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FORMATION OF AWNS IN WHEAT LINES WITH INTROGRESSIONS FROM *Aegilops* SPP. CAUSED BY NOVEL REGULATORY GENES

Awns play a significant role in the plant physiology and are a well-known morphological marker in wheat. Awnedness in wheat is regulated by three inhibitors – Hd, B1, and B2, but awn promoters are still largely unknown. The present study is aimed at analysis of the expression level of awn development regulatory genes orthologs, TaDL, TaTOB1, TaETT2, and TaKNOX3, in Triticum aestivum, genome substitution amphidiploids AABB$S^{sh}S^{sh}$ and AABBUU, and derived lines with introgressions from Aegilops sharonensis and Ae. umbellulata.

Expression of four mentioned genes was detected in the lemma of all strains studied, and the role of these genes in awn development was assumed. In awned introgression lines, expression of all studied genes differed from mid-parent value: it was present in parent genotypes and absent in derived lines. Non-additive expression of four studied genes in introgression lines is considered to be the possible reason that caused development of nonparental awned phenotype. The presence of two products resulting from TaTOB1 cDNA amplification, one of which contained fourth intron and another lacking it, is considered to be the result of two mRNA presence due to different TaTOB1 homoeoalleles expression.

Keywords: wheat, amphidiploids, awn development, genes-inhibitors.

Grasses have a specific flower structure that is typical for this taxon. Stamens and the carpel are covered with sepal homologs – the lemma and the palea. Some of the Gramineae species have awns, needle-like elongations of the lemma, which are considered to be a modified leaf blade [1]. Awns are a well-known morphological marker in wheat, and they play a major role in transpiration and photosynthesis of the spike [2–4].

Molecular mechanisms controlling awn development are still largely unknown. All currently identified and sequenced genes encode transcription factors. Two of them are positive regulators of awn development in rice, the *DL* gene encoding YABBY and the *OsETT2* gene encoding ARF family transcription factors [5]. Negative regulators of awn development include the rice *TOB1* gene and the barley *Hd* gene encoding YABBY and KNOX transcription factors respectively [6–8]. Although the gene regulatory network of awn development has not been established yet, some of the interactions in this network are known. It is believed that there must be some interplay between the KNOX and TOB1 transcription factors, as *tob1* rice mutants do not express the KNOX gene *OSH1*, a marker of indeterminate undifferentiated cells [6]. Positive regulation downstream from *DL* to *OsETT2* may also exist, because in awnless rice cultivar elongation of awns caused by de-repression of *OsETT2* is suppressed by the *dl* mutation [5].

Despite increasing knowledge on regulation of awn development in rice and barley, little is known about this process in wheat. Three well-known genes that are dominant inhibitors of awnedness are *Hd* (4AS), *B1* (5AL), and *B2* (6BL) (cited for [3]). For today, none of these genes were sequenced, and

polymorphisms causing differences in phenotypes are unknown. Previously, the wheat *Hd* candidate, *Wknox1a*, was cloned and sequenced, but polymorphisms underlying *Hd* allelic diversity have not been identified [7]. Awnedness is a recessive character. Any of the three genes mentioned inhibits awn development variously. The availability of only two dominant genes among three represses awn development fully [9]. It has been demonstrated now that in common wheat there are not only genes-inhibitors but also genes – promoters of awn development [10]. Moreover it has been demonstrated that awnedness may be a dominant character in diploid and tetraploid wheats [11], though genes – promoters of awn development are not identified until now. It becomes apparent that except for long ago identified genes, inhibitors of awn development in wheat other regulatory genes can interact with them. However, hitherto molecular mechanisms of ontogenetic events that lead to awn development is unknown [8,12].

Four awned common wheat lines with introgressions from *Aegilops* spp. were obtained by crossing the awnless *T. aestivum* variety Aurora (genome AABBDD) with a genome-substitution amphidiploids. One of them was terminally awned Aurosis (genome AABBS^{sh}S^{sh}), in which the D wheat subgenome of Aurora variety is substituted with the S^{sh} genome of *Ae. sharonensis*. The other was the semi-awned amphidiploid Aurolata (AABBUU with the *Ae. umbellulata* genome U substituting the D subgenome) [13]. The available of awn development promoters in chromosomes of 6-th homoeologous group was demonstrated by us earlier for *Ae. umbellulata* [14] and *Ae. sharonensis* [15]. The Aurora cultivar contains dominant inhibitor of awn development *B1* in chromosome 5A (cited for [4]), so appearance of awn or awn-like sprouts in genome substitution amphidiploids is unexpected phenomenon and can be recorded as novel character. The awned phenotype in the introgression lines can be considered as novel or non-parental one, because among components of initial crosses Aurosis x Aurora and Aurolata x Aurora the awned phenotypes were absent. According to actual views on processes witch take place in genomes of hybrid origination (introgression) appearance of novel phenotype compared to phenotype expected from the phenotypes of initial cross components are to be connected with changes in gene expression, whose products play a part of ontogenetic regulators [15–17]. The aim of our investigation was to ascertain the available or absence of difference in expression of genes that are related to awn development in introgression wheat lines compared to the components of initial crosses, Aurora cultivar and genome substitution amphidiploids.

