



Allelic composition of the *TaCwi-A1* and *TaSus2-2B* genes affecting grain weight in the collection of winter wheat cultivars

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Abstract

Grain yield is closely associated with kernel weight. Cell wall invertase (CWI) and sucrose synthase (SUS) are one of the most important enzymes for sink tissue development and carbon partition, and has a high association with kernel weight. Allelic composition of the *TaCwi-A1* and *TaSus2-2B* loci was tested in 79 winter wheat cultivars using a co-dominant markers CWI21-CWI22, which amplified 404 or 402-bp and Sus2-185/589H2- Sus2-227/589L2, which amplified 423 or 381-bp fragments in different wheat accessions respectively. Some samples carried the mutation in the *TaCwi-A1* locus that negatively affects thousand-kernel weight (TKW) were shown to have TKW higher than the cultivars and lines that do not have this mutation in their genomes and despite the significant differences in TKW (from 39,4 to 59,8 g), all investigated varieties possess *Hap-L* haplotype. It can be attributed to the fact that the *TaCwi-A1* and *TaSus2-2B* are only two of the genes associated with kernel weight and its allelic composition analysis cannot explain all phenotypic variances.

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Introduction

Wheat is one of the most important crops cultivated in the world. Increasing grain yield is of great importance for breeders to meet the needs of wheat for increasing human populations. Grain yield is closely associated with thousand-kernel weight (TKW) (1).

A large number of quantitative trait loci (QTLs) have been reported to control grain yield. Two of them are the *TaCwi-A1* and *TaSus2-2B* loci, encoding a cell wall invertase (Cwi) and sucrose synthase 2 respectively, that catalyze the conversion of sucrose to glucose and fructose (2-4). *Cwi* gene is located on chromosome 2A and comprises 7 exons and 6 introns (5). The point mutation in the fourth intron (AT→AG) results in an altered transcription of the fifth exon that leads to a different polypeptide formation and TKW reduction (6).

Based on sequencing in 61 wheat accessions, three single-nucleotide polymorphisms (SNPs) were detected in *TaSus2-2B*. These formed two haplotypes (*Hap-H* and *Hap-L*). It has been shown that the varieties and lines with *Hap-H* haplotype have a larger TKW compared with varieties and lines with *Hap-L* haplotype (7).

The objective of this study was to investigate the allelic diversity of the *TaCwi-A1* and *TaSus2-2B* loci and evaluate its association with kernel weight in the collection of winter wheat varieties and lines used in Belarusian breeding.

Materials and Methods

Allelic diversity of the *TaCwi-A1* and *TaSus2-2B* loci has been investigated in a collection of 79 varieties of wheat used for the breeding process in Scientific and practical center of NAS of Belarus on agriculture (Zhodino). Thousand kernel weight measurements were conducted in the laboratory of winter wheat of the institution mentioned above.

DNA was isolated from grains by the method proposed by Plaschke et al. (8). Isolation was carried out from two kernels for each cultivar or line.

An analysis of the allelic composition of the *TaCwi-A1* gene was conducted according to the method proposed by Ma et al., 2012, with modifications (6).

An analysis of the allelic composition of the *TaSus2-2B* gene was conducted according to the method proposed by Jiang et al., 2011, with modifications (7).

A composition of the PCR reaction mixture in a volume of 12.5 μ l was the following: 1 \times Taq polymerase buffer "A" without MgCl₂; 1.5 mM MgCl₂; NTP 0.2 mM; 0.25 nM primers; 0.5 EA-Taq polymerase; 50 ng of DNA. For an analysis the primers and PCR reagents produced by "Primetehc" (Minsk) were used.

The visualization of amplified fragments was carried out after a separation by electrophoresis in TAE buffer using the GelDoc 2000 gel documentation system.

Results and Discussion

79 winter wheat cultivars were analyzed using two pairs of complementary dominant functional markers: CWI21-CW22 for investigation of allelic composition of *TaCwi-A1* gene and Sus2-185/589H2- Sus2-227/589L2 for investigation of allelic composition of *TaSus2-2B* gene.

