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HvFT3 & *HvOS2*: HOW COULD WINTER BARLEY FLOWER WITH LITTLE OR NO COLD?

Arantxa Monteagudo[1], Ernesto Igartua[1], Bruno Contreras-Moreira[1,2], M. Pilar Gracia[1], Ana M. Casas[1]

[1]Aula Dei Experimental Station (EEAD-CSIC), Avda. Montañana 1005, E-50059 Zaragoza, Spain; [2]Fundación ARAID, Zaragoza, Spain.

Background

Winter barleys need to have completed a period of cold (vernalization) before the day is long enough to promote flowering, which is regulated by the interactions between the activators *HvVRN1* and *HvFT1* and the repressor *HvVRN2* (Fig.1). The promoter under short days, *HvFT3*, has an adaptive role in Mediterranean conditions in winter plants that have not satisfied their vernalization requirement[1]. *HvOS2*, ortholog of *FLC*, is a flowering repressor also downregulated by vernalization in barley[2]. In this study, we characterize the role of *HvFT3* and *HvOS2* as possible players in the flowering pathway under incomplete or null vernalization. We provide new information on the complex mechanism of flowering in suboptimal conditions. As rising winter temperatures will reduce vernalization potential in many regions, winter cultivars will have to be adjusted to new conditions. *HvFT3* and *HvOS2* could have a role in adaptation to non-inductive conditions for winter barley, and in the development of new ideotypes for future climatic conditions.

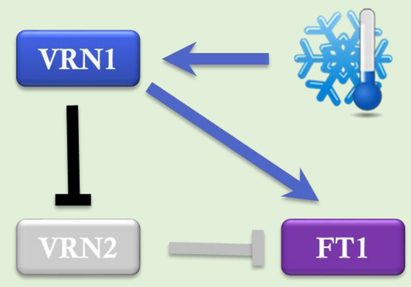


Fig. 1. Scheme of interactions between major flowering time genes in barley. Winter barley is sown in autumn and, during the winter, *HvVRN1* is induced by cold temperatures, down-regulating *HvVRN2* and allowing *HvFT1* expression. Under long photoperiod, *HvVRN2* is expressed and represses *HvFT1*.

Hispanic
(Winter cv. with dominant *HvFT3*)
&
Barberousse
(Winter cv. with recessive *HvFT3*)

Experiment 1

natural photoperiod

Sequential sowings growing under natural photoperiods without vernalization.

Plants were sown from Feb 18th to Apr 15th, 2015, and were grown in a glasshouse until sampling for gene expression analyses on May 19th.

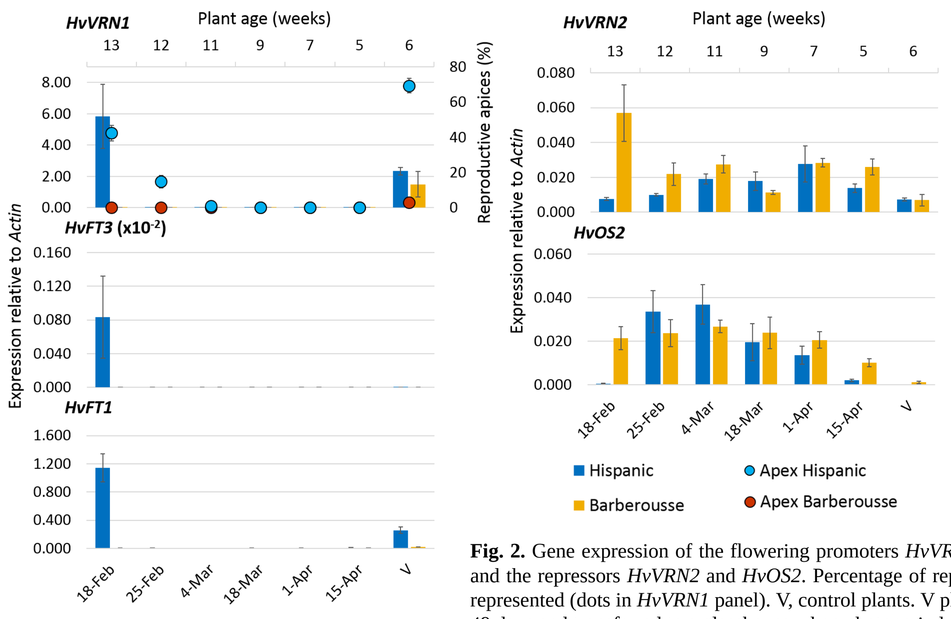
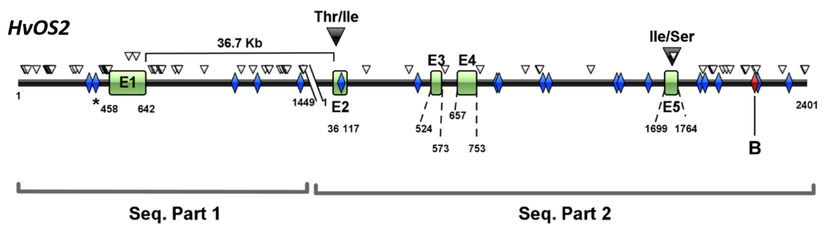