In this study we show that expression of four wheat genes, *Wknox1a* (*TaKNOX3*), *TaTOB1*, *TaETT2*, and *TaDL* takes place in the lemma of bread wheat, where they can regulate awn development similarly to the known rice and barley orthologs. Expression of these genes was also detected in two amphidiploids, Aurosis (AABBS^{sh}S^{sh}) and Aurolata (AABBUU). Next, we show that in the introgression lines (ILs) obtained from crossing amphidiploids with a bread wheat cultivar, expression of the studied genes does not occur in the lemma of the plants which have the novel awned phenotype but is observed in the plants with parental phenotypes, which indicates the role of the studied genes in the emergence of a novel phenotype. Finally, we show that *TaTOB1* produces two transcripts, one of which contains intron and the other is intronless, which may result from the expression of different *TaTOB1* homoeoalleles.

Materials and methods

For this study, we used the common wheat *Triticum aestivum* L. (2n=6x=42, AABBDD, seed number studied (N) is 5) variety Aurora, the genome substitution amphidiploids Aurolata (2n=6x=42, AABBUU, N = 2) with the tetraploid component AABB from Aurora and UU genome from *Aegilops umbellulata* Zhuk., and Aurosis (2n=6x=42, AABBS^{sh}S^{sh}, N = 3) with the S^{sh}S^{sh} genome from *Ae. sharonensis* Eig.

Hexaploid wheat lines with introgressions from *Aegilops* species were obtained by crossing amphidiploids with the recurrent parent cultivar Aurora, followed by the series of backcrosses until BC_4 to restore fertility of hybrids. Starting from BC_4 lines were left for self-pollination for more than 20 generations. The introgression wheat lines employed in this study were: Aurosis derivatives res 122-2 (N = 3) and res 133-1 (N = 5), Aurolata derivatives res 201 (N = 2), F04 1352 lata (N = 3), res 191 (N = 7), and res 193 (N = 6).

Tissue samples were collected from the studied plant materials 10 days after fertilization, only lemmas were taken to provide the tissue specificity of the expression detection. Total RNA was extracted according to [19] with minor modifications. The stage of HMW RNAs separation was omitted.

The reverse transcription reaction was conducted using oligo(dT) priming with RevertAid Reverse Transcriptase (Thermo Fisher Scientific, Lithuania) in accordance with the manufacturer's instruction. After this, cDNA was amplified in PCR with the

reaction conditions previously described [20]. Amplification products were separated in 2 % agarose gel on sodium borate (SB) buffer with OGeneRuler Ultra Low Range DNA Ladder (Thermo Fisher Scientific, Lithuania) as a marker. Visualization of products was performed using ethidium bromide staining [21].

Fragments of the gel containing bands of interest were cut with a sterile scalpel, DNA was then purified using Corning Costar Spin-X columns (Sigma, USA) as described in [22]. The nucleotide sequences were determined by using the Sanger sequencing method on an automated capillary sequencer (Applied Biosystems 3130, USA).

Primers were designed to the coding parts of the genes with the Primer-BLAST service [23]. Sequences of the genes were found in GenBank database: *TaDL* (AB470269.1), *TaETT2* (AY376129.1), *TaKNOX3* (AB182943.1), and PlantTF database *TaTOB1* (Traes_2BL_8BEA9CE1B.1). Primers for actin, used as a reference gene, were taken from [24].

Analysis of electrophoregrams, including determination of amplicon molecular weight and quantification of band DNA content, was performed by the Gel Analyzer program (http://www.gelanalyzer.com/) with the calibration to the per band DNA quantity in the ladder used ($r > 0.9$). All obtained data on studied gene expression were normalized to the reference actin gene.

Statistical analysis was performed using the Shapiro-Wilk test, Student t-test with the Bonferroni correction, and the Kruskal-Wallis test (H – empiric criterion volume) [25,26].

Results and discussion

All plants used in our study were classified into four distinct phenotypes – awnless, awned, semi-awned, and terminally awned. The awnless *T. aestivum* variety Aurora has 3-5 mm awns on the top flowers, and 2-4 mm awns on the middle. This phenotype is similar to that of genotypes with two or three dominant awnedness suppressors (5-10 mm on the top, <5 mm in the middle of the spike) [1]. The terminally awned amphidiploid Aurosis ($AABBS^{sh}S^{sh}$) has 14-23 mm long awns on the terminal flowers, while middle flowers are awnless (2-4 mm). Introgression lines (ILs) res 122-2 and res 133-1 derived from Aurosis x Aurora crosses are awned. The awns are 69-72 mm on the middle flowers and 50-54 mm on the top in res 122-2, and 89-93 mm in the middle and 48-50 mm on the top in res 133-1 (Fig. 1). The phenotype of the awned ILs resembles that of bread wheat with three recessive awnedness suppressors described earlier (60-70 mm top, 65-75 mm middle) [1].

