Identification and characterization of two novel *VRN-B3* alleles in Russian common wheat

Berezhnaya A.A. [1,2]*, Kiseleva A.A.[2], Salina E.A. [2]

[1] Novosibirsk State University, Novosibirsk, Russia;
[2] Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia
- e-mail: al.berezhnaya1@gmail.com

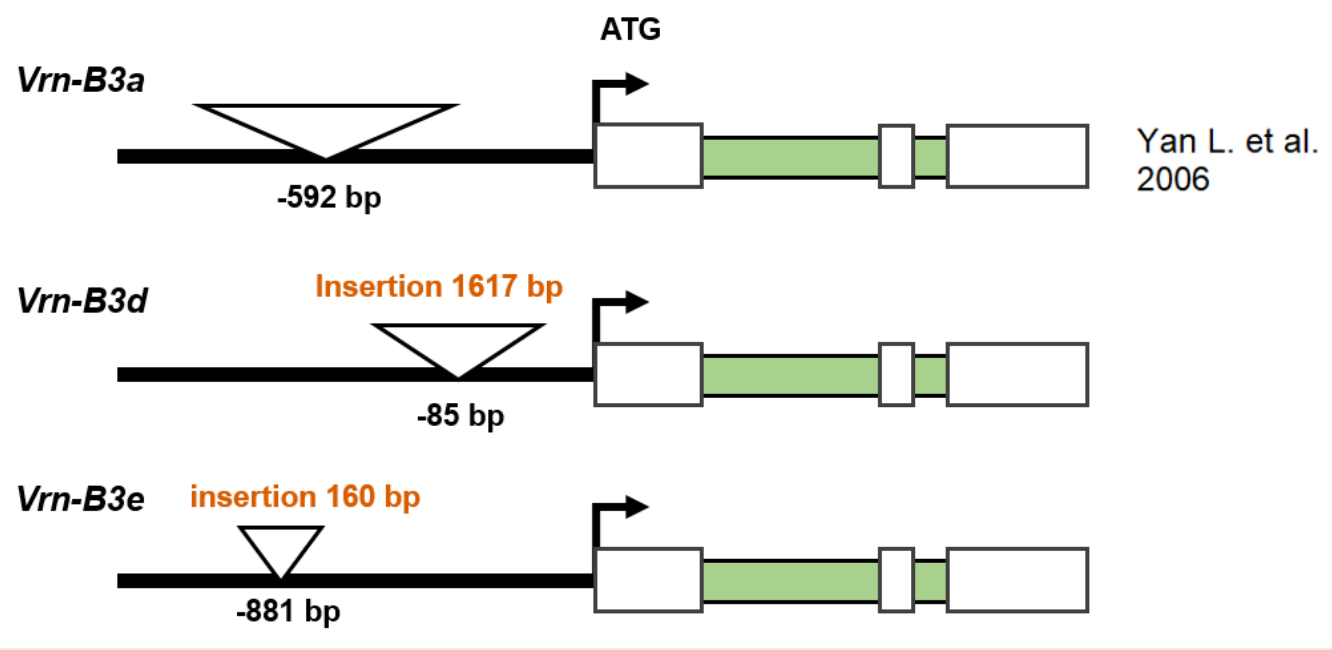
The yield potential of cultivated plants largely depends on heading dates. The *Vrn-B3* wheat gene is one of the key elements in the heading time pathway. Nevertheless, there are only a few studies attempted to identify allelic variations of this gene. Those alleles that were found in previous studies are extremely rare among cultivated varieties.
All the previously discovered alleles had certain insertions in their promoter region. These variations showed the ability to change the expression level of the gene affecting the heading time.

In this study, common wheat accessions from Russia adapted to the environments of the Siberia region Russia were tested for the allelic diversity of the *VRN-B3* gene. The results can be used to research genes regulating heading and for obtaining new wheat varieties with certain heading and flowering times.

Two novel *VRN-B3* alleles were discovered, both are characterized by insertions of different lengths in the gene promoter

The 94 spring wheat varieties were tested for allelic diversity within the *VRN-B3* locus. Genotyping results using previously reported primer sets indicated that 83 out of 94 winter wheat cultivars had the recessive *vrn-B3* allele. The PCR screening also showed the presence of the dominant *Vrn-B3a* allele (Kuibyshevskaya-2, Kazachka varieties).