Fig. 2. Gene expression of the flowering promoters *HvVRN1*, *HvFT3* and *HvFT1*, and the repressors *HvVRN2* and *HvOS2*. Percentage of reproductive apices is also represented (dots in *HvVRN1* panel). V, control plants. V plants were vernalized for 49 days and transferred to a glasshouse when photoperiod was 13 h.

Hispanic plants advanced faster than Barberousse's towards flowering. That result was associated with higher expression levels of *HvVRN1*, *HvFT3* and *HvFT1*, and lower of *HvVRN2* and *HvOS2* in Hispanic. Only the Hispanic plants sown under the shortest photoperiods reached heading time in the course of the experiment.

The allelic variants for 4 of the 5 genes studied were well known for Hispanic and Barberousse. We partially sequenced the gene *HvOS2* to identify posible polymorphisms that could explain the differences of expression found. Two of five SNPs found within the coding sequence cause amino acid substitutions (T66I and I150S in Fig. 3). The second one, found in Hispanic, could affect protein function. A high number of predicted VRN1 regulatory sites where identified throughout the gene sequence.

Fig. 3. Gene sequence for *HvOS2* and polymorphisms between Barberousse and Hispanic identified by sequencing genomic DNA PCR-amplified overlapping fragments. Two parts of the gene were sequenced for both varieties. White triangles: synonymous change of aminoacid or intron variant. Black triangles: non-synonymous polymorphism. Diamonds: predicted VRN1-target sites from Deng et al. (2015)[3]. Blue diamonds: sites are conserved. Red diamond, site appears only in Barberousse (B).



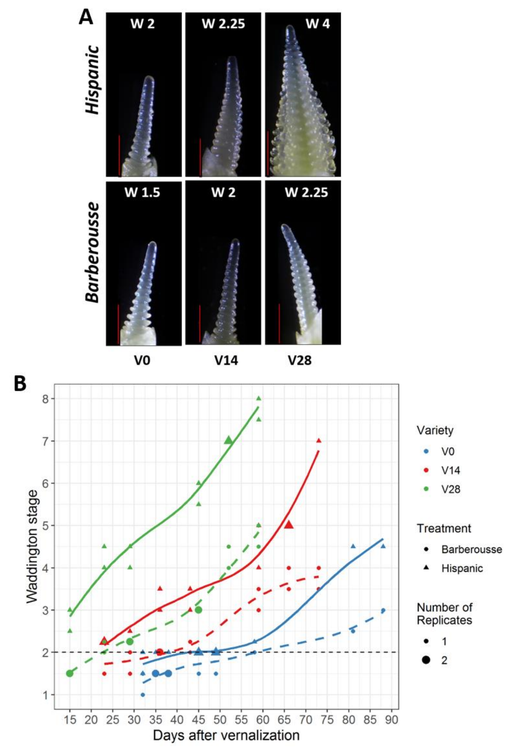
The resulting sequences have been deposited at the European Nucleotide Archive as part of project **PRJEB27962**.

