Non-brittle rachis 1-A (*Btr1-A*) gene in di- and hexaploid wheat species

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Abstract: Spike brittleness is one of the key domestication traits in *Triticum* species. In the recent studies it was found that the *Non-brittle rachis 1-A* (*Btr1-A*) gene involved in the regulation of the brittle/non-brittle spike trait. Here we investigated the genetic variability of the *Btr1-A* gene from 13 accessions of *Triticum monococcum* L., *T. urartu* Thum. ex Gandil., *T. boeoticum* Boiss., *T. macha* Decapr. et Menabde, *T. aestivum* ssp. *petropavlovskyi* (Udacz. et Migusch.) N.P. Gontsch., *T. spelta* L., *T. spelta* ssp. *yunnanense* (King ex S.L. Chen) N.P. Gontsch., *T. vavilovii* (Thum.) Jakibz. and *T. tibetanum* Shao. The *Btr1-A* sequences for *T. aestivum* ssp. *petropavlovskyi* and *T. vavilovii* were obtained for the first time. Hexaploid wheat accessions analyzed were characterized by a 2-bp deletion in the *Btr1-A* coding region (positions 291-299). The presence of this deletion leads to the formation of a nonfunctional protein (97 instead of 196 amino acids). Additional investigations are required to establish the potential relationship between *Btr1-A* and other genes that regulate the brittle/non-brittle spike trait in *Triticum* species.

Key words: wheat; *Triticum*; spike morphology; brittle rachis; *Btr1-A* gene.

1. Introduction

Currently, wheat (*Triticum* L.) is an important cereal in the agriculture, one of the first that was domesticated (Purugganan, Fuller, 2009). Along with spike shape, threshability and spring growth habit, spike brittleness is one of the key domestication traits (Goncharov, 2012). It has been shown that the mutation of the *Non-brittle rachis 1-A* (*Btr1-A*) gene controls the brittle/non-brittle spike trait in *Triticum* species (Zhao et al., 2019). Pourkheirandish et al. (2018) found that the non-synonymous change at the coding region of *Btr1-A* (G to A, A119T) leads to non-brittle rachis formation in the diploid einkorn wheat *Triticum monococcum*, compared to its wild progenitor *Triticum boeoticum* with a brittle rachis spike. In cultivated polyploid wheat species, the *Btr1-A* gene contains a 2-bp deletion in the coding region, forming a premature stop codon and resulting in a non-functional protein (Zhao et al., 2019). Moreover, the transgenic wheat lines created by Zhao et al. (2019) made it possible to establish that the *Btr1-A* gene was associated with spike density, grain size and grain yield. In the present study we investigated the genetic variability of the *Btr1-A* gene from di- and hexaploid wheat species including four endemics.

2. Materials and methods

The germplasm of di- and hexaploid wheat species was grown under standard greenhouse conditions. The brittle/non-brittle spike trait was determined visually. The list of wheat accessions analyzed in this study is presented in Table 1. Total DNA was isolated from 100 mg of leaves using a DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's protocol. The *Btr1-A* sequences of *T. monococcum* and *T. boeoticum* (MG596311-MG596321) and whole genome sequences (WGS) of *Triticum dicoccoides* (LSYQ02000006), *Triticum aestivum* (OETA01178479) (B-genome), *T. aestivum* (OETA01219489) and *Aegilops tauschii* (NWVB01000003) (D-genome) were used to design genome-specific primers to amplify the *Btr1-A* gene from di- and hexaploid wheat species. The primer pair Btr-A1-F/ Btr-A1-R 5′-CGAGCTTGACCT CATGTAAC-3′/ 5′-CTACTGCATCATCAGTCCATC-3′ amplifies partial upstream and downstream parts and the coding region of *Btr1-A*. PCR was performed in a 20-μl volume containing 20 ng of genomic DNA, 10 mM Tris-HCl (pH 8.9), 1 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.5 μM primers, and 0.25 U of Taq DNA polymerase. PCR products were separated by agarose gel electrophoresis and purified using a QIAquick Gel Extraction Kit (QIAGEN). For all wheat accessions purified PCR products were sequenced. Sequencing reactions were performed with 20 ng of the PCR product and an ABI BigDye Terminator Kit on an ABI 3130XL Genetic Analyser (Applied Biosystems) in the SB RAS Genomics Core Facility (http://www.niboch.nsc.ru/doku.php/corefacility). Nucleotide and amino acid sequences were aligned using AliView v. 1.18.1.

3. Results and discussion

We obtained the *Btr1-A* gene sequences for three diploid and five hexaploid wheat species (Table 1). For diploid wheat accessions, the length of the *Btr1-A* sequences obtained was 902 bp. In the case of all hexaploid wheat accessions investigated, the length of the gene sequences was 899 bp (a 1-bp deletion in the upstream and a 2-bp deletion in the coding region of the *Btr1-A* gene compared with diploids).

