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Beta-amylase gene variability in introgressive wheat lines

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Abstract:	Variability of the beta-amylase gene in bread wheat, artificial amphidiploids, and derived introgression wheat lines was analyzed. Variation in homeologous beta-amylase sequences caused by the presence of MITE (Miniature Inverted-Repeat Transposable Element) and its footprint has been identified in bread wheat. The previously unknown localization of MITE in Triticum urartu and T. aestivum L. beta-amylase gene has been found. These species have a MITE sequence in the third intron of beta-amylase, as opposed to Aegilops comosa and a number of other Triticeae species, which have it in the fourth intron. These two MITEs from Ae. comosa and T. aestivum were shown to have low identity scores. Miosa, an artificial amphidiploid, which has the M genome from Ae. comosa was shown to lose the MITE sequences. This loss might be caused by genomic shock due to allopolyploidization.
Response to Reviewers:	Dear Editor We are very grateful to for helpful comments of the reviewers. We have corrected everything that was suggested. -the paper was improved with details added to introduction and discussion -suggested citations added -specific comments from reviewer #1 in attached file corrected -word ‘localised’ was changed to ‘located’ -the paper rechecked for bugs Unfortunately, we couldn’t improve our work with the help of P-MITE database advised, because it has no bread wheat sequences, as opposed to Nucleotide NCBI. Thank you for your time and consideration.

Figure

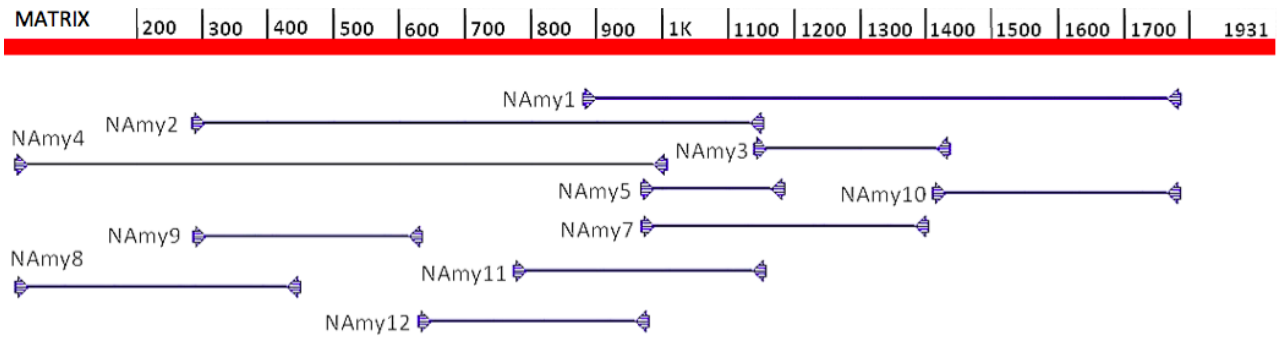

Fig. 1. Localization of primer sequences (lines flanked by arrows) on *T. aestivum* beta-amylase gene sequence JP213065.1 (upper line).

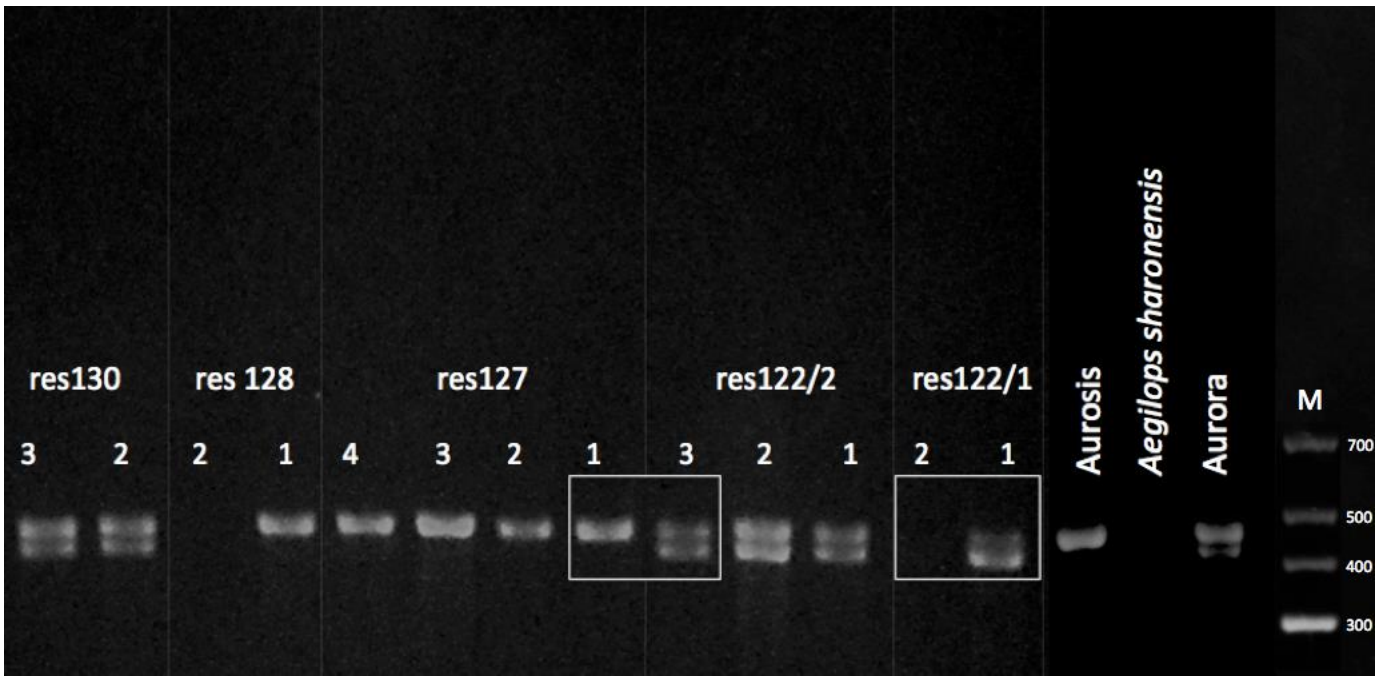

Fig. 2. The absence of one or both NAmy5 parental components. The upper component, mentioned as 1, is heavy; lower component, mentioned as 2, is light. Variability types 1,2/1 and 1,2/0 are marked by rectangles. Different individuals from the res introgressive lines are specified by the numbers (1-4).

M – molecular marker GeneRuler Low Range DNA Ladder (ThermoScientific, Lithuania).

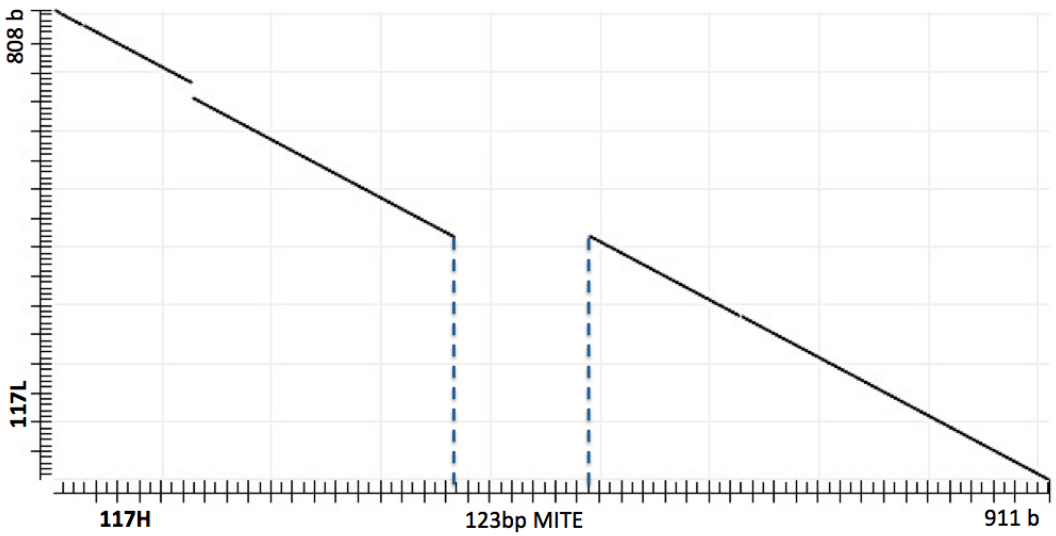

Fig. 3. Dot-plot of NAmy3 heavy (117H (KU987830), abscissa) and light (117L (KU987831), ordinate) res117 amplicons sequences. The MITE sequence in the 117H is highlighted by the dotted line.

