Studying the regulation of epicuticular wax biosynthesis in barley using isogenic *WIN1/win1* lines generated by site-directed mutagensis

Kolosovskaya E.V.*[1,2], Gerasimova S.V.[1,2], Korotkova A.M.[1], Hertig C.[3], Morozov S.V.[4], Chernyak E.I.[4], Domrachev D.V.[4], Vikhorev A.B.[1,2], Shmakov N.A.[1], Kochetov A.V.[1,2], Kumlehn J.[3], Khlestkina E.K.[1,2,5]

[1]Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia
[2]Novosibirsk State University, Novosibirsk, Russia
[3]Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
[4]Novosibirsk Institute of Organic Chemistry, SB RAS, Novosibirsk, Russia
[5]Vavilov Institute of Plant Genetic Resources, Saint Petersburg, Russia

*e-mail: kolosovskaya@bionet.nsc.ru

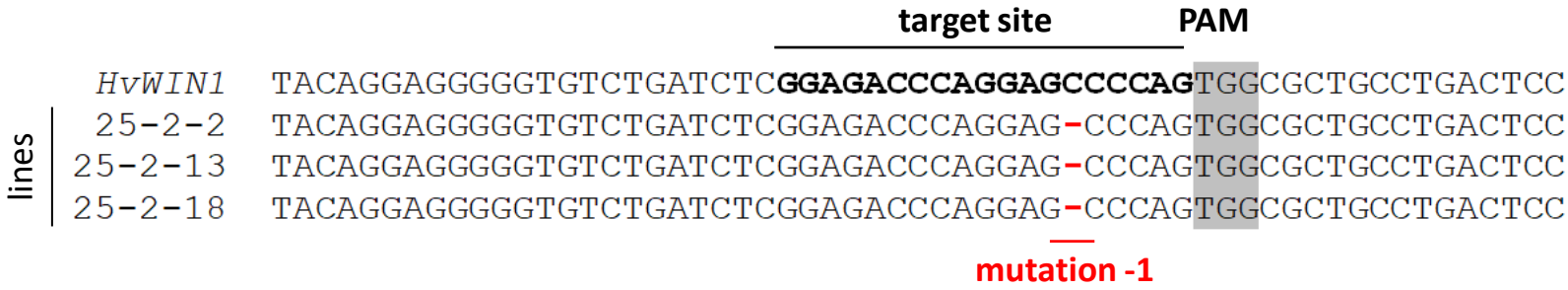
Supported by the RSF (16-14-00086).