Since the awnless variety Aurora could carry at least two dominant awnedness inhibitors in the A and B subgenomes, and Aurosis may get them from Aurora, the appearance of the awned phenotype in their progeny is difficult to be explained by usual inheritance pattern of this trait. At least one out of two dominant awnedness suppressors inherited from Aurora must have changed to recessive ones or be deleted in the lines [14].

The semi-awned amphidiploid Aurolata (AABBUU) has long 25-42 mm awns on the top

Fig. 1. Awned lines with introgressions from *Ae. sharonensis* res 122-2 and res 133-1 and their parents, the *T. aestivum* variety Aurora and the amphidiploid Aurosis

flowers and shorter 6-11 mm awns on the middle flowers. The awnedness phenotype of ILs res 191 and res 193 derived from the Aurolata x Aurora crosses is similar to Aurolata, but phenotype of two other ILs, res 201 and F04 1352 lata, differs from the parental ones (Fig. 2). Lines res 201 and F04 1352 lata are awned, they have long 71-76 mm awns on the middle flowers and 38-40 mm awns on the top of the spike. The semi-awned phenotype of Aurolata and two derived lines is similar to that of genotypes with the single *B1* dominant suppressor (25-35 mm top awns, 5-20 mm middle) [1].

Aurolata has an awnedness promoter from *Ae. umbellulata*, which is partially suppressed by Aurora's awnedness inhibitors located in the A and B genomes [4,15]. As a result of the promoter action, Aurolata develops long awns on the terminal flowers. However, for the awned lines to appear in the progeny of Aurolata x Aurora crosses, the action of at least two dominant awnedness suppressors inherited from Aurora should be conquered. The appearance of awned ILs in the Aurolata x Aurora and Aurosis x Aurora progeny is unlikely to be a result of random mutations. We assume that the cause of this phenomenon is the changes that occur at the epigenetic level.

genome substitution amphidiploids Aurosis and Aurolata. For the three genes, *TaKNOX3*, *TaDL*, and *TaETT2*, the expression was detected by the presence of one amplicon on the electrophoregram, and for *TaTOB1* gene two amplicons with different size were found (Fig. 3, Fig. 4).

Expression levels of all studied genes in the three parent genotypes, Aurora, Aurosis, and Aurolata, were similar. No differences between the expression levels of *TaKNOX3* gene in the variety Aurora and the two amphidiploids were observed ($H = 0$), as well as between the expression levels of *TaDL* gene ($H = 4.46$) and *TaTOB1* gene ($H = 0.2$ and $H = 3.9$ for light and heavy amplicons, respectively). Some difference was found between three genotypes studied in the expression levels of the *TaETT2* gene ($H = 5.36$, $p = 0.05$), but the samples were too small to perform further analysis. In awned ILs res 122-2 and res 133-1, res 201, and F04 1352 lata derived from cross between the bread wheat variety Aurora and the genome substitution amphidiploids, no expression of *TaKNOX3*, *TaDL*, *TaETT2*, and *TaTOB1* was detected.

The wheat ortholog of barley *HvKNOX3* (*Hd*) gene, *TaKNOX3*, was previously described by

Fig. 2. Lines with introgressions from *Ae. umbellulata*: semi-awned res 191 and res 193, and awned res 201, their parents the *T. aestivum* variety Aurora and the genome substitution amphidiploid Aurolata

To find out whether the studied genes are expressed in the lemma of our plants, we conducted end-point detection RT-qPCR using total RNA isolated from the lemma. We found that expression of all the studied genes takes place in the lemma from the middle flowers of the 10 days after flowering (DAF) spike of the bread wheat variety Aurora and

Takumi et al. [7] as *Wknox1-a*, the candidate for wheat *Hd*. We found that expression of *TaKNOX3* was present in the lemma of the awnless bread wheat variety Aurora, the terminally awned amphidiploid Aurosis (Fig. 3), and in the semi-awned amphidiploid Aurolata (Fig. 4), but no expression was observed in ILs res 122-2 and

res 133-1 derived from Aurosis and Aurora crosses (Fig. 3), and in ILs res 201 and F04 1352 lata derived from Aurolata and Aurora crosses (Fig. 4).

Expression of the *TaTOB1* gene was observed in Aurora and the two amphidiploids, and its expression was detected by the presence of two bands on the electrophoregram. No amplification product was detected in derived introgression lines (Fig. 3, Fig. 4).