```
AuH   8    GGCAACTACCTCACCGAGAGGGGAAGTTTTTCCTGTCATGGTACTCCAACAAACTGATCA  67
           ||| |||||||||| ||||||||| ||||||||||||||||||||||||||||||||||||
AuL   9    GGC-ACTACCTCACAGAGAGGGG-AGTTTTTCCTGTCATGGTACTCCAACAAACTGATCA  66

AuH   68   AGCACGGTGACAAGGTCTTGGACGAAGCAAACAAGGTCTTCTTGGGATGCAGGGTGCAGC  127
           ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
AuL   67   AGCACGGTGACAAGATCTTGGACGAAGCAAACAAGGTCTTCTTGGGATGCAGGGTGCAGC  126

AuH   128  TGGCAATCAAAGTAAGTACCATCCTACAGATCTCAGTATATTATAGTAGACAAGATTGCA  187
           |||||||||||||||||||||||||| |||||||||||                    |
AuL   127  TGGCAATCAAAGTAAGTACCATCCTATAGATCTCAGT--------------------GA  165

AuH   188  AAAATCATGATTCAGTTGAAAAGTTGTGCCCCAGATAAACAACAAATAAATGCACAACCC  247
           ||||||||||||||| |||| |||||||||||   |||||||||||||| ||||| |||| |
AuL   166  AAAATCATGATTCATTTGAGAAGTTGTGCCT--GATAAACAACAAA-AAATGTACAAACT  222

AuH   248  CACCATAATGTAGGTGTATAAGCTTACGGAATGAGGAAGTATATACAACTCACATTGCTT  307
           |||  |||  |||  ||| ||||||||  |||  |||||||||||||| ||||||||||||
AuL   223  CACTCTAACATAGA-GTACAAGCTTAAAGAAACAGGAAGTATATACGACTCACATTGCTT  281

AuH   308  AATCTAGAATGTAATGAGACATGATCCAATGTTTGGTTGCAGATCTCTGGCATTCACTGG  367
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AuL   282  AATCTAGAATGTAATGAGACATGATCCAATGTTTGGTTGCAGATCTCTGGCATTCACTGG  341

AuH   368  TGGTACAGA  376
           |||||||||
AuL   342  TGGTACAGA  350
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Fig. 4. Aligned sequences of light (AuL, KU987825) and heavy (AuH, KU987826) NAmy5 amplicons of *T. aestivum*. Larger rectangle highlights sequence that is absent in AuL, but present in AuH. Smaller rectangle highlights MITE footprint. In this locus MITE is localized in *Ae. comosa*, but not in *T. aestivum* beta-amylase gene sequence.

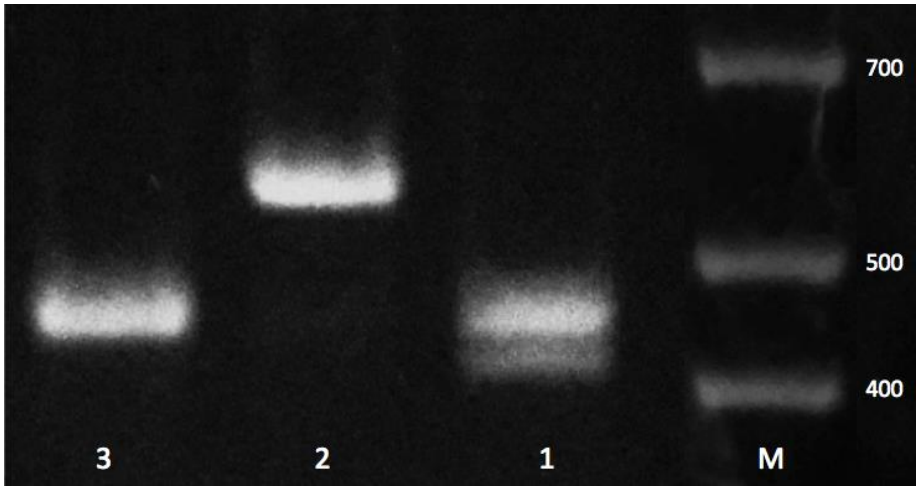

Fig. 5. NAmy5 components of common wheat variety Aurora (1), diploid species *Ae. comosa* (2), and amphidiploid Miosa (3). *Ae. comosa* amplification component is heavier than components of other studied plants.

M – molecular marker GeneRuler Low Range DNA Ladder (ThermoScientific, Lithuania).