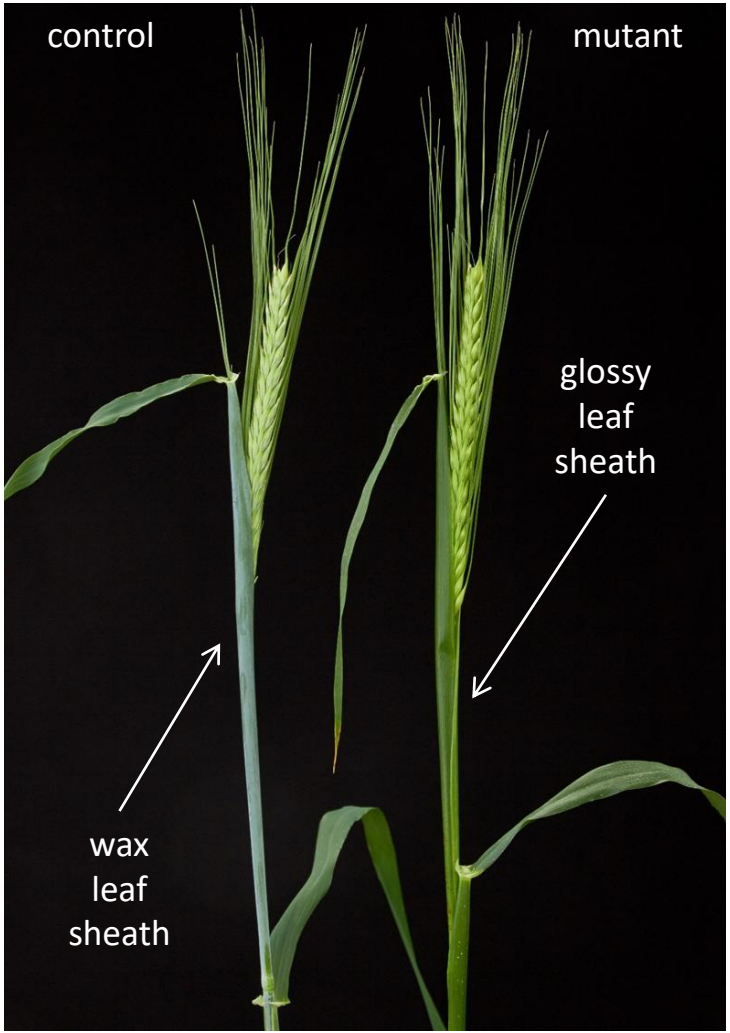
Novosibirsk, 2021

Isogenic lines with contrasting phenotypes are a perfect experimental model system for the elucidation of gene functions.

In the present work, the poorly studied **barley (*Hordeum vulgare*) *WAX INDUCER 1* (*HvWIN1*) gene** was chosen as the target. This gene belongs to the large family of plant-specific **APETALA2/ETHYLENE-RESPONSIVE FACTORS (AP2/ERF)**.

Primary (M1=T0) barley mutants (cv. «Golden Promise») were obtained by targeted knockout of the *HvWIN1* gene using the Cas9/gRNA system. Six T-DNA-free mutant lines harboring different homozygous mutations in the *HvWIN1* gene were selected in the M3 generation.