CWI22 yields 402-bp PCR fragment in varieties with the *TaCwi-A1a* allele, and CWI21 yields a 404-bp fragment in those with the *TaCwi-A1b* allele (Fig. 1). A mean for the TKW trait was 51,4 g. 9 (11%) investigated cultivars and lines carried the *TaCwi-A1b* allele that can lead to the TKW reduction. It was revealed that the Co 207 line and the varieties Balaton, Dromos, Perfect, and Cubus have a mutation in the fourth intron

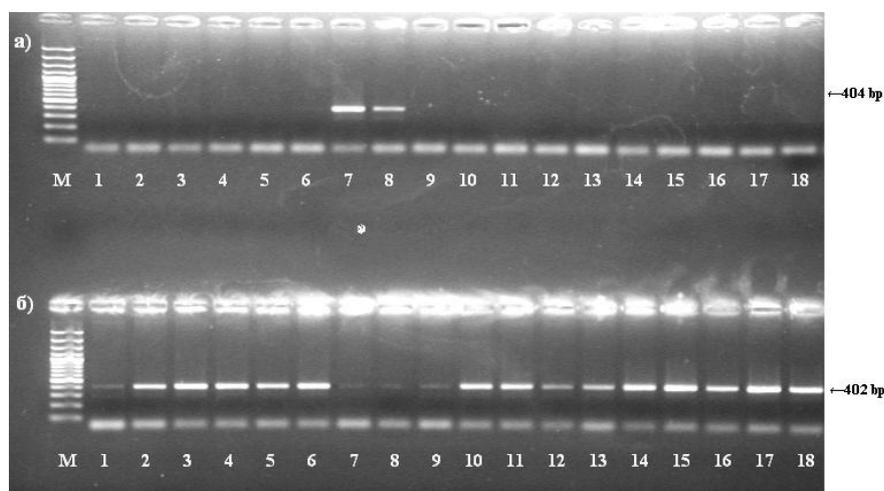


Figure 1. PCR fragments amplified with the markers CWI21 (a) and CWI22 (b) in 9 winter varieties: GeneRuler 100 bp Plus DNA Ladder (M). Varieties: Khorevytsya (1-2), Junona (3-4), Faur (5-6), Balaton (7-8), Miranda (9-10), Khvest (11-12), Blagodarna (13-14), Dobrochyn(15-16), Zagrava odes'ka (17-18).