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AuH          83   TCTTGGACGAAGCAAACAAGGTCTTCTTGGGATGCAGGGTGCAGCTGGCAATCAAAGTAA  142
                  ||||||||||| ||||| |||||||||||||||||||| || ||||||||||||||||||||
Ae. comosa   1    TCTTGGACGAGGCAAATAAGGTCTTCTTGGGATGCAGAGTACAGCTGGCAATCAAAGTAA  60

AuH          143  GTACCATCCTACAGATCTCAGTATATTATA------------------------------  202
                  |||||||||||  ||||||||||  ||||||
Ae. comosa   61   GTACCATCCTATAGATCTCAGTACATTATACTCCCTCCGATCCAAAATAAGCGTCGTGGT  120

AuH          203  ------------------------------------------------------TAGTAGA  262
                                                                        |||||||
Ae. comosa   121  TTTACTTCAAAACTCTCACTCAAACCACGATGCTTATTTTGGATCGGAGGGAGTAGTAGA  180

AuH          263  CAAGATTGCAAAAATCATGATTCAGTTGAAAAGTTGTGCCCCAGATAAACAACAAATAAA  322
                  |||||||  ||||||||||||||| |||| ||||||||||   |||||||||||| |||
Ae. comosa   181  CAAGATTAGAAAAATCATGATTCAATTGAGAAGTTGTGCCT--GATAAACAACAAA-AAA  240

AuH          323  TGCACAACCCCACCATAATGTAGGTGTATAAGCTTACGGAATGAGGAAGTATATACAACT  382
                  ||||||| | |||  |||  |||   || ||||||| |||| |||||||||||||| |||
Ae. comosa   241  TGCACAAACTCACTCTAACATAG---TACAAGCTTAAGGAAAGAGGAAGTATATACGACT  300

AuH          383  CACATTGCTTAATCTAGAATGT-AATGAGACATGA                          383
                  |||||||||||||||||| || ||||||||||||
Ae. comosa   301  CACATTGCTTAATCTAGAACGTAAATGAGACATGA                          336
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Fig. 6. Aligned sequences of NAmy5 *T. aestivum* heavy (AuH, KU987826) and *Ae. comosa* (KU987827) amplicons. *Stowaway*-MITE sequence, which is present in *Ae. comosa* but not in in Aurora is highlighted.

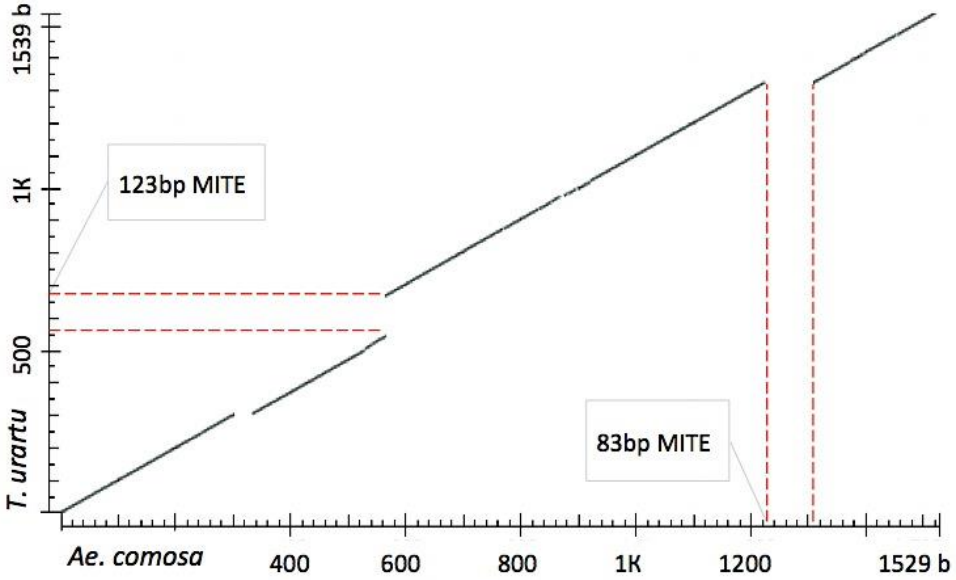

Fig. 7. Dot-plot of *Ae. comosa* (AY821690.1, abscissa) and *T. urartu* (GQ847677.1, ordinate) partial beta-amylase gene sequences. Gaps in the line correspond to deletions in the sequences. Deletions that are marked with dotted lines are MITEs.

Beta-amylase gene variability in introgressive wheat lines

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Abstract: Variability of the beta-amylase gene in bread wheat, artificial amphidiploids, and derived introgression wheat lines was analyzed. Variation in homeologous beta-amylase sequences caused by the presence of MITE (Miniature Inverted-Repeat Transposable Element) and its footprint has been identified in bread wheat. The previously unknown location of MITE in *Triticum urartu* and *T. aestivum* L. beta-amylase gene has been found. These species have a MITE sequence in the third intron of beta-amylase, as opposed to *Aegilops comosa* and a number of other Triticeae species, which have it in the fourth intron. These two MITEs from *Ae. comosa* and *T. aestivum* were shown to have low identity scores. Miosa, an artificial amphidiploid, which has the M genome from *Ae. comosa* was shown to lose the MITE sequences. This loss might be caused by genomic shock due to allopolyploidization.