A wheat ortholog of the *OsETT2* gene, *TaETT2*, was found to be expressed in the lemma of Aurora, Aurosis and Aurolata, while *TaETT2* expression was not observed in awned ILs res 122-2 and res 133-1 derived from Aurosis and Aurora crosses (Fig. 3), as well as in lines res 201 and F04 1352 lata derived from Aurolata and Aurora crosses (Fig. 4).

The expression profile of the wheat ortholog of the rice gene *DL*, *TaDL*, in the studied genotypes was similar to that of *TaETT2*. This gene is expressed in Aurora, Aurosis, and Aurolata, and no expression is observed in awned ILs res 122-2, res 133-1, res 201, and F04 1352 lata (Fig. 3, Fig. 4).

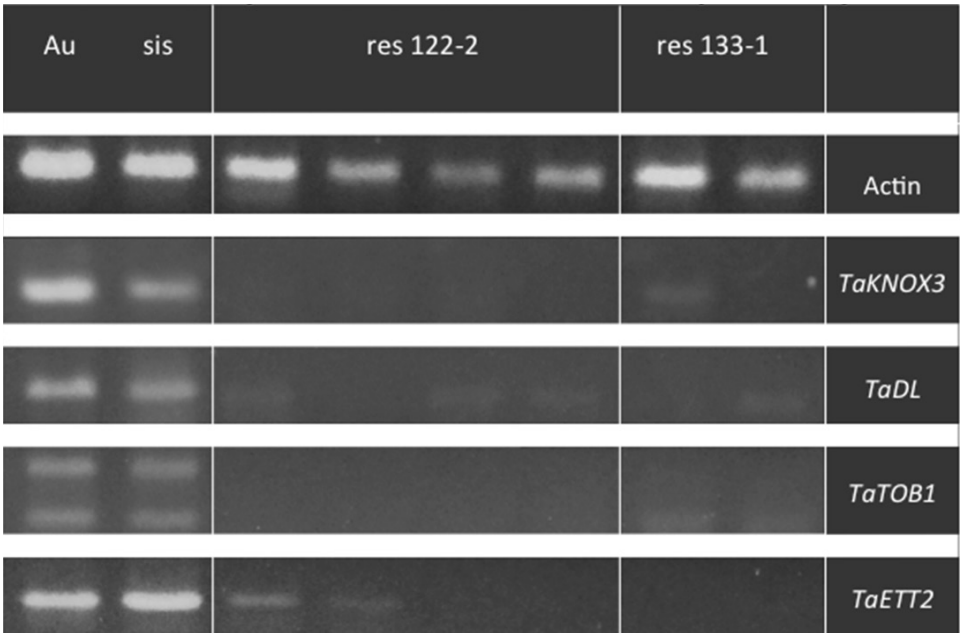

Fig. 3. Expression of the awn development regulatory genes *TaKNOX3, TaDL, TaTOB1, TaETT2* in the lemma of Aurora (Au), Aurosis (sis) and derived ILs (res 122-2 and res 133-1)

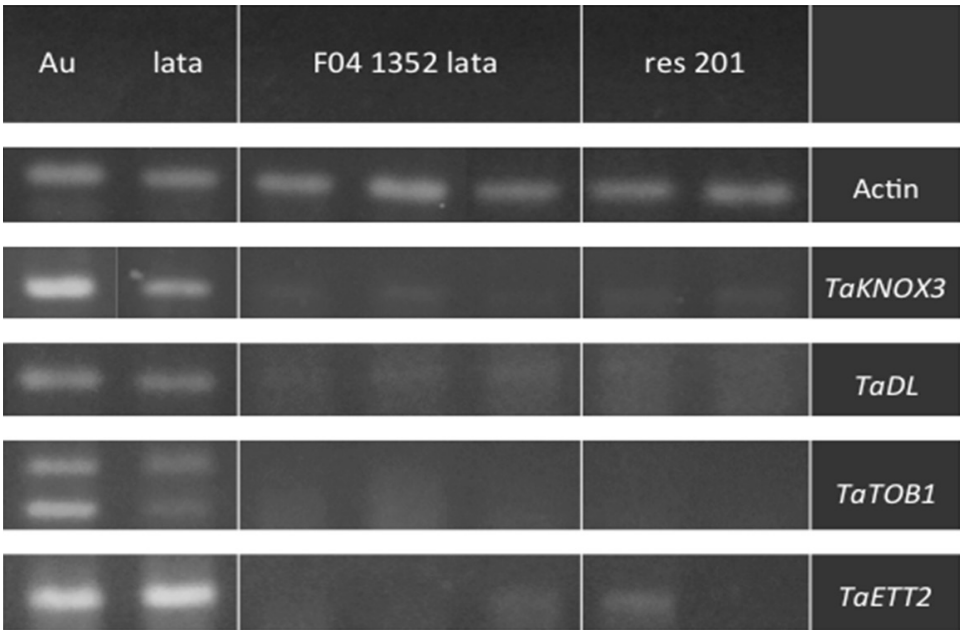

Fig. 4. Expression of the awn development regulatory genes *TaKNOX3, TaDL, TaTOB1, TaETT2* in the lemma of Aurora (Au), Aurolata (lata) and derived ILs F04 1352 lata and res 201

