Copy number variation of the *Vrn-A1b* allele in emmer and spelt wheat

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DOI 10.18699/ICG-PlantGen2019-39

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Abstract: In the present study the copy number variation of *VRN1* was analyzed in accessions of tetraploid wheat *T. dicoccum* and hexaploid wheat *T. spelta*. For this purpose, a PCR approach based on end-point quantification was developed and the results obtained were confirmed in a qPCR assay. The duplication of the *vrn-Ab.3* and *Vrn-A1b.2* alleles in *T. dicoccum* and *T. spelta* has been shown for the first time. Furthermore, in accessions of *T. spelta* the duplication of *Vrn-A1b.2* was strongly associated with the awnless spikes and *Vrn-B1c* genotype, indicating a likely common origin of these accessions. Variation of the *Vrn-A1b.2* haploid copy number can provide additional advantages in manipulation of flowering time of wheat.

Key words: wheat; emmer; spelt; flowering time; copy number variation; genetic diversity.

1. Introduction

The *VRN1* genes are major determinants of such agronomically valuable traits of wheat as growth habit and flowering time. The dominant alleles of these genes contain mutations within the promoter region and in the first intron, determining spring growth habit and early flowering of wheat.

The hexaploid wheat of *T. spelta* belongs to the *Triticum dicoccum* lineage and was derived from hybridization events between the domesticated hulled tetraploid wheat *T. dicoccum* and free-threshing hexaploid wheat *T. aestivum*. The dominant *Vrn-A1b.2* allele is one of major determinants of the spring growth habit in *T. dicoccum* and *T. spelta* (Muterko et al., 2016).

Previously the copy number variation (CNV) of the recessive *vrn-A1* allele was shown for hexaploid wheat (Diaz et al., 2012, Würschum et al., 2015, Muterko and Salina 2017, Muterko and Salina 2018). However, this polymorphism was not analyzed in tetraploid wheat and hexaploid wheat with the dominant *Vrn-A1* alleles. In the present study, the CNV of *VRN1* genes was investigated in accessions of tetraploid wheat *T. dicoccum* and spring accessions of hexaploid wheat *T. spelta*.

2. Materials and methods

Total nucleic acids were extracted from seedlings and leaves using CTAB lysis buffer, chloroform extraction and isopropanol precipitation.

PCR was carried with the VRN1ex4F (agttgcagcaactggagcag) and VRN1ex6R (tattctcctcctgcagtgac) primer pair. Both primers are annealed on all three homeologous *VRN1* genes, producing fragments of similar lengths (470, 476 and 468 bp fragments for the *VRN-A1*, *VRN-B1* and *VRN-D1* genes). However, since the polymorphism of these fragments is localized within the A-tract rich region this accompanied the modulation of curvature of the DNA molecules. The DNA template was fragmented by temperature and PCR was carried out only for 24 cycles to quantify target amplicons at the exponential phase of amplification.

The horizontal electrophoresis was carried out on half of microscopic glass slides at 6–8 °C in ultrathin (0.5 mm) non-denaturing polyacrylamide gels in discontinuous buffer system Tris-HCl / Tris-Borate. Gels were stained in ethidium bromide and visualized under UV light. The ratios of peak intensities of the *VRN-A1* (A1), *VRN-B1* (B1) and *VRN-D1* (D1) fragments were used to quantify the relative copy number of the corresponding genes. In this approach the principles of isotachophoresis are used to preconcentration of DNA fragments and obtain very thin bands, producing the sharpened peaks of fluorescence intensity, which are suitable for proper quantification.

Quantitative PCR was performed using TaqMan probes for the *VRN-A1* and *CONSTANS* genes (Würschum et al., 2015). Sanger sequencing was carried out using a BigDye Terminator v3.1 sequencing kit with subsequent analysis on an ABI 3130xl Genetic Analyzer (SB RAS Genomics Core Facility).

3. Results and discussion

The *VRN1*-ratio test optimized as described in the Methods section was applied to the accessions of *T. dicoccum* and *T. spelta* carrying the dominant *Vrn-A1* allele and found variation in *VRN-A1* copy number, but not in *VRN-B1* or *VRN-D1*.

The A1/B1 ratio for accessions of *T. dicoccum* with the recessive (intact) *vrn-A1* as well as the dominant *Vrn-A1a*, *Vrn-A1b.2*, *Vrn-A1e* and *Vrn-A1k* alleles was 0.8, indicating a single haploiod copy of *VRN-A1*. However, in two accessions from Israel and Palestine, the intensity ratio of the *VRN-A1* PCR fragments to *VRN-B1* results in an average value of 1.6 (1.5–1.7), suggesting a duplication of *VRN-A1*. To confirm this assumption, the qPCR was carried out, where the *CONSTANS* genes were used, as the endogenous control, instead of *VRN-B1*. The results of both *VRN1*-ratio test and qPCR were consistent (Figure 1). Sequence analysis of the *VRN-A1* promoter region showed that both of these *T. dicoccum* accessions carry *vrn-A1b.3*.