Key words: bread wheat, artificial amphidiploids, transposons, MITE, genomic shock.

Acknowledgements
We are pleased to thank George Fedak (Ottawa, Canada) for helpful remarks and careful review.

Introduction

Wheat is an important model organism for fundamental research of the allopolyploidy phenomenon. *Triticum aestivum* L. species is allohexaploid, it has three subgenomes – A, B, and D, the latter has been incorporated recently (8000 y. a.). Subgenome D can be replaced with the genomes of other related species, such as species of the *Aegilops* genus (Zhirov and Ternovskaya 1984), *Secale* (Ma and Gustafson 2008), and *Thinopyrum* (Han et al. 2004) in order to create amphidiploids. As of today, most angiosperms are known to be polyploid or paleopolyploid (Masterson 1994; Soltis et al. 2009). Therefore, artificial wheat amphidiploids may be used as a model plants to study the evolution of plant genomes. In addition, they can be predecessors in the development of introgressive lines. These lines have genes, transferred from the wild relatives, that provide resistance to diseases (such as powdery mildew and leaf rust) and abiotic stresses (frost and drought) (Feldman and Sears 1981; Olivera and Steffenson 2009).

Changes, caused by a set of events, generally referred to as genomic shock, occur in the genomes of introgressive wheat lines. These changes include elimination of low-copy, repetitive, and non-coding sequences, structural rearrangements, activation of transposable elements, epigenetic modifications, and changes in gene expression (Han et al. 2003; Liu et al. 2009; Feldman and Levy 2009). It is essential to determine the basis of the rearrangements caused by genomic shock and to define their hotspots in the genome. Because if introgression point is the hotspot, there is a high probability of inactivation and loss of alien genetic material, and consequently, the trait of interest. The accumulation of knowledge in this field will help to rationalize the process of gene transfer in the future.

In *Aegilops sharonensis*, the beta-amylase gene is located on $4S^{sh}$ gametocidal chromosome. Gametocidal genes (*Gc*), one of which is located on this chromosome, induce DNA breaks in gametes, which do not have this gene. Thereby, *Gc* genes promote genome rearrangements (Nasuda et al. 1998). The gametocidal chromosome is important tool in chromosome engineering. It is also a subject of research on the genome formation through chromosomal rearrangements promoted by its influence (Endo 2007).

MITEs are non-autonomous small DNA transposons which are the most abundant type of mobile elements in plants. They are highly associated with protein-encoding genes and may regulate their expression through the presence of cis-regulatory elements in their sequences and by generation of MITE-derived small RNAs (Bureau and Wessler 1994; Kuang et al. 2009; Guermonprez et al. 2012). Mobile elements of this type are active, they can transpose throughout the genome resulting in formation of insertional polymorphisms and creation of allelic diversity (Chen et al. 2012). Beta-amylase genes of grasses contain *Stowaway*-like MITE, which can contribute to this gene variability (Mason-Gamer 2007). As the changes in isozyme spectrum of beta-amylase were previously shown in the introgressive lines of *T. aestivum/Ae. sharonensis* (Navalikhina et al. 2014), the goal of this study was to examine the beta-amylase gene sequence variability and to determine its mechanism.

Materials and methods

Plant materials

For this study we used winter bread wheat *T. aestivum* L. (2n=6x=42, AABBDD) variety Aurora, diploid *Ae. sharonensis* Eig (2n=14, $S^{sh}S^{sh}$), amphidiploid Aurosis (2n=6x=42, $AABBS^{sh}S^{sh}$) which has the tetraploid component of Aurora (AABB) and the $S^{sh}S^{sh}$ genome of *Ae. sharonensis* (Zhirov and Ternovskaya 1984). The introgressive wheat lines that were obtained by crossing Aurora and Aurosis and employed in this study, were res115, res117, res118, res121, res122/1, res122/2, res127, res128, res130, res132, res139, res141, res148. Diploid *Ae. comosa* Sm. (2n=14, MM), and amphidiploid Miosa (2n=6x=42, AABBMM) which has A and B genomes of durum winter wheat line Mutico italicum 59h132 (*T. durum* Desf.) and M genome of *Ae. comosa* (Antonyuk et al. 2013), were also employed in this study.

DNA extraction

Genomic DNA was extracted from coleoptile tissue using the CTAB method (Sambrook et al. 1989).