As mentioned before, the expression of *TaTOB1* in the studied genotypes was detected by the presence of two amplicons of different sizes. Agarose gel separation of Aurora cDNA amplification products gave two components, while genomic DNA PCR products separation showed only one amplicon. The first component from cDNA amplification products coincided with the size of the genomic DNA amplicon (232 bp), and the second component was approximately 110 bp lighter (Fig. 5). The presence of two *TaTOB1* amplification products of different sizes on the electrophoregram indicates the presence of two transcripts in the mRNA pool.

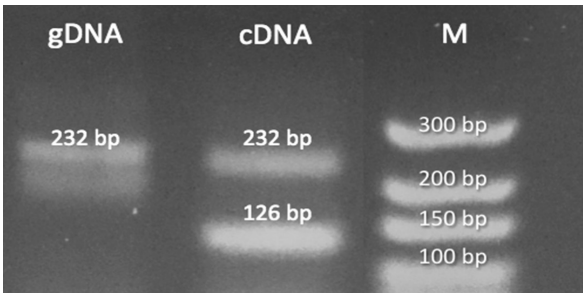

Fig. 5. Amplification products of the *TaTOB1* gene with coding DNA (cDNA) and genomic DNA (gDNA) of the *T. aestivum* variety Aurora. Approximate sizes of amplicons are given. M – molecular marker

In order to find out the reason for the presence of two transcripts we aligned our primer sequences to the sequence of the *TaTOB1-B* homoeoallele, the structure of which had been determined earlier [12]. Primers used in this study for wheat *TaTOB1* amplification are located in two different exons: the forward primer sequence was aligned to the fourth exon sequence, and the reverse – to the fifth exon sequence (Fig. 6). The expected amplification product with cDNA was supposed to be 126 bp, which corresponds to the size of the light amplicon we observed on the electrophoregram. In the genomic sequence, the fourth and fifth exons are separated by the fourth intron 119 bp in length. The second amplification product differs from the light one approximately by 110 bp, and it has the same size as the gDNA amplification product, which leads us to the idea that the heavy amplicon may carry the fourth intron.

To check our assumption about the origin of two *TaTOB1* components, we sequenced the heavy and light amplicons of the bread wheat variety Aurora and the *T. aestivum / Ae. sharonensis* genome substitution amphidiploid Aurosis. The sequences of heavy amplicons of Aurora and Aurosis were identical by 73 %, and the light amplicons shared

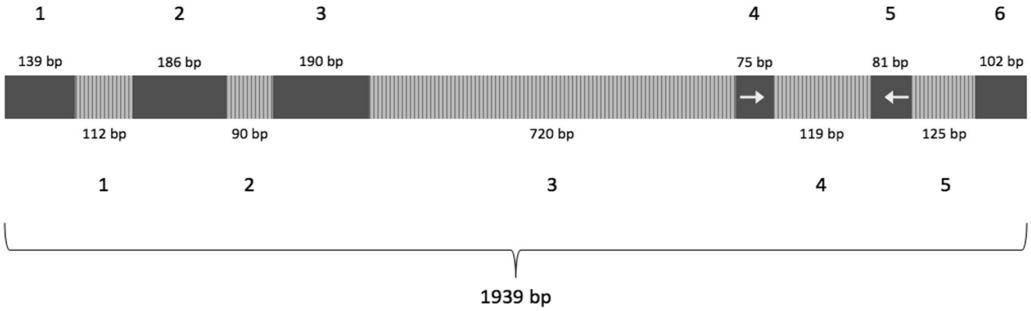

Fig. 6. The exon-intron structure of the *TaTOB1-B* homoeoallele. Exon number and its length are shown in the top rows, and intron number and its length are in the bottom rows. Primers are indicated by two arrows