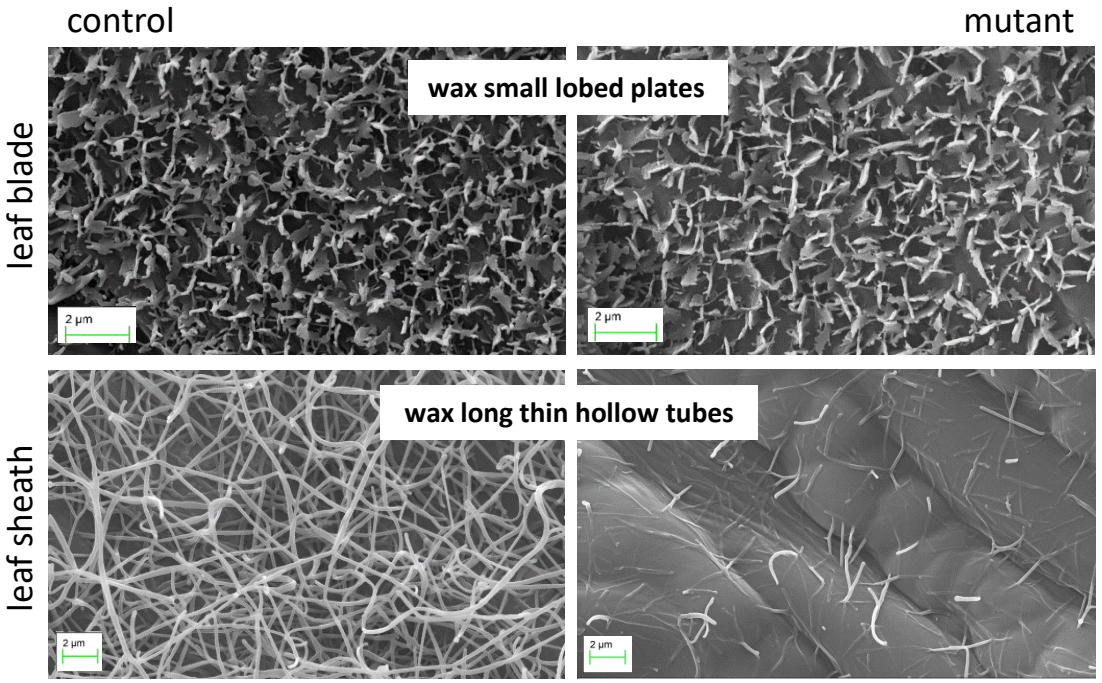


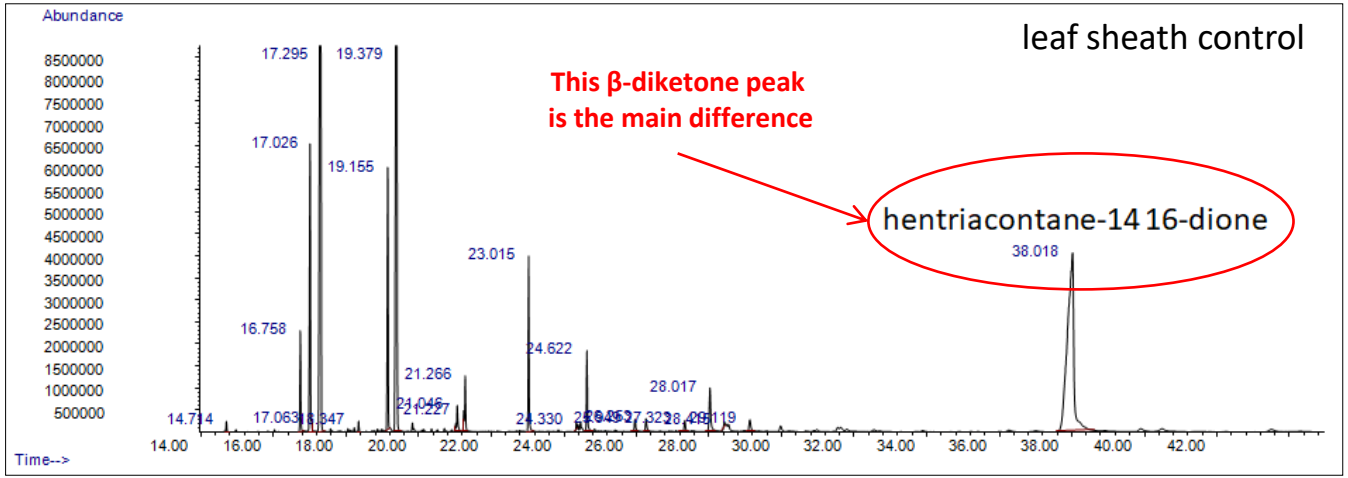


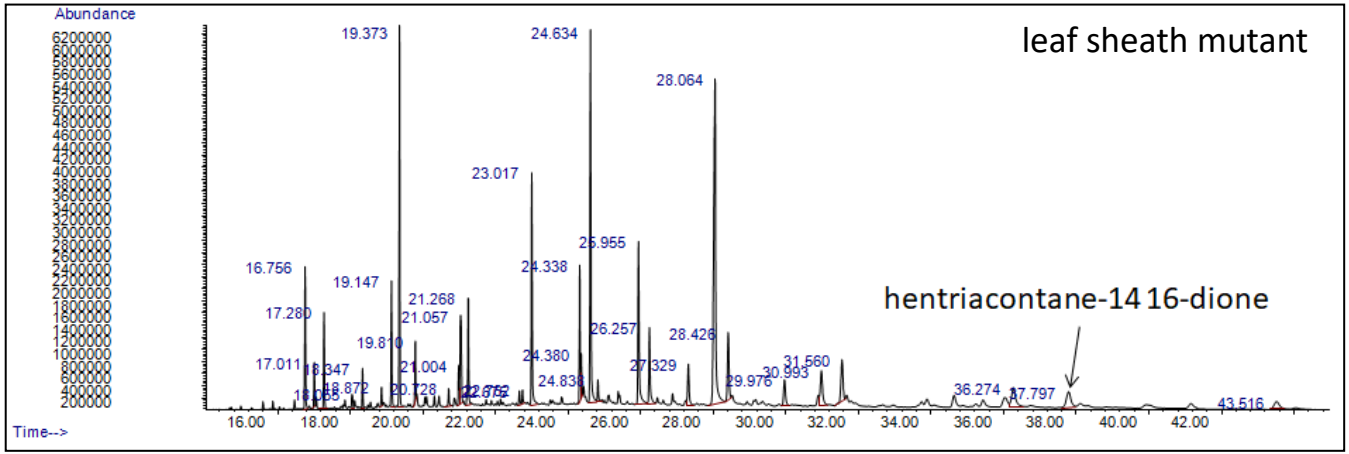
Normally, starting from the booting stage, the surface of the flag leaf sheath of barley is covered with a well-visible wax layer.

Mutant plants and control plants accumulate similar amounts of epicuticular wax on their leaf blades.