Primer design

Specific primers were designed using the service PrimerBLAST based on Transcriptome Shotgun Assembly (TSA) mRNA sequence of *T. aestivum* beta-amylase (GenBank accession number JP213065.1) (Fig. 1). Sequences of primers may be provided on request. Out of twelve designed primer pairs it was not possible to get the result with two (NAmy10 and NAmy11). Amplification using NAmy4 primer pair gave non-specific components, so it was not taken for further analysis.

PCR conditions and gel electrophoresis

Beta-amylase gene sequence variation was studied using gene amplification by PCR. The reaction was performed by the tdPCR (touch-down PCR) method (Korbie and Mattick 2008). Amplification products were separated on 1% agarose gel on sodium borate (SB) buffer. Visualization of products was performed using ethidium bromide (Sambrook et al. 1989).

DNA extraction from gel

Fragments of the gel that contained amplification components studied were excised by scalpel from the gel. DNA was extracted from the gel using a GeneJET Gel Extaction Kit (Fermentas, Lithuania).

Sequence determination and data analysis

The nucleotide sequences were determined using Sanger sequencing method on automated capillary sequencer (Applied Biosystems 3130, USA). Sequences were compared with each other and aligned to the existing sequences from databases using Nucleotide BLAST service (Fig. 1).

Results and discussion

Spectra characteristics

The following patterns of variability were identified from an analysis of Aurora, *Ae. sharonensis*, Aurosis, and derived introgression lines electrophoregrams.

Aurora and Aurosis components were always present in all spectra. They can be single bands (NAmy1, NAmy2, NAmy8, NAmy9, NAmy12), or double (NAmy3, NAmy5, NAmy7). Spectrum of *Ae. sharonensis* always has single component which have the same molecular weight as amplicon of Aurora (Fig. 2). If Aurora has two components in the spectrum, the component of *Ae. sharonensis* usually coincides by weight with the heavy one, except for the NAmy3 amplicon that coincides with the light.

Ae. sharonensis amplicons are always present in spectra, with the exception of the NAmy12. Lack of NAmy12 amplicon can be explained by the differences in the nucleotide sequences of the beta-amylase gene in *Ae. sharonensis* and *T. aestivum*, which correspond to the left and right NAmy12 primers. If the absence had been caused by large-scale changes (insertions or deletions), we would have observed the changes in amplification products of other primers that amplify this part of the gene (NAmy2, NAmy11).

Aurosis always have two components – one from Aurora and another from *Ae. sharonensis*. The exception is the NAmy5 amplicon, where only one component inherent to *Ae. sharonensis* is present, while the component of Aurora is not (Fig. 2).

Introgressive lines that, according to earlier results (Antonyuk et al. 2009), have $4S^{sh}$ chromosome (res117, res118, res127, res141) or its arm, carrying the *β-Amy-*S^{sh}*1* gene (res115), usually have one heavy amplicon in the NAmy5 spectrum inherent to Aurosis and *Ae. sharonensis*. Lines without $4S^{sh}$/4D substitution usually have two NAmy5 amplicons. Presumably, the second light component is formed due to *β-Amy-D1* gene amplification. Therefore, this component is absent in the Aurosis spectrum, because this amphidiploid has no bread wheat D genome.

In Aurora and Aurosis NAmy3, NAmy5, and NAmy7 spectra two amplification products are present. Both of them have the same intensity of ethidium bromide fluorescence. These parental forms components are often kept in derived lines, though sometimes one of them may be lost. Since all plants in this study are expected to be homozygous at all loci, finding the several amplicons can only be explained by the presence of several genes. Therefore, appearance of two amplicons is caused by the presence of three orthologous beta-amylase genes with different sequences in hexaploid genome.

Two types of variability for the studied markers were observed in introgressive lines.

1) Lack of parental component (1/0 variability type) was found in the products of amplification by NAmy1, NAmy2, NAmy8, NAmy9, and NAmy12.

2) The absence of one or both parental components (1,2/1 and 1,2/0 variability types) was observed in the products of amplification by NAmy3, NAmy5, and NAmy7 primers. NAmy3 had one 1,2/0 variability type, as opposed to NAmy5 and NAmy7 that had two variability types – lack of second (light) component and the absence of either components (Fig. 2). Because the region flanked by NAmy7 includes the region flanked by NAmy5, and the difference in light and heavy components molecular weight was found to be similar, it was assumed that the presence of two amplicons in both cases had the same basis. Therefore, for the further analysis we took only NAmy5 amplicons.

