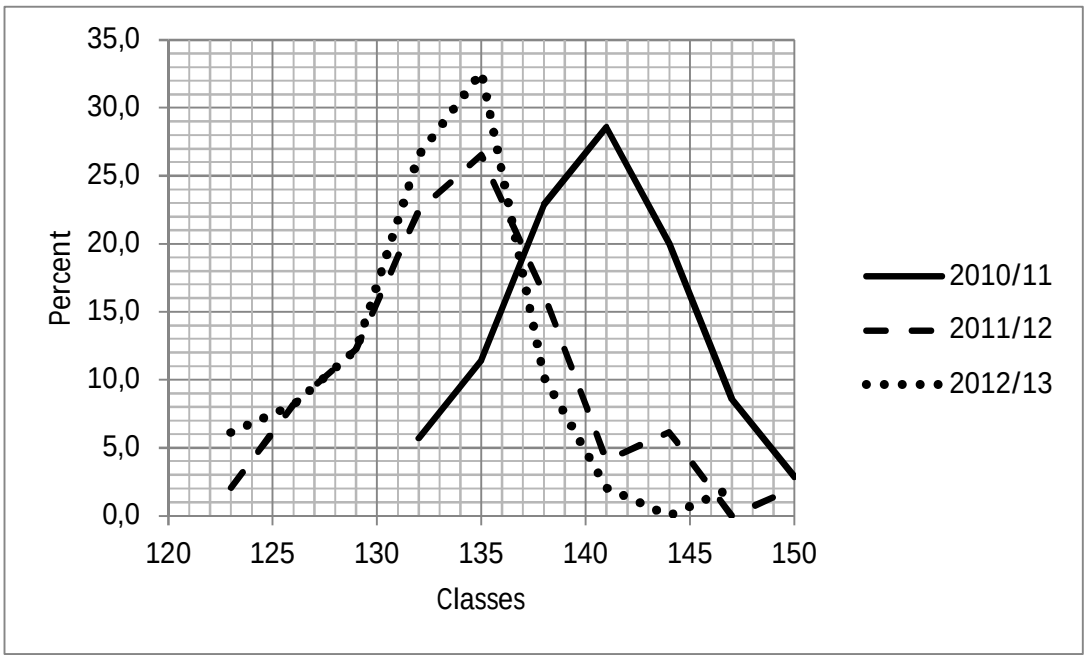

Figure. Distribution of variety-specific levels of CI_g in three seasons.

The results of marker-trait associations (MTAs) in two – three analyzed years are shown in Table 2. Overall 20 alleles of SSR markers were associated with the lighter CI_g while 17 alleles were associated with the darker CI_g. Ten MTAs were found to be significant in two growing seasons. 27 MTAs were shown to be significantly associated with CI_g in one growing season, namely alleles *Xgwm357*$_{119}$, *Xgwm18*$_{192}$, *Xgwm18*$_{196}$, *Xtaglgap*$_{218}$, *Xgwm3*$_{86}$, *Xgwm155*$_{139}$, *Xgwm155*$_{152}$, *Xgwm389*$_{145}$, *Xgwm408*$_{188}$, *Xgwm190*$_{204}$, *Xgwm325*$_{146}$, *Xgwm325*$_{148}$, *Xgwm577*$_{175}$, *Xgwm44*$_{176}$, *Xwmc405*$_{218}$ were found to be significantly associated with the lighter CI_g while alleles *Xgwm357*$_{125}$, *Xtaglgap*$_{215}$, *Xgwm3*$_{79}$, *Xgwm155*$_{145}$, *Xgwm389*$_{142}$, *Xgwm186*$_{102}$, *Xgwm186*$_{129}$, *Xgwm190*$_{208}$, *Xgwm325*$_{142}$, *Xgwm577*$_{137}$, *Xgwm44*$_{187}$, *Xwmc405*$_{222}$ showed to be significantly associated with the darker CI_g.