Experiment 2

12h photoperiod

In experiment 1 we observed that gene expression was dependent on plant age. To determine the involvement of *HvFT3* in flowering with incomplete or null vernalization, experiment 2 was carried out growing the plants in short photoperiods (12h) after different vernalization treatments (0, 14 and 28 days in cold). Plants were sampled at different moments (14, 28, 35 and 49 days after vernalization end), and dissected to determine the stage of the apices (Waddington stage[4]). Days to first internode (DEV31[5]) and awn appearance (DEV49[5]) were also determined.

Hispanic V0 plants reached awn tipping after 126 days, whereas Barberousse did not reach that stage during the entire duration of the experiment (136 days).



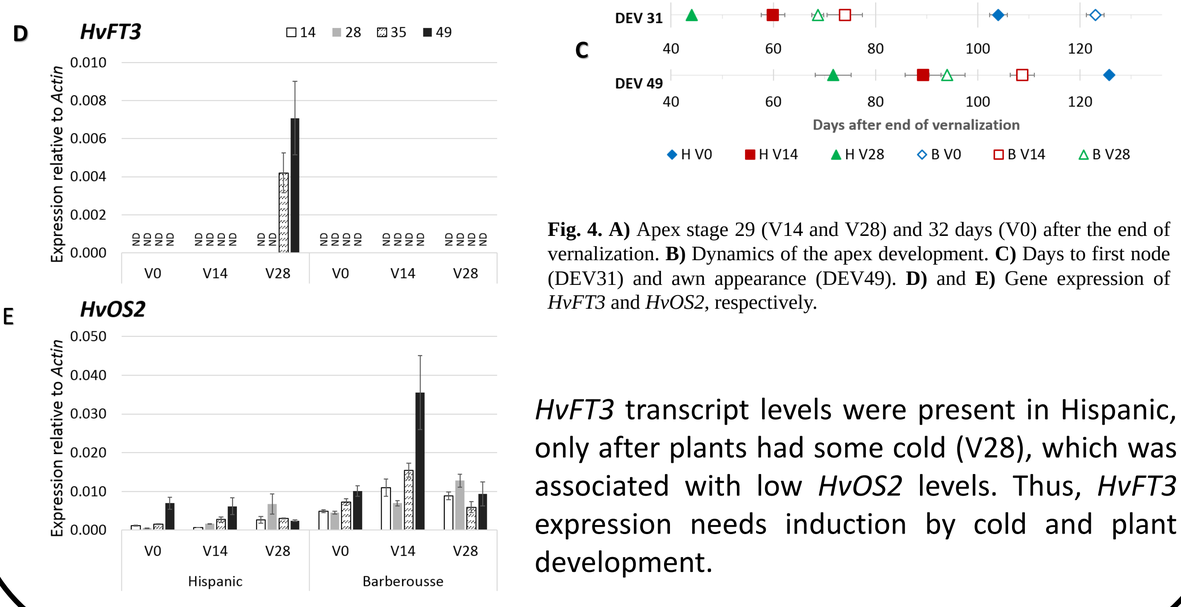


Fig. 4. A) Apex stage 29 (V14 and V28) and 32 days (V0) after the end of vernalization. **B)** Dynamics of the apex development. **C)** Days to first node (DEV31) and awn appearance (DEV49). **D)** and **E)** Gene expression of *HvFT3* and *HvOS2*, respectively.

HvFT3 transcript levels were present in Hispanic, only after plants had some cold (V28), which was associated with low *HvOS2* levels. Thus, *HvFT3* expression needs induction by cold and plant development.

Conclusion

HvFT3 was expressed in a winter cultivar only after some cold exposure, and increasingly with plant age. It is particularly remarkable that the expression of *HvFT3* was correlated with earlier flowering, although it was detected only after the transition from vegetative to reproductive apex had occurred, and with low or no expression of other flowering repressors such as *HvOS2*. The potential change in *HvOS2* protein function, and other polymorphisms in non-coding regions could explain the expression differences found.

HvFT3 and *HvOS2* could have a role in non-inductive conditions for winter barley. Future research could shed light on the role of both genes and its possible relation. This study opens avenues for breeding ideotypes that accelerate flowering under non-optimal conditions.

References

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Acknowledgements

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