Variability caused by the presence of MITE in the beta-amylase gene

In order to establish the molecular mechanism of amplicons variation, we sequenced NAmy3 amplification products of Aurosis (heavy and light components AsisH (KU987832) and AsisL (KU987833)) and introgressive line res117 (res117H (KU987830) and res117L (KU987831)). It was found that heavy components (AsisH and 117H) have 123 nt sequence which is absent in the light (AsisL and 117L) components (Fig. 3). This sequence has 100% identity score to mobile element *Stowaway*-MITE in *Triticum urartu* Thumanjan ex Gandilyan beta-amylase gene sequence (GQ847677.1).

It is known that in plant genomes MITEs are associated with protein-encoding genes. They can be located either near or inside the genes and function as cis- or trans- regulatory elements (Guermonprez et al. 2012). Polymorphism caused by the presence of *Stowaway*-MITE in the fourth intron of beta-amylase genes in Triticeae was previously shown by Mason-Gamer (Mason-Gamer 2007). These genes may contain full or truncated sequence of MITE, excision footprint or empty site. Heavy components of Aurosis and res117 contain full-length MITE sequence which is inherent to *T. urartu*, the donor of wheat A genome, while light components contain empty site.

Variability caused by the presence of MITE footprint in the beta-amylase gene

In order to establish the basis of NAmy5 amplicon variability, amplification products sequences of Aurora (KU987825, KU987826), *Ae. sharonensis* (KU987829), and Aurosis (KU987824) parental forms and plants from two derived introgressive lines res115 (KU987822) and res117 (KU987823) were analyzed. Only Aurora has two components in the spectrum, other plants have a single amplicon. The difference in molecular weights of two components in Aurora is caused by the presence of 21 nt sequence in heavy component that is absent in the light one. This sequence was found to have typical MITE transposon footprint – TAGTA sequence. Therefore, heavy Aurora amplicon contain MITE excision footprint and light amplicon has empty site (Fig. 4) (Mason-Gamer 2005, 2007). The presence of such footprints in beta-amylase genes of grasses was indicated by Mason-Gamer (Mason-Gamer 2005, 2007). Sequenses of *Ae. sharonensis*, Aurosis, and two representatives of the lines also have footprints of *Stowaway*-MITE. They are homologous to Aurora heavy component and sequence identity in reference to AuH is 94% for res115, res117, Aurosis, and is 91% for *Ae. sharonensis*.

To determine the genomic origin of Aurora sequences with transposon footprint and with empty site (AuH and AuL), they were aligned to the beta-amylase gene sequences of *T. urartu* (GQ847677.1, A genome donor), *Ae. tauschii* (AY821695.1, D genome donor), and *Ae. speltoides* (HE565971.1), the genetic material of which is believed to participate in the creation of the B genome. It was found, that beta-amylase sequences of *T. urartu* and *Ae. speltoides* are homologous to AuH amplicon sequence, while AuL is 99% identical to beta-amylase gene fragment of *Ae. tauschii*. We suggest that beta-amylase gene sequence in the D genome (chromosomal location 4DL) corresponds to AuL fragment and contains 21 nucleotide deletion compared to sequences in 5AL and 4AL.

Variability caused by the presence of MITE and MITE footprint in the beta-amylase gene in *Ae. comosa* and Miosa

The results reveal that NAmy5 amplification component of wild wheat *Ae. comosa* (MM) differs from that of Aurora in molecular weight (Fig. 5). It should be noted that amphidiploid Miosa (AABBMM) had only one component that coincides by molecular weight with that of Aurora. Theoretically Miosa should have had two spectrum components – heavy from durum wheat, which corresponds to beta-amylase gene on chromosome 4A, and heavier one from *Ae. comosa* that is inherent to the M genome. However, Miosa had a single amplification component that was 95% homologous to the Aurora heavy component.

Sequences of *Ae. comosa* (KU987827) and Miosa (KU987828) NAmy5 amplicons were compared with the AuH (KU987826) Aurora amplicon. It was found that *Ae. comosa* amplicon sequence differs from AuH by 83 nt sequence (Fig. 6). This sequence is *Stowaway*-MITE, as demonstrated by alignment with GenBank *Ae. comosa* (AY821690.1) sequence in which this transposon is annotated. The degree of homology between the sequence of MITE element in the GenBank sequence and obtained sequence is 100%. Since Miosa is an artificial amphidiploid, it is likely that the loss of the MITE transposon sequence is associated with the elimination of high-copy sequences or with the activation and movement of the element, because these phenomena are often associated with genomic shock caused by allopolyploidization (Jackson and Chen 2010; Feldman and Levy 2009; Ma and Gustafson 2008; Han et al. 2005).