Table 2 displays the mean values for CI_g with their standard errors (Sx) at *$p \leq 0.05$ and **$p \leq 0.01$ significance. Bold regular/italic font indicates significant plus/minus associations. During two growing seasons the darker CI_g was significantly associated with alleles *Xgwm18*$_{186}$, *Xgwm155*$_{141}$, *Xgwm155*$_{147}$, *Xgwm325*$_{128}$, *Xgwm325*$_{150}$ while the lighter CI_g showed stable associations in two – three growing seasons with alleles *Xgwm18*$_{188}$, *Xgwm155*$_{143}$, *Xgwm186*$_{115}$, *Xgwm325*$_{115}$,

2. Associations of SSR alleles with grain colour (Cl_g), revealed in field experiments in three growing seasons

Locus	Allele	Cl_g		
		2010/11	2011/12	2012/13
		Mean ± Sx	Mean ± Sx	Mean ± Sx
Xgwm357 (1A)	116	–	133,8 ± 3,23	134.8 ± 2,80
	119	141,1 ± 2,86	**138,3 ± 3,83***	–
	121	141,2 ± 2,74	–	–
	123	139,9 ± 1,77	133,3 ± 1,22	131,6 ± 1,31
	125	137,7 ± 0,98	***132,5 ± 0,71****	130,8 ± 0,85
	128	140,4 ± 1,31	132,7 ± 1,16	130,1 ± 1,67
	134	137,6 ± 2,53	133,3 ± 2,54	127,8 ± 1,62
Xgwm18 (1B)	186	138,1 ± 1,73	***128,7 ± 1,44***	***128,3 ± 2,41****
	188	139,1 ± 0,64	**134,2 ± 0,71****	**132,9 ± 0,83***
	192	139,9 ± 0,76	**132,8 ± 1,08***	130,8 ± 1,14
	196	–	**135,0 ± 0,87****	131,6 ± 1,21
Xtaglgap (1B)	215	138,7 ± 1,62	***129,9 ± 1,43****	130,9 ± 2,33
	218	139,2 ± 0,82	**134,0 ± 0,61****	132,0 ± 0,71
	235	–	–	–
	238	139,7 ± 1,20	133,3 ± 1,51	128,8 ± 1,40
	265	–	–	–
Xgwm095 (2A)	110	–	–	–
	120	138,2 ± 1,49	135,6 ± 2,65	133,7 ± 3,03
	122	139,3 ± 0,78	132,4 ± 0,66	130,3 ± 0,80
	128	138,1 ± 2,89	132,7 ± 0,90	130,8 ± 0,95
	130	–	134,1 ± 1,38	133,2 ± 1,66
Xgwm3 (2D)	75	–	–	–
	77	138,4 ± 2,54	132,5 ± 1,66	129,1 ± 1,87
	79	137,8 ± 1,43	***130,4 ± 1,74****	129,9 ± 2,39
	81	139,9 ± 1,84	134,4 ± 1,18	131,1 ± 0,55
	83	–	132,4 ± 1,70	130,8 ± 0,85
	86	138,9 ± 1,15	**134,2 ± 0,93***	132,6 ± 1,03
	88	140,2 ± 1,42	134,1 ± 0,77	132,0 ± 1,15
Xgwm155 (3A)	129	–	–	–
	135	–	–	–
	137	–	–	–
	139	138,6 ± 1,14	**138,1 ± 2,43****	130,7 ± 2,22
	141	136,8 ± 3,56	***130,7 ± 2,31****	***126,2 ± 2,67****
	143	**143,7 ± 2,23***	***132,8 ± 0,94****	**132,2 ± 0,83***
	145	138,3 ± 2,01	***132,5 ± 1,26****	130,3 ± 1,77
	147	***138,3 ± 1,27****	***132,1 ± 0,79****	132,1 ± 1,39
	149	140,1 ± 0,69	***133,2 ± 0,79****	**133,1 ± 0,99****
	152	–	**136,9 ± 3,20***	134,3 ± 1,07