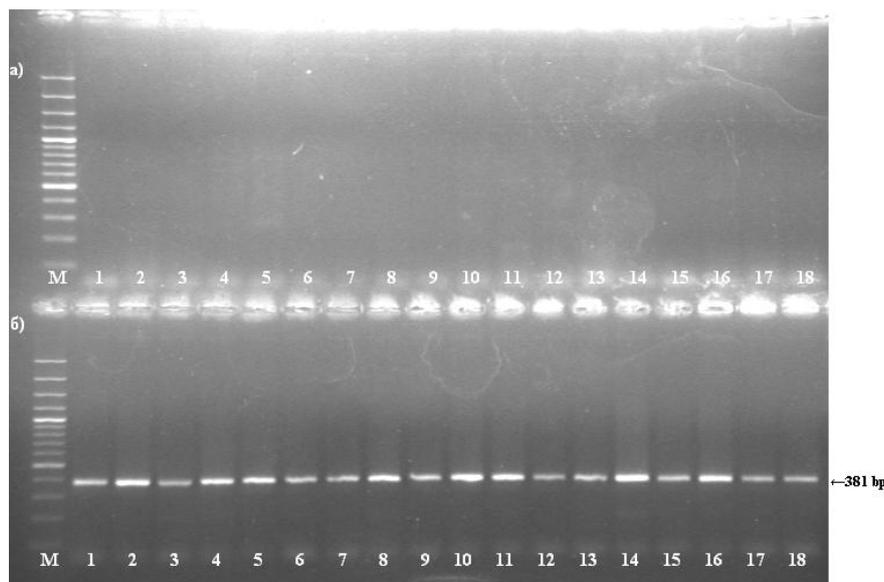


Figure 2. PCR fragments amplified with the markers Sus2-185/Sus2-589H2 (a) and Sus2-227/Sus2-589L2 (b) in 9 winter varieties: GeneRuler 100 bp Plus DNA Ladder (M). Varieties: Khorevytsya (1-2), Junona (3-4), Faur (5-6), Balaton (7-8), Miranda (9-10), Khvest (11-12), Blagodarna (13-14), Dobrochyn(15-16), Zagrava odes'ka (17-18).

(AT→AG) of this locus and the TKW that is less than the average value (47,9; 49,7; 51,0; 50,2 and 50,3 g respectively). The TKW of the cultivars Akord, Acratos, Catalus, and FT Wonder that also carry this mutation was higher than the average and amounted to 56,6; 55,5; 53,1 and 56,9 g respectively (Table 1).

The TKW of 70 cultivars and lines that do not carry a mutation in the *TaCwi-A1* locus (*TaCwi-A1a* allele) was in the range of 39,4 to 59,8 g and its average value was 51,3 g. The mean TKW for 9 varieties and lines that have a mutation in the *TaCwi-A1*

locus was 52,4 g. The range of values varied from 47,9 to 56,9 g.

Some samples carried the mutation in the *TaCwi-A1* locus were shown to have TKW higher than the cultivars and lines that do not have this mutation in their genomes.

Sus2-185/589H2 yields 423-bp PCR fragment in varieties with the *Hap-H* haplotype, and Sus2-227/589L2 yields a 381-bp fragment in those with the *Hap-L* haplotype (Fig. 2). Despite the significant differences in TKW, all investigated varieties are *Hap-L*.

Table 1. Thousand-kernel weight and allelic composition of the *TaCwi-A1* and *TaSus2-2B* genes in winter wheat varieties

No	Name of variety or line	Country of origin	Thousand-kernel weight, g	Allelic composition of <i>TaCwi-A1</i> locus	Allelic composition of <i>TaSus2-2B</i> locus
1	Elegiya (sample 1)	Belarus	52,9	<i>TaCwi-A1a</i>	<i>Hap-L</i>
2	Elegiya (sample 2)	-/-	53,1	-/-	-/-
3	Al'batros odesskiy	Ukraine	48,0	-/-	-/-
4	Bagira	-/-	52,1	-/-	-/-
5	Barvina	-/-	55,2	-/-	-/-
6	Blagodarna	-/-	56,5	-/-	-/-
7	Borvij (sample 1)	-/-	40,9	-/-	-/-
8	Borvij (sample 2)	-/-	50,3	-/-	-/-
9	Bunchuk	-/-	44,3	-/-	-/-
10	Vidrada	-/-	56,7	-/-	-/-
11	Vil'shana	-/-	53,4	-/-	-/-
12	Gerta	-/-	51,5	-/-	-/-
13	Godual'nytsya odes'ka	-/-	57,7	-/-	-/-
14	Dobrochyn	-/-	52,5	-/-	-/-
15	Zagrava odes'ka (sample 1)	-/-	46,2	-/-	-/-
16	Zagrava odes'ka (sample 2)	-/-	51,2	-/-	-/-
17	Zamozhnist'	-/-	51,7	-/-	-/-
18	Istina odes'ka	-/-	45,7	-/-	-/-
19	Kalita	-/-	43,2	-/-	-/-
20	Karmen	-/-	47,6	-/-	-/-
21	Kozachiy ataman	-/-	52,8	-/-	-/-
22	Lord	-/-	55,1	-/-	-/-
23	Mironivs'ka storichna	-/-	49,8	-/-	-/-
24	Naysel	-/-	50,2	-/-	-/-
25	Odesskaya 200	-/-	54,5	-/-	-/-
26	Podolyanka	-/-	59,8	-/-	-/-
27	Polevik	-/-	51,9	-/-	-/-
28	Pochayivka	-/-	55,9	-/-	-/-
29	Prydesnyans'ka napivkarlykova	-/-	59,4	-/-	-/-
30	Roksolana	-/-	44,5	-/-	-/-
31	Sagaidak	-/-	58,5	-/-	-/-
32	Selyanka odesskaya	-/-	51,8	-/-	-/-
33	Slavna	-/-	56,0	-/-	-/-
34	Turunchuk	-/-	51,0	-/-	-/-
35	Uzhynok	-/-	46,7	-/-	-/-
36	Unikum	-/-	44,9	-/-	-/-
37	Utes	-/-	56,1	-/-	-/-
38	Khvest	-/-	47,4	-/-	-/-
39	Khorevytsya (sample 1)	-/-	55,5	-/-	-/-
40	Khorevytsya (sample 2)	-/-	59,5	-/-	-/-
41	Elik	-/-	52,7	-/-	-/-
42	Epokha odes'ka	-/-	46,4	-/-	-/-
43	Yavorina	-/-	47,5	-/-	-/-
44	Yaroslavna	-/-	47,1	-/-	-/-