Location and characterization of MITEs in beta-amylase genes of Triticeae

It was found, that in wheat and *Ae. sharonensis* beta-amylase gene sequences, MITE elements are not present in the locus, amplified by NAmy5, in which MITE is located in *Ae. comosa*. Therefore, we aligned *T. urartu* (GQ847677.1) and *Ae. comosa* (AY821690.1) beta-amylase sequences to demonstrate different location of MITEs in the beta-amylase genes of these species. We have shown that the elements are located in different loci. In *T. urartu* beta-

amylase sequence MITE is located in the locus (545-668 nt) that corresponds to locus 564 nt in *Ae. comosa* with empty site. By contrast, *Ae. comosa* MITE is located in locus (1224-1308 nt), that corresponds to 1325 nt in *T. urartu* sequence (Fig. 7). Analysis of exon-intron structure of the two beta-amylase gene sequences reveals that *T. urartu* and *Ae. comosa* MITEs are located in two different introns of the gene. Sequence from 1190 to 1482 nt, which contains 83 bp MITE, in *Ae. comosa* corresponds to fourth intron of the gene. On the contrary, *T. urartu* locus 564 nt, where its MITE element is found, corresponds to the third intron (from 515 to 927 nt) of beta-amylase gene. Considering that *T. urartu* is believed to be wheat A genome donor, we can expect same MITE location in the bread wheat A genome.

Our findings contradict the study by Mason-Gamer, who pointed out that in *Triticinae* MITEs are located in the fourth intron of beta-amylase gene. The author have shown that during the evolution of *Triticinae* MITEs underwent multiple deletions and insertions in the fourth intron of the beta-amylase gene (Mason-Gamer 2007). We demonstrated, however, that insertions have occurred at different loci – in addition to the fourth intron element could have been inserted in the third.

Comparison of *T. urartu* and *Ae. comosa* MITE sequences reveal that they have a low percentage of homology. Thus, they differ in length – 83 bp in *Ae. comosa* and 123 bp in *T. urartu*. Transposable element of *Ae. comosa* has two deletions (21 and 19 nucleotides) compared with *T. urartu* MITE. The degree of homology between the two sequences is 79%, mainly due to conserved TIRs and adjacent sequences.

Two MITEs in *T. urartu* and *Ae. comosa* beta-amylase genes are different elements. They vary by location, length, and have low homology degree. We can assume, that these transposons have different origin, and their insertions occurred independently during evolution of grasses. Another version is that MITEs of *T. urartu* and *Ae. comosa* have one common predecessor, which transposed within beta-amylase gene and undergone truncation.

Since MITEs are relatively short transposable elements, they tend to form stable single-strand secondary structures in genomes (Guermonprez et al. 2012). To calculate probabilities and to visualize the formation of hairpins by MITE sequences of *Ae. comosa* and *T. urartu*, we created a model using Predict a Secondary Structure Web Server. Gibbs free energy for the formation of these structures at 27 °C is negative and constitutes -88.1 and -41.7 kJ/mol for *T. urartu* and *Ae. comosa* MITEs respectively. Thus, hairpin structures formed by these transposons are quite stable.

In order to find sequences homologous to identified MITEs in the genomes of other plants, we performed the search using the NCBI BLAST tool. We found sequences 1100225-1100368 nt, 1469199-1469325 nt and 1147197-1147312 nt on *T. aestivum* 3B chromosome (FN564429.1), which had a high degree of homology (84-96%) with MITE of *T. urartu*. These sequences were annotated as *Pan Stowaway*-like MITE transposable elements. A similar search for *Ae. comosa* MITE sequence returned 88% sequence identity with *Stowaway* transposable element in *Peridictyon sanctum* (*Festucopsis sancta* (Janka) Melderis) beta-amylase gene (AY821714). According to 80-80-80 rule (Wicker et al. 2007), we classified both *T. urartu* and *Ae. comosa* MITEs as *Stowaway*-like elements. Transposable element of *T. urartu*, was defined to belong to *Pan* family.

Compliance with Ethical Standards

Maksym Antonyuk declares that he has no conflict of interest.

Anastasiia Navalikhina declares that she has no conflict of interest.

Tamara Ternovska declares that she has no conflict of interest.

This article does not contain any studies with human participants or animals performed by any of the authors.

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Dear Editor

We are very grateful to for helpful comments of the reviewers. We have corrected everything that was suggested.

- the paper was improved with details added to introduction and discussion
- suggested citations added
- specific comments from reviewer #1 in attached file corrected
- word ‘localised’ was changed to ‘located’
- the paper rechecked for bugs

Unfortunately, we couldn’t improve our work with the help of P-MITE database advised, because it has no bread wheat sequences, as opposed to Nucleotide NCBI.

Thank you for your time and concideration.