Identification of an AP2/ERF Transcription Factor Controlling the Synthesis of Barley Epicuticular Wax

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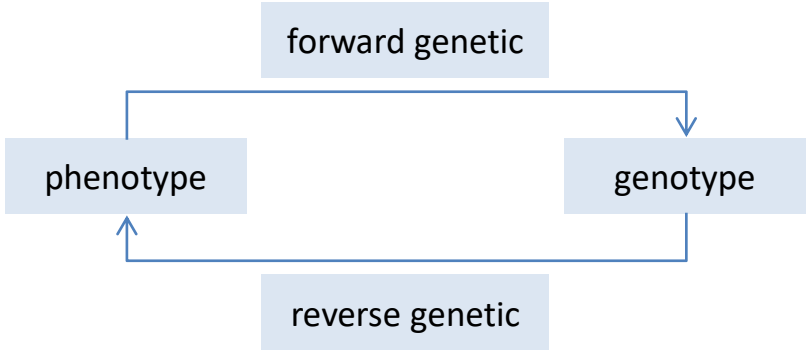
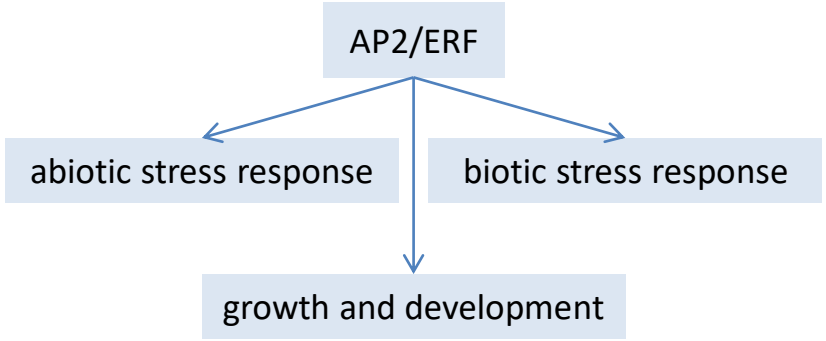
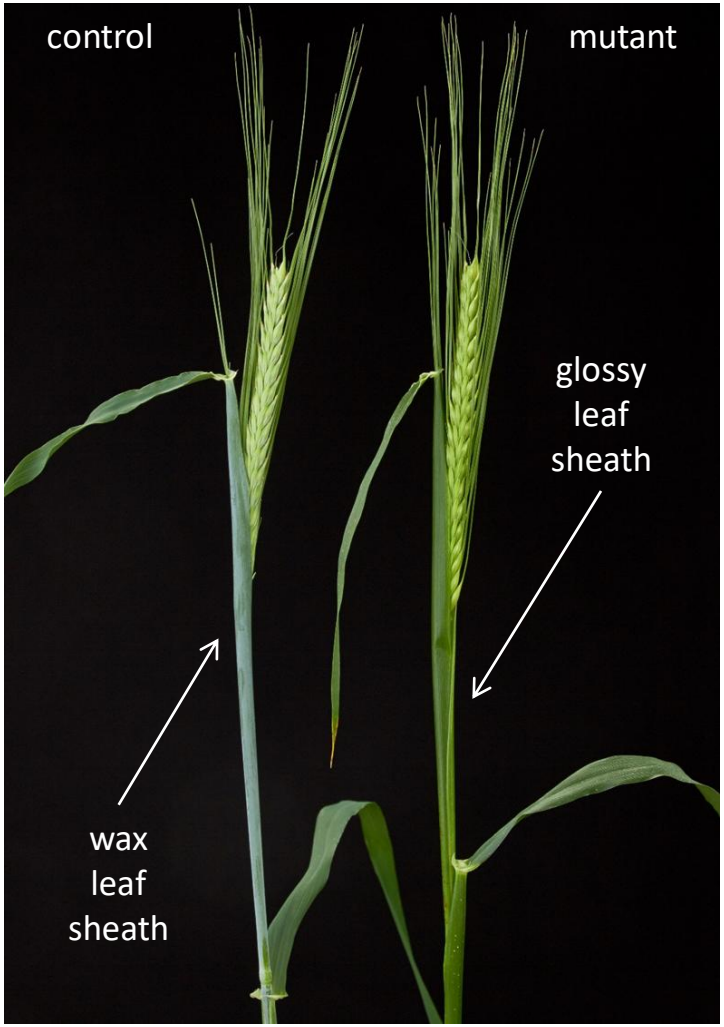