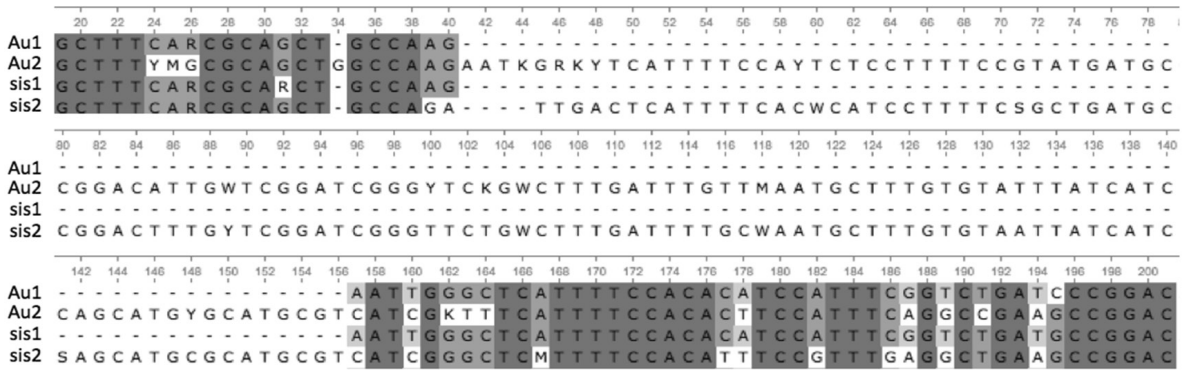

Fig. 7. Aligned sequences of *TaTOB1* amplification products of the *T. aestivum* variety Aurora (Au1 is the light product, Au2 is the heavy one) and the *T. aestivum / Ae. sharonensis* genome substitution amphidiploid Aurosis (sis1 is the light product, sis2 is the heavy one)

85 % homology. Aurora heavy amplicon had 117 bp unique sequence located between two sequences shared with the Aurora light amplicon, whereas the Aurosis heavy amplicon had a 112-bp insert between two flanking sequences shared with the Aurosis light amplicon (Fig. 7). We suppose that the 117-bp sequence in the Aurora heavy amplicon, and the 112 bp sequence in the Aurosis heavy amplicon are sequences of the fourth intron in one of the *TaTOB1* transcripts.

Two Aurolata derived ILs, res 193 and res 191, whose phenotype is similar to that of Aurolata (semi-awned) showed no difference in the expression of all the four studied genes compared to the parents (Table).

indicates their likely role in the regulation of awn development in wheat and studied amphidiploids, similar to the role their orthologs play in related Gramineae species.

Introgression lines res 122-2, res 133-1, F04 1352 lata, and res 201 derived from crossings of one of the amphidiploids (Aurosis or Aurolata) with the *T. aestivum* variety Aurora do not show expression of the four studied genes (Fig. 3, Fig. 4). As Aurora and Aurosis (the parents of the ILs res 122-2 and res 133-1) show the presence of all four gene expressions, the absence of their expression in the derived lines can be regarded as a non-additive gene expression. The same applies for ILs F04 1352 lata and res 201, as their parents

Table. **Expression levels[1)] of the studied awnedness regulators in the parents (Aurora, Aurolata) and derived ILs res 191 and res 193 with the parental phenotype**

Gene	parents (Aurora, Aurolata)	res 191	res 193	t[2)] for comparison	
				parents–res 191	parents–res 193
TaKNOX3	44.2±14.8	43.4±31.0	44.8±23.2	0.06	0.04
TaTOB1	48.3±24.0	37.0±21.5	38.0±19.1	0.83	0.82
TaETT2	57.5±21.5	30.2±16.6	34.9±16.7	2.38	1.97
TaDL	52.8±8.0	31.6±12.5	32.1±13.3	3.20	3.20

[1)] Values of expression levels of all genes are calculated as ng/band and normalized to actin
[2)] 0.01 = 3.43 for *df* = 12 (line 191) and 0.01 = 3.50 for *df* = 11 (line 193) with the Bonferroni correction for two comparisons

For the *TaKNOX3* gene, expression levels were similar in Aurora, Aurolata and derived ILs res 193 (t = 0.04) and res 191 (t = 0.06). For the light component of *TaTOB1*, no difference was found between the expression levels in Aurora, Aurolata and derived ILs res 193 (t = 0.82) and res 191 (t = 0.83), and the presence of the heavy component was found to be variable in the studied lines. No difference in the expression levels was found between Aurora, Aurolata and derived IL res 191 for the *TaETT2* gene (t = 2.38) and *TaDL* gene (t = 3.2), as well as between expression levels of these genes in the parental genotypes and IL res 193 (t = 1.97 and t = 3.2 for *TaETT2* and *TaDL*, respectively).

The presence of four orthologs of the awnedness regulatory genes, *TaTOB1*, *TaDL*, *TaETT2*, and *TaKNOX3*, in the genome of *T. aestivum* was described previously [12]. Three of them are orthologs of rice awn regulators *DL*, *TOB1*, and *ETT2*. *TaKNOX3* is regarded as a barley *Hd* (*HvKNOX3*) ortholog described previously as *Wknox1a* [5–7]. Here, we show that the expression of *TaTOB1*, *TaDL*, *TaETT2*, and *TaKNOX3* takes place in the lemma of the *T. aestivum* variety Aurora and genome substitution amphidiploids *T. aestivum / Ae. sharonensis* (Aurosis) and *T. aestivum / Ae. umbellulata* (Aurolata) (Fig. 3, Fig. 4). The tissue specificity of expression of these genes

Aurora and Aurolata have equally detectable expression levels of the genes studied. The ILs have an awned phenotype differing from the parental phenotypes and their expression level of four studied genes, which orthologs are known to regulate awn development, differs from the mid-parent value. Non-additive expression of regulatory genes is considered to be one of the main causes of the emergence of new phenotypes in hybrids and plants with alien introgressions in their genomes [16–18]. So, we assume that non-additive expression of the studied orthologs of awnedness regulators may have caused the development of the non-parental phenotype in awned introgression lines. Also, the non-additive expression of studied genes may be the feature of more extensive transcriptome changes.

