Transfer of genes controlling of agronomic important traits from artificial hexaploid wheat into common wheat gene pool

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INTRODUCTION

Since the beginning of 20th century the enrichment of common wheat gene pool by utilization of wheat wild and cultivated relatives became the one of the ways to increase genetic diversity of wheat. According to the Catalogue of Gene Symbols for Wheat (http://wheat.pw.usda.gov) most genes for resistance to powdery mildew, leaf, stem and yellow rust etc. have been transferred into the common wheat gene pool from species of genera *Aegilops*, *Triticum*, *Secale* and other related species. In some case it was possible to transfer loci for abiotic stress tolerance (i.e. drought, heat, soil toxicities etc) [1]. However there are few publications describing the transfer of genes for improvement of end-use quality and grain characteristics. The simplest way of gene transfer is to utilize synthetic wheats with hexaploid number of chromosomes and subgenome *DD* as the genetic bridge between wild relatives and common wheat [2]. In most cases the amphidiploids are of *Aegilops tauschii* (donor of subgenome *DD*) and donors of *AABB* subgenomes *Triticum turgidum* or *Triticum durum* etc. Only rare reports are of utilization in such kind of crosses of amphidiploids with genome formula A^tA^tGGDD in which donors of genome A^tA^tGG derived from tetraploid wheats of *Timopheevii* group [3, 4]. This work presents the results of the study of the set of introgression lines of amphidiploid *Triticum militinae* (A^tA^tGG) x *Aegilops tauschii* (*DD*) named *Triticum miguschovae* [5] over a period of the last ten years in order to reveal lines with useful traits, decreases in the number and sizes of alien introgressions, observe the number and character of introgressions and study the interaction of alien genetic material incorporated in genome of common wheat.

MATERIAL AND METHODS

Materials. The materials of investigation were amphidiploid *Triticum miguschovae* (*Triticum militinae* (A^tA^tGG) x *Aegilops tauschii* (*DD*)), two common wheat varieties Kavkaz and Bezostaya 1, and 62 common wheat introgression lines.

Methods. DNA extraction was performed from young freeze-dried leaves using a CTAB method [6]. PCR of microsatellites and their visualization were done according to the method of Roder *et al* [7]. For mass screening the products of amplification were separated in 2% agarose gels followed by ethidium bromide staining and UV visualization.

RESULTS AND DISCUSSIONS

Genotyping of amphidiploid *Triticum miguschovae* using SSR markers was not previously done, so the first step of our investigation was to study polymorphism of those markers between genome of *Triticum miguschovae* and recurrent varieties Kavkaz and Bezostaya 1. For this propose we used 102 markers according to recommendation of John Innes Genome Laboratory (UK) where this genotyping was performed (table 1). The markers randomly covered all chromosomes of three subgenomes of wheat *A*, *B* and *D*. For each chromosome 4-6 markers were tested, located on both the long and short arms of the chromosomes (table 1). Most of the tested SSR primers amplified only one fragment with the exception of psp3001, psp3030, gwm165, gwm129, gwm205, gwm130 and gwm455.