The **reverse genetic** approach allows one to identify the unknown gene functions. Site-directed mutagenesis provides broad opportunities for reverse genetics. **One of the directions in this area is the use of the Cas9/gRNA system to study the functions of regulatory genes.**

In the present work, the poorly studied **HvWIN1 barley (Hordeum vulgare) gene** was chosen as the target. This gene belongs to the large family of plant-specific **APETALA2/Ethylene-responsive factors (AP2/ERF)**.

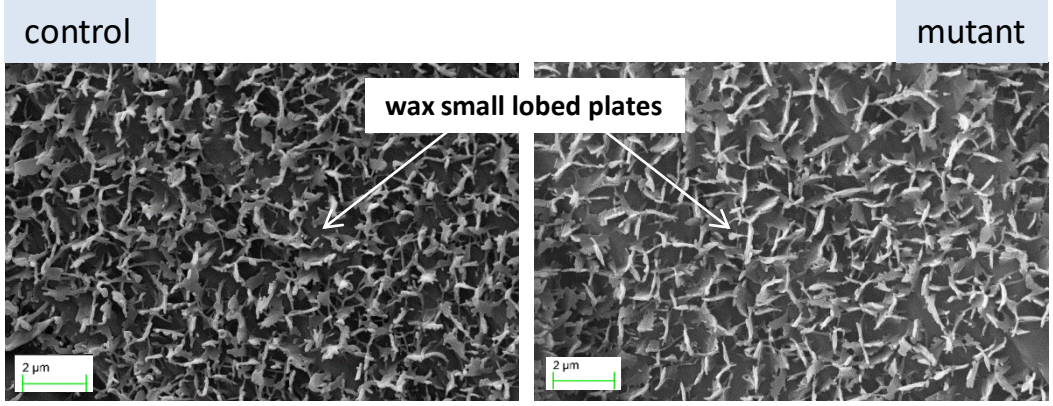


Primary (M1=T0) barley mutants (cv. «Golden Promise») were obtained by targeted knockout of the HvWIN1 gene using the Cas9/gRNA system. Three T-DNA-free mutant lines harboring different homozygous mutations in the HvWIN1 gene were selected in the M4 generation.

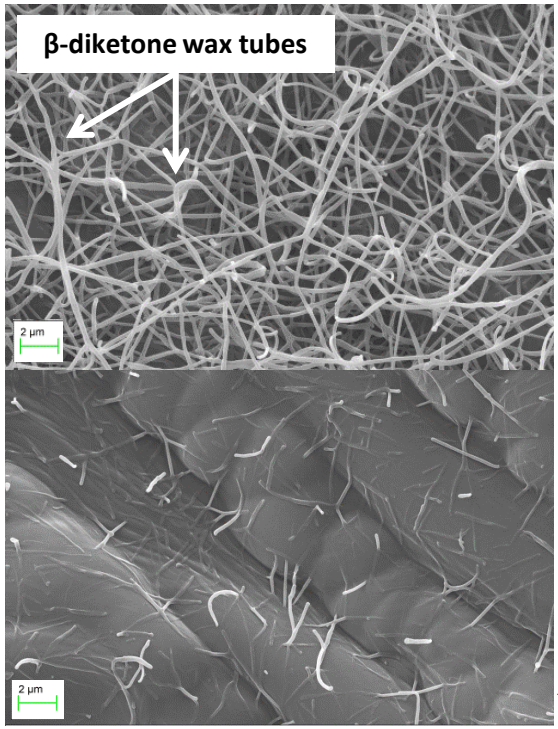
Normally, starting from the booting stage, the surface of the flag leaf sheath of barley is covered with a well-visible wax layer.