***HvWIN1* gene mutants exhibit a recessive phenotype of epicuticular wax deficiency on the leaf sheaths.**



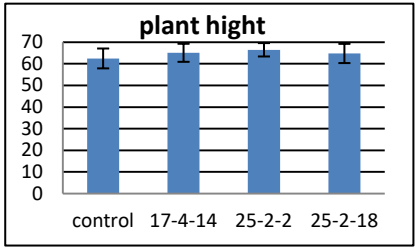


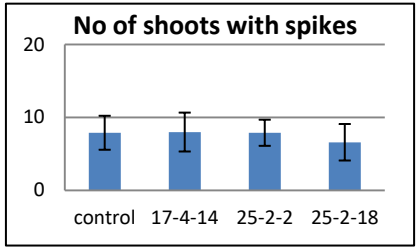


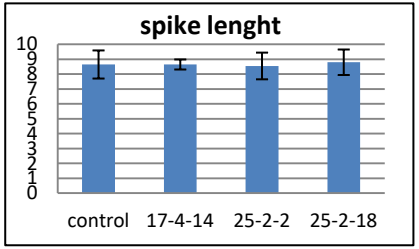
The biochemical composition of the affected wax were studied using scanning electron microscopy and gas chromatography coupled with mass spectrometry.

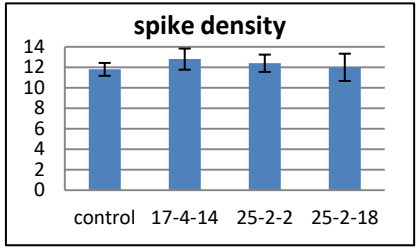
Wax measurements showed that leaf sheath epicuticular wax of mutant plants differs remarkably from that of the wild-type in both total amount and composition. In particular, the amount of β-diketones is significantly reduced in mutants.

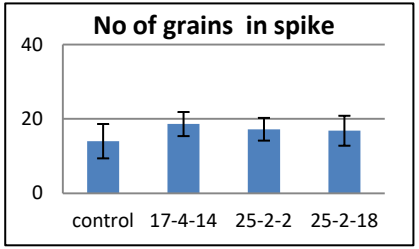
The long thin, hollow tubes on the uppermost barley leaf sheaths are attributable to the β-diketones, most of which is hentriacontane-14,16-dione.