Table 1. SSR markers of wheat chromosomes, used in the present investigation

Chromo-some	Markers
1A	psp3001, gwm164, barc083, wmc093[np], gwm135[np]
1B	barc008*, psp3000, gwm011*, psp3100*, wmc044
1D	gwm337, gdm111*, gwm458, gwm642*
2A	gwm636, wmc177, gwm095*, psp3088*, gwm445,barc005 *
2B	gwm257*, wmc154, barc167*, gwm338*
2D	gwm455, barc095, barc168, gwm539, wmc018*
3A	psp3001*, barc045,gwm369, gwm674, gwm155*, wmc264
3B	psp3001 *, gwm285 *, gwm493 *, psp3030*, barc164*
3D	gdm072 *, gwm161, gwm456, gwm003, gwm383
4A	gwm165, barc106, barc184, dupw004*, gwm610[np].
4B	wmc047*, barc163*, gwm107*, gwm165[np], gwm495*, psp3030*
4D	wmc457 [np], gdm129, wmc285, wmc331, psp3007, gwm165
5A	barc056 * barc141 [np], gwm129, gwm205*, barc151, gwm126 [np]
5B	barc109, gwm159 *, gwm234 *, gwm213*, barc140, gwm408 [np]
5D	barc143 *, gwm190, gwm205*, barc110, barc144*, gwm583*
6A	barc171, gwm334*, dupw167, gwm570*l, psp3071
6B	barc198, gwm193 *, psp3009*, wmc105, barc134*, gwm219*
6D	barc173, gwm469, barc096, barc175 *
7A	barc108, psp3001, gwm130, wmc168*, barc029 *, dupw254
7B	barc072, gwm046, gwm333[np], psp3033
7D	gwm130, barc214*, gwm295, barc076 [np], psp3123, psp3113

* - absence of product of amplification in *T.miguschovae*, np – non polymorphic marker

If we take into account null alleles, the percent of polymorphic markers for chromosomes of subgenome A^t

of *Triticum miguschovae* was 85%, for chromosomes of subgenome *G* – 92% and for chromosomes of subgenome *D* – 94%. The high level of polymorphism of those microsatellite markers which we have tested allowed us to apply them for the screening of the 62 common wheat introgression lines in order to reveal the number and character of the introgressions in their genome. The results of this testing are presented in table 2.

Table 2. The number and character of introgressions revealed by application of SSR markers of chromosomes of *Triticum miguschovae*