Table 1 continued

Locus	Allele	Cl_g

		2010/11	2011/12	2012/13
		Mean ± Sx	Mean ± Sx	Mean ± Sx
Xgwm389 (3B)	117	138,4 ± 0,97	133,2 ± 0,98	130,4 ± 0,93
	119	–	132,3 ± 1,32	130,0 ± 1,47
	134	139,1 ± 1,72	133,5 ± 1,29	132,4 ± 1,50
	136	137,4 ± 1,93	132,6 ± 2,44	131,6 ± 2,56
	138	140,3 ± 1,23	133,5 ± 1,40	131,6 ± 2,17
	142	–	***131,8 ± 0,50****	131,8 ± 1,02
	145	–	**137,1 ± 1,75****	–
Xgwm165/1 (4A)	185	136,8 ± 3,08	133,0 ± 1,00	131,3 ± 0,43
	189	–	–	–
	191	137,2 ± 1,38	132,7 ± 0,98	131,0 ± 1,27
	193	140,6 ± 1,22	133,3 ± 0,90	131,0 ± 1,26
	195	139,3 ± 0,57	132,8 ± 0,91	131,8 ± 0,89
Xgwm186 (5A)	102	139,2 ± 1,01	***132,3 ± 0,71****	130,7 ± 0,68
	107	–	134,4 ± 3,26	–
	113	**141,2 ± 2,74***	***132,2 ± 0,88****	132,8 ± 2,99
	115	**139,6 ± 0,25***	**138,7 ± 2,50****	–
	125	139,1 ± 2,31	133,5 ± 3,09	131,6 ± 4,44
	129	***134,1 ± 1,16****	134,2 ± 1,41	–
	135	139,0 ± 3,38	134,0 ± 1,73	130,0 ± 1,63
	139	–	132,3 ± 3,22	–
	142	**139,2 ± 1,30***	132,7 ± 0,78	–
Xgwm408 (5B)	148	–	–	–
	162	–	–	–
	178	–	132,4 ± 0,42	–
	185	–	–	–
	188	138,4 ± 0,87	134,2 ± 0,80	131,4 ± 0,87
	192	139,0 ± 2,42	131,5 ± 2,61	131,4 ± 3,63
Xgwm190 (5D)	204	**141,5 ± 0,96****	134,1 ± 0,80	132,7 ± 1,12
	208	***137,6 ± 0,70****	132,5 ± 0,69	130,4 ± 0,70
	210	–	–	–
Xgwm325 (6D)	115	–	**134,3 ± 1,19****	**133,7 ± 0,99***
	120	–	–	–
	128	–	***129,0 ± 1,03****	***127,9 ± 2,44****
	134	–	–	–
	138	–	–	–
	142	***136,1 ± 1,51****	–	–
	144	138,5 ± 0,94	132,6 ± 0,97	131,7 ± 1,25
	146	140,9 ± 1,45	**134,9 ± 1,03****	132,4 ± 1,19
	148	**140,7 ± 0,94***	134,8 ± 2,86	127,1 ± 4,00
	150	–	***127,4 ± 1,66****	***128,8 ± 1,14****

Table 1 continued

Locus	Allele	Cl_g

		2010/11	2011/12	2012/13
		Mean ± Sx	Mean ± Sx	Mean ± Sx
Xgwm577 (7B)	137	137,6 ± 2,25	***128,2 ± 1,37***	128,4 ± 2,44
	152	–	132,2 ± 2,14	–
	171	***136,1 ± 1,44****	**133,5 ± 0,83****	131,9 ± 1,07
	173	**139,7 ± 0,76***	**133,6 ± 0,66****	131,3 ± 0,70
	175	–	**137,0 ± 2,97****	134,7 ± 4,16
Xgwm437 (7D)	105	–	–	–
	107	138,9 ± 0,87	132,8 ± 0,71	132,3 ± 0,90
	109	139,6 ± 1,00	133,3 ± 0,83	129,8 ± 0,83
	113	138,1 ± 3,71	136,0 ± 4,20	–
Xgwm44 (7D)	176	143,0 ± 4,61	**136,0 ± 1,08***	132,1 ± 1,63
	178	–	–	–
	180	139,5 ± 2,18	135,3 ± 2,89	–
	183	139,2 ± 0,67	132,7 ± 1,02	131,8 ± 1,46
	185	138,3 ± 0,80	133,0 ± 0,72	130,8 ± 0,76
	187	–	***129,7 ± 2,30****	130,4 ± 5,02
Xbarc126 (7D)	138	–	134,9 ± 2,04	130,8 ± 0,76
	142	–	–	–
	146	–	–	–
	152	–	–	–
	156	139,2 ± 0,90	132,7 ± 0,80	131,2 ± 0,97
	158	–	–	–
	160	138,9 ± 3,23	–	–
	162	–	–	–
	164	138,0 ± 0,95	134,0 ± 0,97	130,2 ± 1,05
	166	140,7 ± 1,51	131,7 ± 0,89	131,4 ± 1,07
Xwmc405 (7D)	210	–	–	–
	212	–	–	–
	216	136,1 ± 1,88	–	–
	218	140,2 ± 0,98	**133,1 ± 0,68***	131,9 ± 0,81
	220	137,6 ± 1,06	133,5 ± 0,87	131,0 ± 0,94
	222	139,2 ± 2,23	***128,0 ± 2,29****	–

*Significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; all significant deviations are shown in bold font, among them regular/italic indicate increasing or reducing, respectively, of the studied value depending on alleles associated with this value; Mean – average values; Sx – standard error; a dash means not available data

Xgwm577$_{173}$. SSR alleles *Xgwm155*$_{149}$, *Xgwm186*$_{113}$, *Xgwm186*$_{142}$, *Xgwm577*$_{171}$ showed alternative effect on the value of CI_g.