Aurolata-derived ILs res 193 and res 191 do not differ from the parent phenotype. They do not show any differences in the expression levels of four studied genes compared to parents, as opposed to awned lines F04 1352 lata and res 201 with the non-parental phenotype. The correlation of the expression profiles with the phenotype of Aurolata-derived ILs confirms our assumption that changes in the regulatory gene expression may have caused the development of a novel phenotype in the awned F04 1352 lata and res 201 lines. So, we suggest that the studied genes are involved in the regulation of awnedness in the

studied plant material, as changes in their expression is correlating with the changes in awnedness.

It is largely known that the *Hd* gene is the awnedness inhibitor in wheat, and the dominant allele of this gene suppresses awn development [1]. In barley, the dominant allele of the wheat *Hd* ortholog, *HvKNOX3*, causes awn development impairment due to its overexpression in the lemma, which is also considered to be an inhibitory effect [27]. We can expect that in wheat an inhibitory action of the dominant *Hd* allele on awn development is associated with high levels of this gene expression similarly to barley. As *TaKNOX3* is the candidate for the wheat *Hd* gene, we suppose that its high expression should suppress awn development.

High expression of the *TaKNOX3* gene, which, according to our assumption, suppresses awnedness, is found in the lemma of the three parent genotypes – awnless Aurora, terminally awned Aurosis, and semi-awned Aurolata. Proceeding from this assumption, the removal of *TaKNOX3* inhibitory action in ILs res 122-2, res 133-1, res 201, and F04 1352 lata may have caused the emergence of awned non-parental phenotype in these lines. The existing correlation between the expression of the *Hd* candidate, the *TaKNOX3* gene, and awnedness in studied genotypes, may show that high expression of *TaKNOX3* gene suppress awn development.

Previously it was shown that rice *TOB1* gene is a negative regulator of awn development [6]. We suppose, that similarly to *TOB1* in rice, the wheat ortholog *TaTOB1* may suppress awn development in the studied genotypes. Our results fit into this hypothesis, as *TaTOB1* expression is absent in awned lines but present in the parent genotypes, that is, the lack of its expression is associated with the awnedness.

Expression of the *TaTOB1* gene produces two transcripts, as two products for this gene cDNA amplification are found in the *T. aestivum* variety Aurora and two studied amphidiploids Aurosis and Aurolata (Fig. 5). In Aurora the size of the heavy amplicon, which has 117 bp insert, coincides with the genomic DNA amplification product (Fig. 7). The length of the insert in Aurora is about the predicted size of the fourth intron in the *TaTOB1-B* and *TaTOB1-D* homoeoalleles, which is 119 bp [12]. We suggest that two different *TaTOB1* transcripts in Aurora differ by the presence of the intron sequence, and the 117 bp insert found in the heavy amplicon is the fourth intron of the *TaTOB1* gene. The two transcripts may be a result of expression of two different *TaTOB1* gene homoeoalleles, *TaTOB1-B* and *TaTOB1-D*, as their fourth intron length is about the size of the insert. The sequence of the Aurosis heavy amplicon has a set of features distinguishing it from the Aurora's heavy amplicon. Firstly, they differ by the size of the insert, Aurosis amplicon has a 113 bp insert sequence with a 4 bp deletion in the 5' region of the predicted fourth intron (Fig. 7). Secondly, the 3' region of the Aurosis fourth exon sequence contains a dinucleotide inversion AG > GA compared to the light amplicons and Aurora's heavy amplicon. As Aurosis and Aurora have a different third genome – the S^{sh} genome of *Ae. sharonensis* in Aurosis and the D genome in Aurora, their heavy amplicons appear to be a result of expression of different homoeoalleles, *TaTOB1-*S^{sh} in Aurosis and *TaTOB1-D* in Aurora. Proceeding from this, the light amplicons of both genotypes are the products of *TaTOB1-B* transcription.

Amplicons originating from the *TaTOB1-*S^{sh} and *TaTOB1-D* homoeoalleles have features that may be the reason why they have retained the fourth intron. Firstly, canonical splicing site GT–AG sequence is not found on the borders of the introns in heavy amplicons derived from *TaTOB1-*S^{sh} and *TaTOB1-D*. Secondly, their sequences have A > C SNP in the border sequence of the fifth exon compared to the light amplicons (Fig. 7). Both changes could contribute to the ability of the splicing complex to process the fourth intron from the *TaTOB1-*S^{sh} and *TaTOB1-D* pre-mRNA. The retained fourth intron may disrupt the function of TaTOB1 proteins translated from *TaTOB1-*S^{sh} and *TaTOB1-D* leaving *TaTOB1-B* the only homoeoallele that gives a functional product.