Line	Introgressions of chromosomes of subgenomes*		
	A^t	***G***	***D***
4/8163	*2A* ^{tr}	*2G* ^{tr}	*2D* ^{tr}
5/8164	*2A* ^{tr}	*2G* ^{tr}	*2D* ^{tr}
6/8165	*1A* ^{tr},*4A* ^{tr}	*1G*^{tr},*5G*	*5D* ^{tr}
7/8166	*3A* ^{tr}		*1D* ^{tr}
8/8167	*1A* ^{tr}, *4A* ^{tr}	*5G*	*5D* ^{tr}
9/8168	*1A*^{tr}, *2A*^{tr}, *7A*^{tr}		*1D*^{tr},*2D*^{tr},*5D*^{tr},*6D*^{tr}
10/8169	*3A* ^{tr}	*5G* ^{tr}	*1D* ^{tr}
11/8170	*3A* ^{tr}	*5G* ^{tr}	*1D* ^{tr}, *5D* ^{tr}
13/8172	*3A* ^{tr}, *4A* ^{tr}		*1D* ^{tr}, *5D* ^{tr}
15/8174	*1A* ^{tr}, *4A* ^{tr}		*2D* ^{tr}, *5D* ^{tr}
17/8176	*2A* ^{tr}, *4A* ^{tr}		*4D* ^{tr}, *6D* ^{tr}
23/8182	*4A* ^{tr}, *5A* ^{tr}	*2G* ^{tr}	*1D*^{tr},*2D*^{tr},*4D*^{tr},*7D*^{tr}
24/8183			*1D* ^{tr}, *5D* ^{tr}
25/8184	*3A* ^{tr}, *5A* ^{tr}		*5D* ^{tr}
27/8186	*3A^t* tr		*6D* tr
34/8193	*2A* ^{tr}, *4A* ^{tr}	*2G*	*4D* ^{tr}
42/8201			*2D* ^{tr}, *4D* ^{tr}, *6D* ^{tr}
72/8231	*4A* ^{tr}		
73/8232	*4A* ^{tr}	*5G*	*5D* ^{tr}
74/8233		*2G*^{tr}	*5D* ^{tr}
75/8234			*2D* ^{tr}
76/8235	*2A* ^{tr}, *5A* ^{tr}		*3D* ^{tr}
77/8236			*1D* ^{tr}, *3D* ^{tr}
82/8241			*2D* ^{tr}, *4D* ^{tr}
83/8242	*3A* ^{tr}		*1D* ^{tr}, *2D* ^{tr}
84/8243		*5G* ^{tr}	*1D* ^{tr}
85/8244	*3A* ^{tr}, *4A* ^{tr}		*2D* ^{tr}, *4D* ^{tr}, *6D* ^{tr}
86/8245	*3A*^{tr},*4A*^{tr},*6A*^{tr}		
87/8246	*1A*^{tr}, *3A*^{tr},*5A*^{tr}		
88/8247	*3A* ^{tr}, *4A* ^{tr}		
89/8248	*3A* ^{tr}		*4D* ^{tr}
92/8251		*4G*^{tr},*7G*	*2D* ^{tr}
93/8252	*5A* ^{tr}		*4D* ^{tr}
94/8253	*3A* ^{tr}, *5A* ^{tr}		*5D* ^{tr}
95/8254	*4A* ^{tr}, *5A* ^{tr}		*4D* ^{tr}, *5D* ^{tr}
96/8255	*4A* ^{tr}, *6A* ^{tr}	*5G* ^{tr}	*5D* ^{tr}
97/8256	*4A*^{tr}		*5D* ^{tr}, *6D* ^{tr}
98/8257			*2D* ^{tr}, *4D* ^{tr}, *5D* ^{tr}
99/8258	*3A* ^{tr}		*2D* ^{tr}
102/8261	*4A* ^{tr}		*5D* ^{tr}
103/8262	*3A* ^{tr}		*1D* ^{tr}, *2D* ^{tr}
104/8263			*2D* ^{tr}
105/8264	*5A* ^{tr}	*5G* ^{tr}	*5D* ^{tr}, *6D* ^{tr}
106/8265			*5D* ^{tr}, *6D* ^{tr}
107/8266			*6D* ^{tr}
108/8267	*3A* ^{tr}, *4A* ^{tr}	*1G?,5G*	
109/8268	*2A* ^{tr}	*2G*	*2D* ^{tr}, *4D* ^{tr}
112/8271	*4A* ^{tr}, *5A* ^{tr}		*2D* ^{tr}, *5D* ^{tr}, *7D* ^{tr}
114/8273	*2A* ^{tr}, *4A* ^{tr}	*2G*	*2D* ^{tr}, *4D* ^{tr}
115/8274	*2A* ^{tr}, *3A* ^{tr}		*4D* ^{tr}
116/8275	*3A* ^{tr}, *4A* ^{tr}		*2D* ^{tr}, *7D* ^{tr}
117/8276		*5G*	*2D* ^{tr}, *3D* ^{tr}
118/8277	*3A* ^{tr}		*2D* ^{tr}
119/8278	*1A*^{tr}, *4A*^{tr}		*2D* ^{tr}, *5D* ^{tr}
122/8281	*6A* ^{tr}		*1D* ^{tr}, *4D* ^{tr}
123/8282	*3A*^{tr},*4A*^{tr},*7A*^{tr}		*4D* ^{tr}, *5D* ^{tr}
124/8283			*3D* ^{tr}, *4D* ^{tr}
125/8284			*4D* ^{tr}
126/8285		*5G*, *7G*^{tr}	*4D* ^{tr}, *5D* ^{tr} ,*6D* ^{tr}
127/8286	*3A* ^{tr}		*5D* ^{tr}, *6D* ^{tr}
128/8287	*4A* ^{tr}	*7G*^{tr}	*4D* ^{tr}
1552		*7G*^{tr}	*2D* ^{tr}

* - tr – identified translocation (however for chromosomes of subgenome *DD* these are assumed to arise from recombination); ? – not sure.