Among studied 17 microsatellite markers we have found 37 alleles of *Xgwm357*, *Xgwm18*, *Xtaglgap*, *Xgwm3*, *Xgwm389*, *Xgwm155*, *Xgwm186*, *Xgwm190*, *Xgwm325*, *Xgwm577*, *Xgwm44* and *Xwmc405* mapped on 1A, 1B, 2D, 3A, 3B, 5A, 5D, 6D, 7B

and 7D chromosomes associated with the value of CI_g. Khlestkina et al. [11] has mapped a total of 35 microsatellite markers on the homoeologous group 1 chromosomes. The genes *Bg, Rg1, Rg3* and the smokey-grey glume color gene were mapped between the markers *Xgwm1223* and *Xgwm0033* at the distal ends of the short arms of the homoeologous group 1 chromosomes. In our study we have detected alleles of the microsatellite marker *Xgwm18*-1BL to be significantly associated with the value of CI_g during two growing seasons. At the same time alleles $Xgwm357_{119}$ and $Xgwm357_{125}$ detected at the chromosome 1AS were shown to be associated with CI_g during one growing season, thus insufficient amount of data prevented us from drawing any conclusions. Additionally Khlestkina et al. [11] reported a total of 8 microsatellite markers to be mapped on the chromosome 7D. The major gene loci *Pl* (purple leaf), *Pc2* (purple culm), and *Pan1* (purple anthers) were mapped in a region, about 15 cM distal from the centromere on chromosome 7DS. Among four microsatellite markers located at the chromosome 7D used in our study we have found markers *Xgwm44* and *Xwmc405* to be significantly associated with CI_g during one growing season. In the mentioned region there was previously mapped the gene *Rc-D1* in charge for anthocyanin pigmentation of coleoptile [10].

We have detected that microsatellite marker *Xtaglgap*-1B hasn't shown stable association with QTL for CI_g in all years analyzed. In studies carried out by Landjeva et al. [16] it was noted that the presence of allele $Xtaglgap_{244}$ was strictly correlated with the red glume colour gene *Rg-B1b* in Bulgarian wheat cultivars. Khlestkina et al. [11] has found an association between *Rg-B1b* and $Xtaglgap_{250}$. Additionally she suggested that besides 250 bp, alleles of 241, 244 and 247 bp of *Xtaglgap* marker may be spesific for *Rg-B1b* in different wheat collections.

Himi et al. [9] reported that pigmentation of wheat grain is controlled by the *R* gene on the end region of the long arms of wheat chromosomes 3A, 3B, and 3D. In our study we have found markers *Xgwm155*-3A, *Xgwm389*-3B significantly associated with CI_g during one – two growing seasons. Microsatellite marker *Xgwm155*-3A had the highest number of alleles associated with CI_g when compared with the other studied markers. Alleles of microsatellite markers *Xgwm18*-1B,

Xgwm155-3A, *Xgwm186*-5A, *Xgwm325*-6D, *Xgwm577*-7B which have shown stable associations in two growing seasons are located near to QTLs identified for the first time to be significantly associated with CI_g.

Conclusions

In this study the analysis of microsatellite markers associated with grain colour (CI_g) was performed in order to identify the important regions involving in this trait. As a result, 37 marker trait associations (MTAs) were found to be significant in one – three growing seasons, of which 20 MTAs were significantly associated with the lighter CI_g while 17 MTAs showed association with the darker Cl_g. Our study showed that the significant MTAs were located on wheat chromosomes 1B, 3A, 5A, 6D and 7B. The performed association analysis provides useful information for breeding of Ukrainian bread wheat.

References

1. *Цевма В. М.,* Інструментальне визначення сортоспецифічних характеристик кольору зерна пшениці / *В.М.Цевма, О.М. Хохлов* // Зб. наук. праць СГІ. – 2007. – Вип. 10 (50). – С. 116-135.

2. *Хохлов О.М.* Застосування програми аналізу зображень ImageJ в дослідженнях об’єктів сортовивчення / *О.М. Хохлов* // Виноградарство і виноробство. Міжвідомчий тематичний науковий збірник. – Спец. випуск. – Одеса. – 2009. – С.179-185.