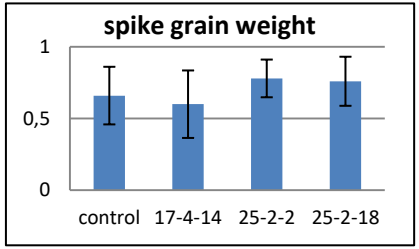


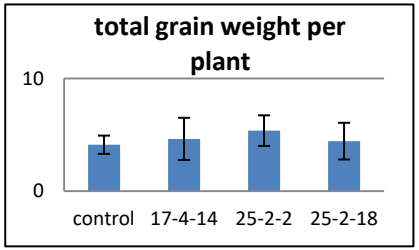


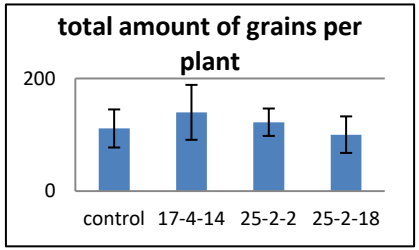


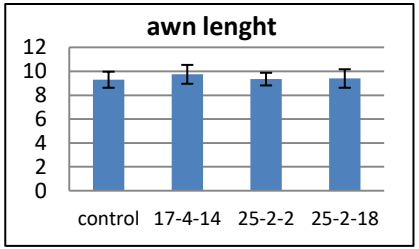


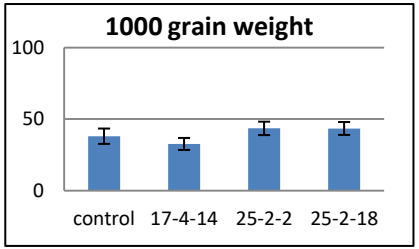


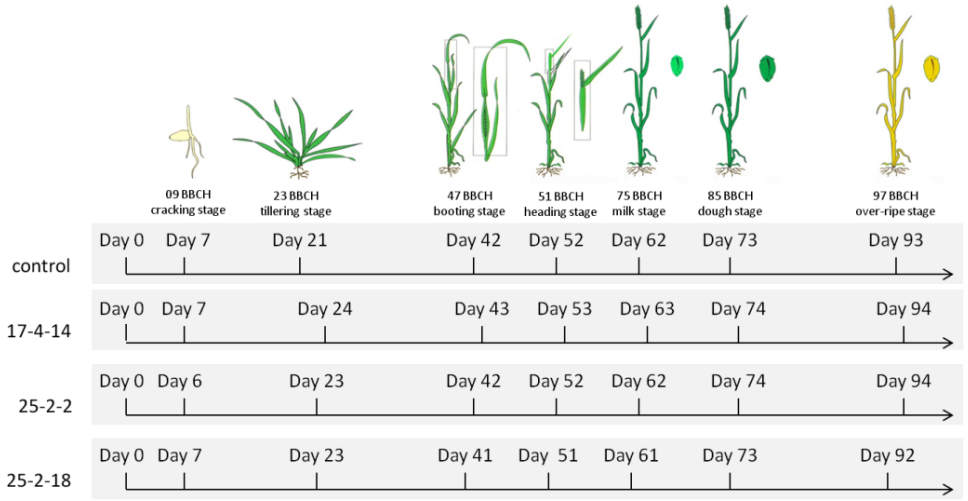








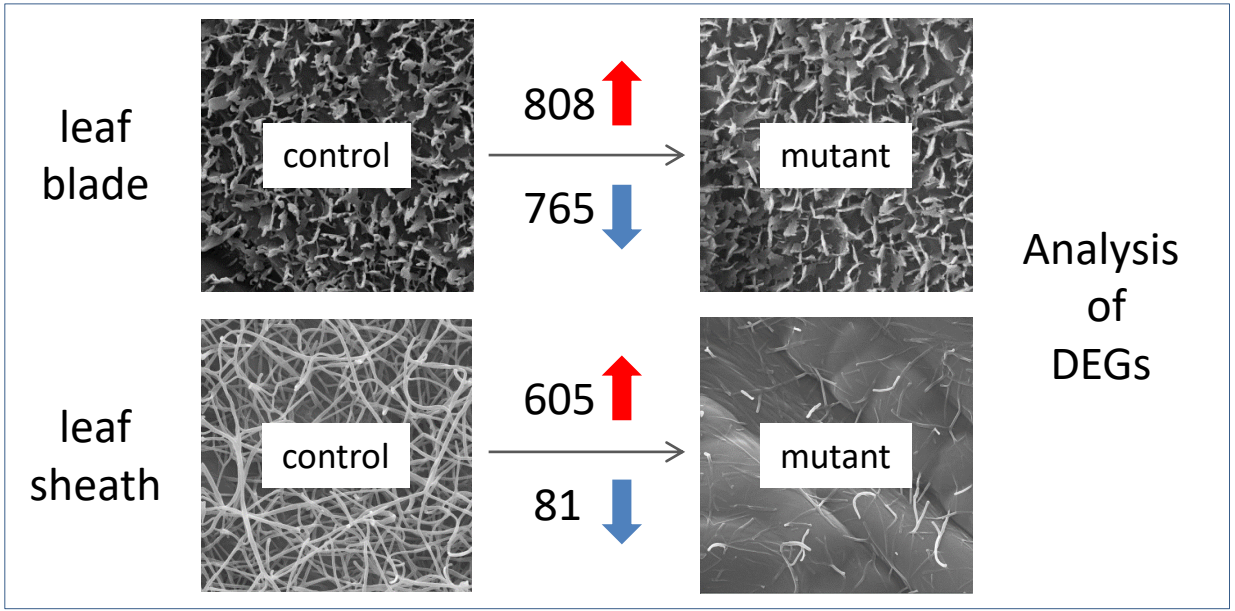




To assess the possible pleiotropic effects of its loss-of-function, a comprehensive phenotyping of the *WIN1*/*win1* pair of isogenic lines was performed.

As a result, developmental stages and major agricultural traits proved not affected by the mutation.

In order to identify genetic mechanisms of phenotype formation, a comparative transcriptome analysis of leaf blades and leaf sheaths of *win1* mutant and wild-type plants was performed.



up-regulated

down-regulated

Among the genes showing reduced expression in the mutant leaf sheath were those previously known to be associated with the synthesis of epicuticular wax components.

Conclusion

The nature of the mutant phenotype and the data of comparative transcriptome analysis suggest that the transcription factor *HvWIN1* normally regulates the formation of the cuticular layer at the surface of leaf sheaths of upper barley leaves.