The *VRN1*-ratio test divided accessions of *T. spelta* with the *Vrn-A1b* allele into two groups. It one of them, the average

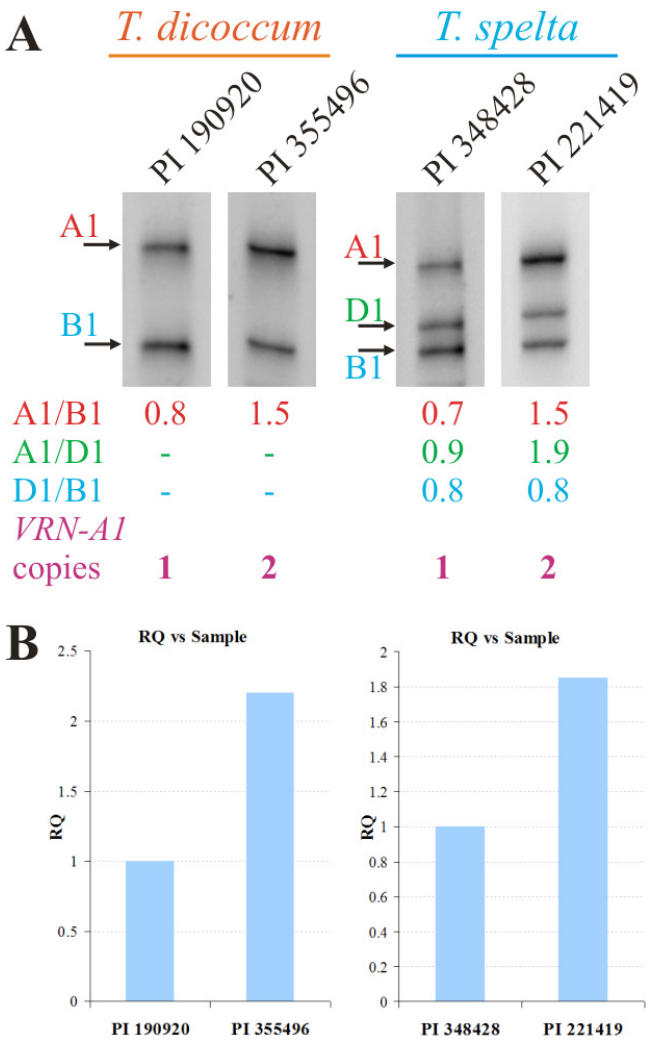

Figure 1. Quantification of the *VRN-A1* copies in accessions of *T dicoccum* and *T. spelta* during the (A) *VRN1*-ratio test and (B) qPCR assays.

A1/B1 and A1/D1 ratios were 0.7 and 1, respectively, while in the other, 1.4 and 2. The D1/B1 ratio for both groups was 0.8. The results obtained assuming single and two haploid copies of *VRN-A1* for these groups, respectively, and the copy number of *VRN-A1* was confirmed using a qPCR assay (see Figure 1).

Interestingly, that duplication of *Vrn-A1b.2* in accessions of *T. spelta* was strongly associated with the *Vrn-B1c* genotype and awnless spikes, while accessions containing a single haploid copy of this allele were awned and carried recessive *vrn-B1* with the *VRN-B1s* haplotype. These observations are likely related to the different origin of the *T. spelta* accessions analyzed. In fact, almost all accessions of *T. spelta* carrying a single haploid copy of *Vrn-A1b.2* originate from Italy (assumed Asturian spelt).

It is known that gene dosage of the dominant *VRN1* alleles positively correlates with early flowering of polyploid wheat. On the other hand, multiplication of the recessive *vrn-A1* allele is associated with later flowering. In any case, the effect of the *Vrn-A1b.2* duplication on phenotype provides additional advantages in the manipulation of flowering time of wheat.

4. Conclusions

Copy number variation of the *VRN1* genes was investigated in accessions of tetraploid wheat *T. dicoccum* and hexaploid wheat *T. spelta*. The duplication of *vrn-A1b.3* in *T. dicoccum* as well as *vrn-A1b.3* and the *Vrn-A1b.2* alleles in *T. spelta* was shown for the first time. In the European population of spring spelt wheat, the duplication of *Vrn-A1b.2* is associated with the awnless spikes and the *Vrn-B1c* genotype.

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Acknowledgements. This research was funded by the Russian Scientific Foundation grant No. 18-74-00080.

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