3. *Breseghello F., Sorrells M. E.* Association analysis as a strategy for improvement of quantitative traits in plants // Crop Science. – 2006. – Vol. 43, № 6. – P. 1323-1330.

4. *Breseghello F., Sorrells M. E.* QTL analysis of kernel size and shape in two hexaploid wheat mapping populations // Field Crops Res. – 2007. – Vol. 101, № 2. – P. 172-179.

5. *Brbaklic L., Trkulja D., Kondic-Spika A., Treskic S., Kobiljski B.* Detection of QTLs for Important Agronomical Traits n Hexaploid Wheat Using Association Analysis. Czech Journal of Genetics and Plant Breeding. – 2013. – Vol. 49, № 1. – P. 1-8.

6. *Campbell K.G., Bergman C. J., Gualberto D. G., Anderson J. A.,* Giroux M. J., *Hareland G., Fulcher R. G., Sorrells M .E., Finney P. L.* Quantitative trait loci associated with kernel traits in a soft x hard wheat cross // Crop Sci. – 1999. – Vol. 39, № 4. – P. 1184-1195.

7. *Havrlentova M., Psenakova I., Žofajova A., Ruckschloss L., Kraic J.* Anthocyanins in wheat seed – a mini review // Nova Biotechnologica et Chimica. – 2014. – Vol. 13, № 1. – doi: 10.2478/nbec-2014-0001.

8. *Himi E., Mares D.J., Yanagisawa A., Noda K.* Effect of grain colour gene (*R*) on grain dormancy and sensitivity of the embryo to abscisic acid (ABA) in wheat // J. Exp. Bot. – 2002. – Vol. 53, № 374. – P. 1569-1574.

9. *Himi E., Nisar A., Noda K.* Colour genes (*R* and *Rc*) for grain and coleoptile unregulate flavonoid biosynthesis genes in wheat // Genome. – 2005. – Vol. 48, № 4. – P. 747-754.

10. *Khlestkina E.K., Pestsova E.G., Röder M.S., Börner A.* Molecular mapping, phenotypic expression and geographical distribution of genes determining anthocyanin pigmentation of coleoptiles in wheat (*Triticum aestivum* L.) // Theor Appl Genet. – 2002. – Vol. 104, № 4. – P. 632-637.

11. *Khlestkina E. K., Pshenichnikova T. A., Salina E. A., Röder M. S., Arbuzova V. S., Börner A.* Microsatellite mapping of genes for coloration of different wheat plant organs on homoeologous groups 1 and 7 chromosomes // Proceedings of the 14th International EWAC Conference, Istanbul, Turkey, 6-10 May 2007. – P. 85-90.

12. *Kolesnyk O. O., Chebotar S. V., Sivolap Yu. M., Tsevma V. M., Khokhlov O. M., Litvinenko M. A.* The courses of nurseries control and variety investigation systems founded on molecular markers // Collection of scientific papers of PBGI-NCCI. – 2013. – Vol. 22 (62). – P. 89-99.

13. *Kuraparthy V., Sood S., Gill B. S.* Targeted genomic mapping of a red seed color gene (*R-A1*) in wheat // Crop Sci. – 2008. – Vol. 48, № 1. – P. 37-48.

14. *Kwack M. S., Kim E. N., Lee H., Kim J., Chun S., Kim K. D.* Digital image analysis to measure lesion area of cucumber anthracnose by *Colletotrichum orbiculare* // J Gen Plant Pathol. – 2005. – Vol. 71, № 6. – P. 418-421.

15. *Landjeva S., Ganeva G., Korzun V., Palejev D., Chebotar S., Kudrjavtsev A.* Genetic diversity of old bread wheat germplasm from the Black Sea region evaluated by microsatellites and agronomic traits // Genetic Resources and Crop Evolution. – 2014. – doi:10.1017/S1479262114000781.9.

16. *Landjeva S., Korzun V., Ganeva G.* Evaluation of genetic diversity among Bulgarian winter wheat (*Triticum aestivum* L.) varieties during the period 1925 – 2003 using microsatellites // Gen. Res. Crop Evol. – 2006. – Vol. 53, № 8. – P. 1605-1614.