Comparative analyses allowed us to determine that the *Btr1-A* gene sequences of *T. monococcum* (PI-266844) and *T. boeoticum* (K-18399) were identical with *T. monococcum* (MG596319, MG596320) and *T. boeoticum* (MG596311-MG596313, MG596315, MG596317-MG596318), respectively. The *T. urartu* (Ig-110784) sequence contained an additional substitution at position 125 (T to A, L42Q) compared to the data presented by Zhao et al. (2019) (Figure 1). The 2-bp deletion in the *Btr1-A* coding region of *T. spelta* and *T. spelta* ssp. *yunnanense* (non-brittle rachis), *T. tibetanum* and

Table 1
Wheat species used in the study and their phenotypes

Species	Accession	Phenotype
Triticum monococcum L.	PI-266844	Non-brittle rachis
T. urartu Thum. ex Gandil.	Ig-110784	Brittle rachis
T. boeoticum Boiss.	K-18399	Brittle rachis
T. macha Decapr. et Menabde	K-31689	Brittle rachis*
T. macha Decapr. et Menabde	K-58671	Brittle rachis*
T. aestivum ssp. *petropavlovskyi* (Udacz. et Migusch.) N.P. Gontsch.	KU502	Non-brittle rachis
T. aestivum ssp. *petropavlovskyi* (Udacz. et Migusch.) N.P. Gontsch.	K-43351	Non-brittle rachis
T. spelta L.	K-53364	Non-brittle rachis
T. spelta ssp. *yunnanense* (King ex S.L. Chen) N.P. Gontsch.	KU506	Non-brittle rachis
T. spelta ssp. *yunnanense* (King ex S.L. Chen) N.P. Gontsch.	KU509	Non-brittle rachis
T. vavilovii (Thum.) Jakibz.	Tri9416	Non-brittle rachis
T. tibetanum Shao	KU510	Brittle rachis*
T. tibetanum Shao	KU515	Brittle rachis*

* the spike is separated from the whole straw, rarely without breaking into spikelets (Dorofeev et al., 1979)

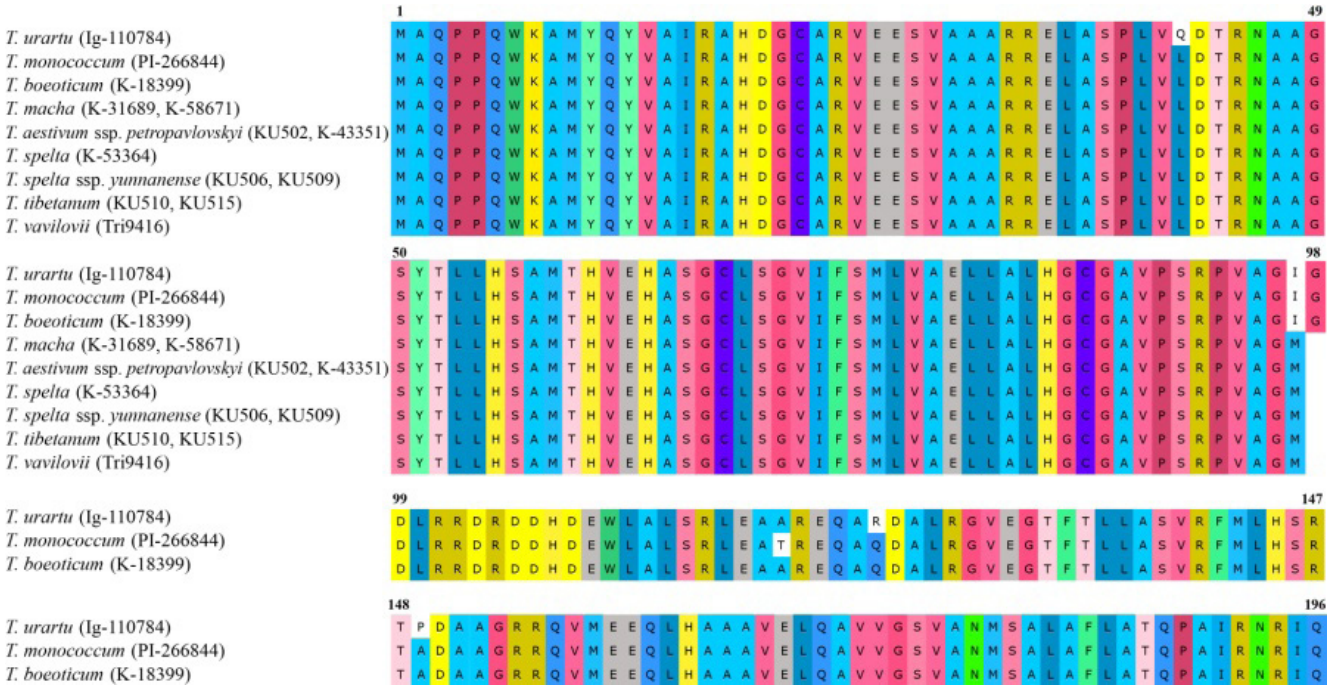

Figure 1. Alignment of the BTR1 protein of the di- and hexaploid wheat accessions analyzed in this study. The length of the BTR1 protein is 196 and 97 amino acids for the diploids and the hexaploids, respectively.

T. macha (brittle rachis) was previously detected by Zhao et al. (2019). The *T. aestivum* ssp. *petropavlovskyi* and *T. vavilovii* accessions with non-brittle rachis that were investigated in this study also contain that deletion. This deletion formed a premature stop codon and resulted in a nonfunctional protein (Figure 1). The 1-bp deletion at position -97 from the start codon for all hexaploid wheat accessions was detected for the first time in the present study.

4. Conclusions

In the present study we investigated the genetic variability of the *Btr1-A* gene from several *Triticum* species including four endemics. All hexaploid wheat accessions were characterized by a 2-bp deletion in the *Btr1-A* coding region, leading to the formation of a nonfunctional protein (97 amino acids instead of 196 in diploids). The *Btr1-A* sequences for *T. aestivum* ssp. *petropavlovskyi* and *T. vavilovii* were established for the first time in this study. Nevertheless, further investigations are required to understand the potential relationship between the *Btr1* and *Q* genes, regulating spike morphology traits such as spike density, brittleness and grain weight.

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Conflict of interest. The authors declare no conflict of interest.