In the previous studies on awnedness regulation in rice, it was shown that *OsETT2* induces awn development, so we expected that the wheat ortholog *TaETT2* plays a similar role as a positive awn development regulator [5]. However, though *TaETT2* is expressed in the bread wheat Aurora, this variety has the awnless phenotype. The reason for this may be the presence of inhibitory genes, *TaTOB1* and *TaKNOX3*, which suppress awn development eliminating the effect of the positive regulator *TaETT2*. If this assumption is correct, then the removal of the inhibitory effect of *TaKNOX3* and *TaTOB1* in the ILs, detected by the absence of their expression, is sufficient to promote development of the awns even in the absence of *TaETT2* promoter expression.

It has been shown that expression of *TaDL* takes place in the midrib region of the wheat lemma, which coincides with the localization of *DL* expression in the rice lemma [28]. Expression of the *DL* gene in the rice lemma is considered to promote awn development [5]. Likewise, we supposed that wheat *TaDL* has a similar role in awn development, and

thus we expected that the presence of its expression would be associated with the awned phenotype of the plants. However, our results were opposite – the presence of *TaDL* expression was detected in the genotypes without pronounced awnedness. This indicates that the expression of the *TaDL* gene in the lemma of the bread wheat variety Aurora and the two genome substitution amphidiploids is not sufficient to promote awn development in these genotypes. In addition, the presence of this gene expression in the lemma of the awned ILs in this study was not required for awn development, because awns were successfully formed without any detected *TaDL* expression. The reason for this may be the same as for *TaETT2* – phenotypic expression of both promoter genes in the parental genotypes is masked by the inhibitors *TaTOB1* and *TaKNOX3*, and the removal of the inhibitory effect of these genes in the ILs is sufficient for awn development.

Conclusion

Association of the non-additive expression of the four studied genes with the awned phenotype in the introgression lines indicates that it could play a role in the development of this non-parental feature. Otherwise, the variation observed in the transcriptome of the studied lines may be a symptom of more extensive changes causing the appearance of the novel phenotype.

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ФОРМУВАННЯ ОСТЕЙ У ПШЕНИЧНИХ ЛІНІЙ З ІНТРОГРЕСІЯМИ ВІД ВИДІВ *Aegilops* ЗА УЧАСТЮ НОВИХ РЕГУЛЯТОРНИХ ГЕНІВ

Ості, голкоподібні подовження лемми, відіграють певну роль у фізіологічних процесах у злаків та є добре відомою й зручною маркерною ознакою у пшениці. Відсутність остей (безостість) у пшениці регулюється трьома генами-інгібіторами – *Hd, B1* та *B2*. Однак молекулярний механізм подій, які б завершувались утворенням остей (остистий фенотип), досі невідомий, як невідомі і гени – промотори розвитку остей у пшениці м'якої. Метою дослідження було проаналізувати експресію генів *TaDL, TaTOB1, TaETT2, TaKNOX3,* що є ортологами регуляторних генів розвитку остей у рису та ячменю, у генотипах *Triticum aestivum* (сорт Аврора), геномно-заміщених амфідиплоїдах Аврозис та Авролата, гексаплоїдних пшеничних лініях з інтрогресіями від *Aegilops sharonensis* та *Ae. umbellulata.*

У леммі пшениці м'якої та амфідиплоїдів було зареєстровано експресію всіх чотирьох генів. Профіль експресії генів *TaDL, TaTOB1, TaETT2* та *TaKNOX3* залежав від фенотипу рослин: усі чотири гени експресуються в геномі Аврори та геномно-заміщених амфідиплоїдів і не експресуються в геномі остистих інтрогресивних ліній, похідних Аврозису та Авролати. Для двох ліній похідних Авролати, які за фенотипом не відрізнялись від Авролати (напівостисті), зареєстровано повний збіг картини експресії за всіма чотирма генами з такою для обох компонентів ініціального схрещування Аврори та Авролати. Збіг появи новітнього фенотипу (остистий колос) проти батьківських зі зміною профілів експресії критичних для розвитку остей генів підтверджує участь досліджуваних генів у розвитку колоса, одночасно вказуючи на наявність деяких епігенетичних ефектів у їхній експресії (неадитивність експресії). Експресія гена *TaTOB1* проявляється двома ампліконами різної маси. За даними секвенування зроблено припущення про наявність різних алелів цього гена за рахунок наявності/відсутності 4-го інтрона в гені.

Ключові слова: пшениця, амфідиплоїди, розвиток остей, гени-інгібітори остей, гени-промотори остей.

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