17. *Li J., Wei H., Hu X., Lu B., Yang W.* Locus *R-D1* conferring red-grain-color in synthetic derivative wheat chuanmai 42 mapped with SSR markers // Molecular Plant Breeding. – 2010. – Vol. 1, № 3. – doi:10.5376/mpb.2010.01.0003.

18. *Martinek P., Jirsa O., Vaculova K., Chrpova J., Watanabei N., Burešova V., Kopecky D., Štiasna K., Vyhnanek T., Trojan V.* Use of wheat gene resources with different grain colour in breeding // Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs. – 2014. – Vol. 64, № 1. – P. 75–78.

19. *McIntosh R.A., Yamazaki Y., Devos K.M., Dubcovsky J., Rogers J., Appels R.* Catalogue of gene symbols for wheat (2003). Available from *http://www.shigen.nig.ac.jp/wheat/* komugi/genes/download.jsp [cited 16 December 2004].

20. *Nalam V., Vales M.I., Watson C. et al.* Map-based analysis of genes affecting the brittle rachis character in tetraploid wheat (*Triticum turgidum* L.) // Theor. Appl. Genet. – 2006. – Vol. 112, № 2. – P. 373–381.

21. *Röder M.S., Korzun V., Wendehake K., Plaschke J., Tixier M.-H., Leroy P., Ganal M.* A microsatellite map of wheat // Genetics. – 1998. – Vol. 149, № 4. – P. 2007–2023.

22. *Röder M.S., Wendehake K., Korzun V., Bredemeijer G., Laborie D., Bertrand L., Isaac P., Rendell S., Jackson J., Cooke R. J., Vosman B., Ganal M. W.* Construction and analysis of a microsatellite-based databasa for European wheat cultivars // Theoretical and Applied Genetics. – 2002. – Vol. 106, № 1. – P. 67–73.

23. *Sivolap, Yu. M.* The use of PCR analysis in genetic and breeding research: a laboratory manual. Kiev: Agrarian Sciences. – 1998. – 159 p.

24. Technical manual. GenePrint® STR Systems (Silver Stain Detection). Promega Corporation. – 2001. – 47 p.

25. *Williams K., Munkvold J., Sorrells M.* Comparison of digital image analysis using elliptic Fourier descriptors and major dimensions to phenotype seed shape in hexaploid wheat (*Triticum aestivum* L.) // Euphytica. – 2013. – Vol. 190, № 1. – P. 99-116.

МІКРОСАТЕЛІТНІ МАРКЕРИ, АСОЦІЙОВАНІ З ОЗНАКОЮ КОЛЬОРУ ЗЕРНА УКРАЇНСЬКИХ СОРТІВ ОЗИМОЇ М'ЯКОЇ ПШЕНИЦІ

О. О. Колесник, С. В. Чеботар, О. М. Хохлов

Аналіз мікросателітних маркерів, пов'язаних із кольором зерна був проведений з метою виявлення важливих регіонів що відповідають за цю ознаку. В результаті 37 асоціацій маркерів з ознакою кольору зерна виявилися значними протягом одного - трьох сільськогосподарських сезонів вегетації, з яких 20 були пов'язані зі світлішим кольором зерна, а 17 показали достовірну асоціацію з темнішим кольором зерна. Наше дослідження продемонструвало, що значні асоціації маркерів були знайдені на хромосомах пшениці 1B, 3A, 5A, 6D і 7B.

Ключові слова: *Triticum aestivum L., мікросателітний аналіз, аналіз асоціацій, колір зерна*

МИКРОСАТЕЛЛИТНЫЕ МАРКЕРЫ, АССОЦИИРОВАННЫЕ С ПРИЗНАКОМ ЦВЕТА ЗЕРНА УКРАИНСКИХ СОРТОВ ОЗИМОЙ МЯГКОЙ ПШЕНИЦЫ

О. А. Колесник, С. В. Чеботарь, А. Н. Хохлов

Анализ микросателлитных маркеров, связанных с цветом зерна был проведен с целью выявления важных регионов отвечающих за этот признак. В результате 37 ассоциаций маркеров с признаком цвета зерна оказались

значительными в течение одного - трех сельскохозяйственных сезонов вегетации, из которых 20 были связаны с более светлым цветом зерна, а 17 показали достоверную ассоциацию с более темным цветом зерна. Наше исследование показало, что значительные ассоциации маркеров были найдены на хромосомах пшеницы 1B, 3A, 5A, 6D и 7B.

Ключевые слова: *Triticum aestivum L., микросателитный анализ, анализ ассоциаций, цвет зерна*