Table 1. cont. Thousand-kernel weight and allelic composition of the *TaCwi-A1* and *TaSus2-2B* genes in winter wheat varieties

No	Name of variety or line	Country of origin	Thousand-kernel weight, g	Allelic composition of <i>TaCwi-A1</i> locus	Allelic composition of <i>TaSus2-2B</i> locus
45	Ariadna	Russia	54,6	-/-	-/-
46	Asket	-/-	42,2	-/-	-/-
47	Bogdanka	-/-	50,6	-/-	-/-
48	Don 95	-/-	55,1	-/-	-/-
49	Donskoy syurpriz	-/-	48,2	-/-	-/-
50	Donskaya polukarlikovaya	-/-	54,8	-/-	-/-
51	Yermak	-/-	52,2	-/-	-/-
52	Yershovskaya 11	-/-	53,8	-/-	-/-
53	Zhemchuzhina Povolzh'ya	-/-	50,1	-/-	-/-
54	Zarnitsa	-/-	50,9	-/-	-/-
55	Koroganka	-/-	48,3	-/-	-/-
56	Levoberezhnaya 1	-/-	55,9	-/-	-/-
57	Novoyershovskaya	-/-	56,6	-/-	-/-
58	Pamyati Kalinenko	-/-	45,0	-/-	-/-
59	Proza	-/-	53,3	-/-	-/-
60	Rostovchanka 3	-/-	45,0	-/-	-/-
61	Sintetik	-/-	56,5	-/-	-/-
62	Yunona	-/-	44,8	-/-	-/-
63	F.594	-/-	39,4	-/-	-/-
64	Emmit	Germany	50,0	-/-	-/-
65	Samurai	-/-	55,3	-/-	-/-
66	Skagen	-/-	56,3	-/-	-/-
67	Faur	Romania	53,2	-/-	-/-
68	Miranda	-/-	55,4	-/-	-/-
69	Lupus	Austria	47,1	-/-	-/-
70	Saturnus	-/-	52,1	-/-	-/-
71	Akord	Ukraine	56,6	<i>TaCwi-A1b</i>	-/-
72	Acratos	Germany	55,5	-/-	-/-
73	Catalus	-/-	53,1	-/-	-/-
74	Cubus	-/-	50,3	-/-	-/-
75	Dromos	-/-	51,0	-/-	-/-
76	Perfect	-/-	50,2	-/-	-/-
77	Balaton	Austria	49,7	-/-	-/-
78	Co 207	France	47,9	-/-	-/-
79	FT Wonder	Canada	56,9	-/-	-/-

It can be attributed to the fact that the *TaCwi-A1* and *TaSus2-2B* are only two of the genes associated with kernel weight and its allelic composition analysis cannot explain all phenotypic variances. The further identification and cloning of QTLs responsible for TKW and development of functional markers are the necessary and perspective direction for an improvement of wheat breeding process.

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