These primer sets failed to amplify a PCR product in the Velut spring wheat cultivar. Therefore, the newly developed specific primer set was used to detect any sequence variations.



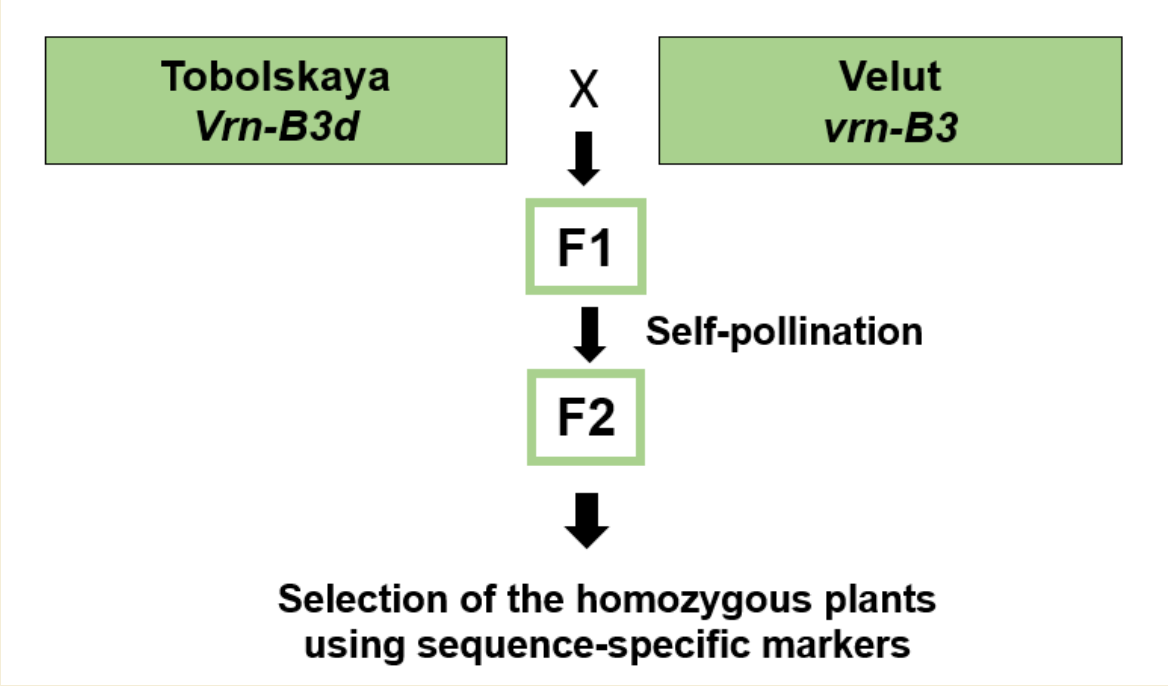
Results indicated that an exact 1617-bp fragment was inserted 85-bp upstream from the start codon. The new *VRN-3* allele was designated as *Vrn-B3d*. It was found that the fragment inserted in the promoter flanked by identical LTRs that are 316-bp in length.

It was also showed that ten cultivars contained an identical insertion of 160 bp located in the promoter region of the *Vrn-B3* gene 881 bp upstream from the start codon.

The *Vrn-B3d* allele: expression level analysis

Two groups of plants derived from the F2 population Velut (new *Vrn-B3d* allele) × Tobolskaya (wild type *vrn-B3*)) were selected to assess the gene expression level of the new *Vrn-B3d* allele. We analyzed F2 hybrids using sequence-specific markers to select the homozygous plants carrying different *VRN-B3* alleles.