The results in table 2 reveal that in the tested lines the number of introgressions ranged from 1 to 7, however 79% of our lines have 2 - 4 introgressions. Most introgressions represent translocations and recombinations with the exception of some introgressions of whole subgenome-*G* chromosomes. The number of introgressions from different subgenomes was different as well as from chromosomes of different homoeologous groups. The most frequently (95 times, 49% of all introgressions) genetic material of chromosomes of subgenome *DD* was substituted by their homologues from genome of *T. miguschovae.* The least frequent such introgressions occurred for chromosomes of subgenome *GG* (25 times; 13%) and the most of them were whole chromosome substitutions. Genetic material of subgenome A^tA^t of *T. miguschovae* substituted their homoeologues 73 times with frequency 38%. In our lines the maximum number of introgressions from particular subgenomes of *T. miguschovae* in genome of one wheat line was 3 for subgenome A^tA^t , 2 for subgenome *GG* and 4 for subgenome *DD*. It can be explained by different rate of divergence of homoeologues subgenomes of *T. aestivum* and *T. miguschovae.* Subgenomes *DD* of *T. aestivum* and *DD* of *T. miguschovae* appeared to be the less divergent, as it was expected, because *Aegilops tauschii* was the donor of this genome for both of these species [5,8]. The most divergent occurred to be subgenome *BB* of *T. aestivum* and subgenome *GG* of *T. miguschovae* despite according to the general opinion *Aegilops speltoides* was the donor of those subgenomes for progenitors of these two groups of wheats [9-11].

The presence in the introgression lines of alien genetic material in the form of translocations and recombinations opens a further perspective for investigation of these lines, because they are one of the

best sources for fine mapping of particular genes, especially QTLs, transferred into the common wheat gene pool from *T. militinae* and *Aegilops tauschii*, and investigation of genomes of related to common wheat species.

Table 3. Number of detected introgressions of *T. miguschovae* in genome of common wheat lines

Subgenome	A^tA^t							
group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	total
Number introgressions	6	9	21	23	9	3	2	73
Subgenome	***GG***							
group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	total
Number introgressions	2	7	0	1	11	0	4	25
Subgenome	***DD***							
group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	total
Number introgressions	12	23	4	19	23	11	3	95
Total	20	39	25	43	43	14	9	193

We first assayed to find links between particular introgressions and grain protein content (GPC), because some lines inherited this characteristic from alien species. It was found out that an increase in GPC links with introgressions of $2A^t$ and *2G* chromosomes. A particular link was found between a particular locus of $2A^tS$ chromosome, marked by wmc 177. The link with this particular locus on the short arm of $2A^t$ chromosome of wheat with increasing of GPC was reported in other investigations as well [12-13], which suggests the existence of a common gene for this trait in both *timopheevii* and *emmer* wheat groups. Smaller influences on increasing GPC were revealed for loci on chromosomes *2GL, 2DL*, and the combinations of $3A^tS$+*5GL* or $3A^tS$+*5GL*+*5DL*.

It was known that long shape of wheat grain is undesirable characteristics for breeders and in some crosses intorgression from wild relatives especially of the *timopheevii* wheat group such type of grain shape appeared often. We studied the links of QTL for this characteristic with particular introgressions and found that this characteristic links with a locus between gwm636 and wmc177 on $2A^tS$ chromosome and particular loci on *2DL* and *5GL*. This analysis showed a way to break the link between long shape of the ear and increasing of GPC, which we have observed in some tested lines.

An increase of grain size in our lines was linked with loci on chromosomes $1A^tS$ and *1DS* of *T. miguschovae,* especially those regions linked with gliadin loci. Another interesting phenomenon of intragenome interactions in *T. militinae* was observed when we analyzed lines with this introgressions of $1A^tS$, which mainly have black glumes, but the parental forms of the *T. militinae* donor of this translocation has yellow glumes. It can be explained that in genome of parental species this characteristic was suppressed, but was expressed only in the introgression lines were not all the genome of this wild relative of common wheat are present.

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