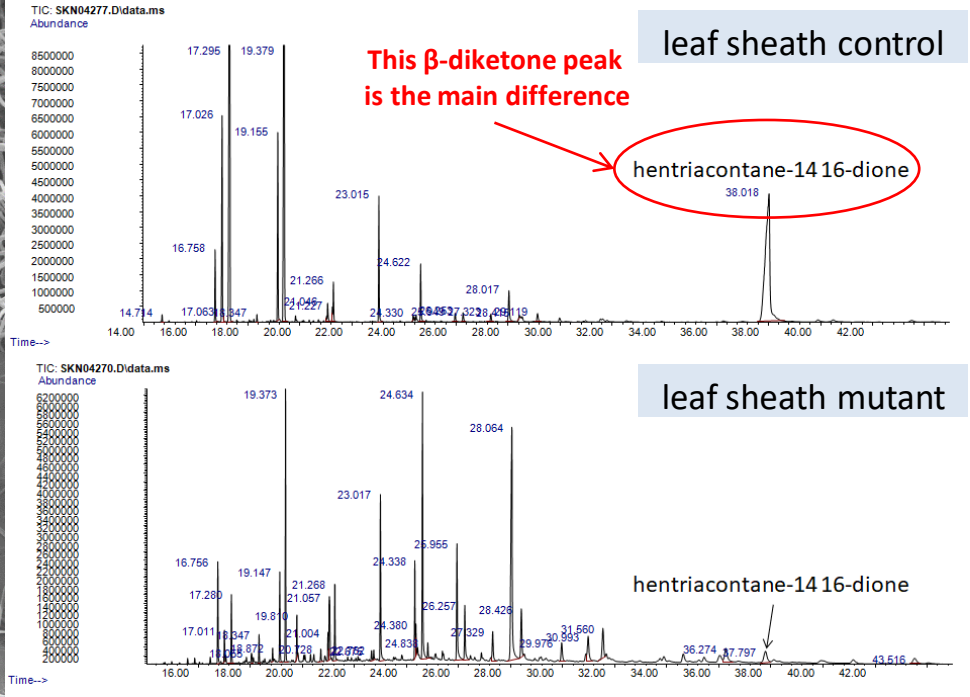
HvWIN1 gene mutants exhibit a recessive phenotype of epicuticular wax deficiency on the leaf sheaths.

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



SEM microphotographs and 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.

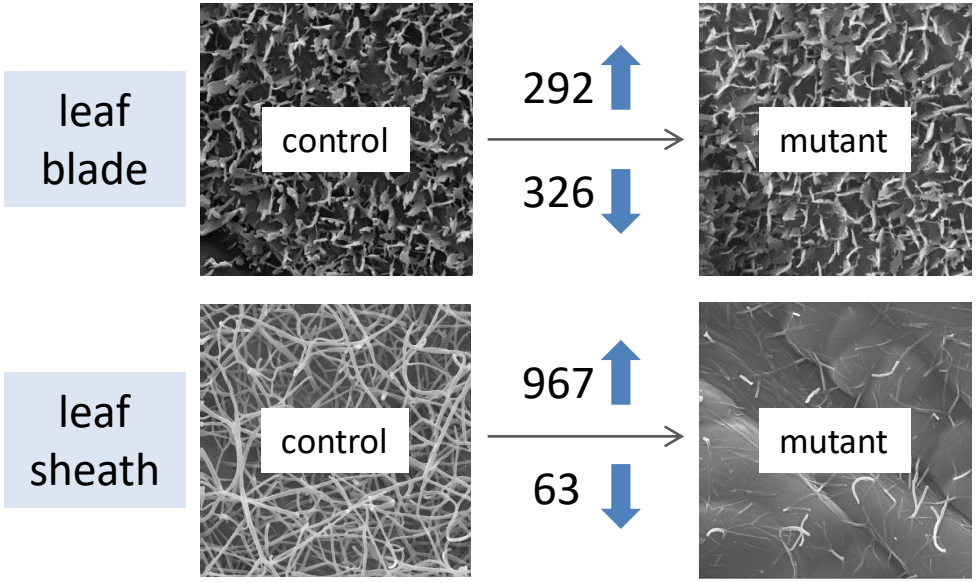




Research was conducted using equipment of The Core Facilities Center “Microscopic analysis of biological objects” at the Central Siberian Botanical Garden SB RAS (Novosibirsk, Russia).

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.

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.



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.