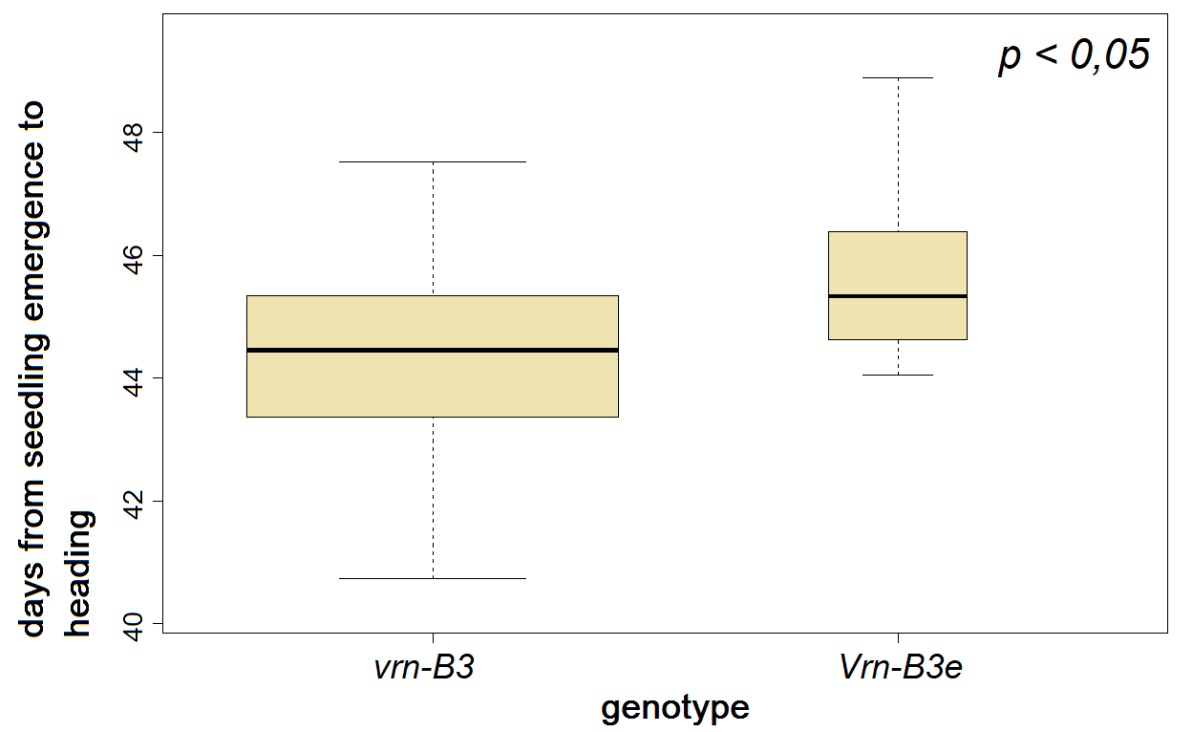
Expression was investigated in leaf tissue sampled at two growth stages (5 leaves unfolded and the 1st node) under short-days conditions (9 h of light).



In thus experiment the expression level of the target gene turned out to be both weak and non-specific. Due to these circumstances, no differences between the new *Vrn-B3d* allele and the wild type *vrn-B3* allele were observed in the study.

Plants with *Vrn-B3e* allele headed later in comparison to the plants with the wild-type allele

To assess the possible effect of the new *Vrn-B3e* allele on heading time, we used field-based phenotype data and measured the differences amongst days to heading between two groups with certain combinations of vernalization and photoperiod alleles (*Ppd-D1b/Vrn-A1a/vrn-D1/vrn-B3* and *Ppd-D1b/Vrn-A1a/vrn-D1/Vrn-B3e*).



We found that cultivars with the combination of *Ppd-D1b/Vrn-A1a/vrn-D1/Vrn-B3e* headed an average of 1.5 days later than cultivars with the combination of *Ppd-D1b/Vrn-A1a/vrn-D1/vrn-B3* under long-day conditions.

Due to the small number of cultivars with the same Vrn-B1 gene alleles, both groups of plants we compared were not homogeneous and contained *vrn-B1, Vrn-B1a* and *Vrn-B1c* alleles. Considering the above, a more detailed analysis may be required to determine the influence the *Vrn-B3e* allele has on heading time.

Conclusion

- Two novel alleles of the *VRN-B3* gene were discovered and characterized
- Among cultivars we examined, ten accessions carried a 160-bp insertion in the promoter region, one variety (Velut) carried a 1617-bp insertion. These alleles were designated as *Vrn-B3e* and *Vrn-B3d*, respectively.
- The qPCR experiment did not show a difference in the transcription levels between *vrn-B3*/*Vrn-B3d* plant groups.
- The field data indicated the plants with the *Vrn-B3e* allele headed later in comparison to the plants with the wild-type allele.
- Sequence-specific markers were developed for identifying the new *VRN-B3* alleles.

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