

WHAT IS *PPDH2* DOING IN WINTER VARIETIES?



Ernesto Igartua*¹, Ildikó Karsai*², Tibor Kiss², Maria Pilar Gracia¹, Ana M. Casas¹

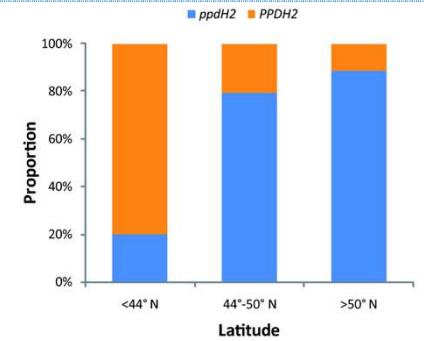
¹ Estación Experimental de Aula Dei-CSIC (EEAD-CSIC), Avda Montañana 1005, 50059 Zaragoza, España

² Centre for Agricultural Research, Hungarian Academy of Sciences, 2462 Martonvásár, Hungary

* these authors contributed equally to this study

INTRODUCTION. Temperatures during barley growing season have been on the rise in Southern Europe over the last 40 years. Under these circumstances, winter cereal farmers are exposed to a difficult choice of cultivars for autumn sowing, from spring cultivars in warm areas to strictly winter cultivars. The choice must take into account winter temperatures and frost probability for the region. Spanish barley landraces (and other Mediterranean materials) present specific combinations of flowering time genes, indicating adaptation to Mediterranean environments. Specifically, most

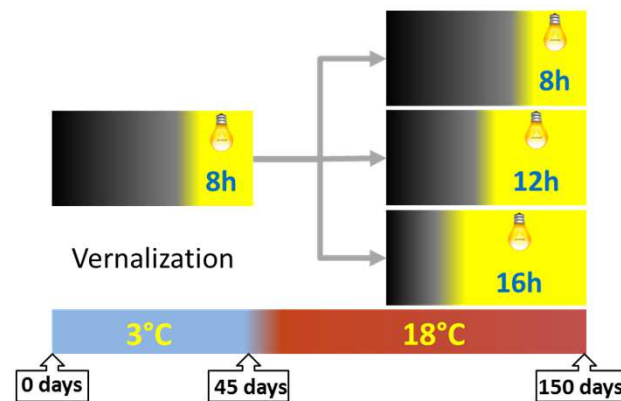
winter landraces carry an active version of the *HvFT3* (*PpdH2*) gene, almost absent from winter cultivars, except in the Mediterranean rim (Casao et al 2011, Fig. 4 reproduced to the right). Several questions arise: why has this allele been kept in lower European latitudes? What effects does it have? To test the effect of allelic variation at *HvFT1* (*VrnH3*) and *HvFT3* (*PpdH2*) on development, we examined the growth of RILs from a biparental population representing four haplotypes of these genes in a winter genetic background.



PLANT MATERIAL. Esterel is a French elite winter genotype, null for *ppdH2* and with an early allele in *VrnH3*. SBCC016 is a Spanish landrace which carries *PPDH2* and a late allele in *VrnH3*. The SBCC016 x Esterel population was described previously (Casas et al. 2011). Twelve F4 recombinant families, representing the different haplotypes for *VrnH3* and *PpdH2* in a winter (*VrnH1/VrnH2* from Esterel) background were selected for this study, three per haplotype, homozygous for the genes of interest.

PHENOTYPING. After vernalization (45 days, 3°C, 8 h light), plants were transferred to three growth chambers under 18°C constant temperature. Three photoperiods were compared: 8, 12 or 16h light. The light source were metal halide lamps.

GENE EXPRESSION. Twenty one days after transfer to the growth chambers, the plants were sampled for RNA extraction (three biological replicates per genotype/treatment). Gene expression was quantified by RT-qPCR for genes *VrnH1*, *VrnH2*, *VrnH3*, *PpdH1* and *PpdH2*, relative to *Actin*.

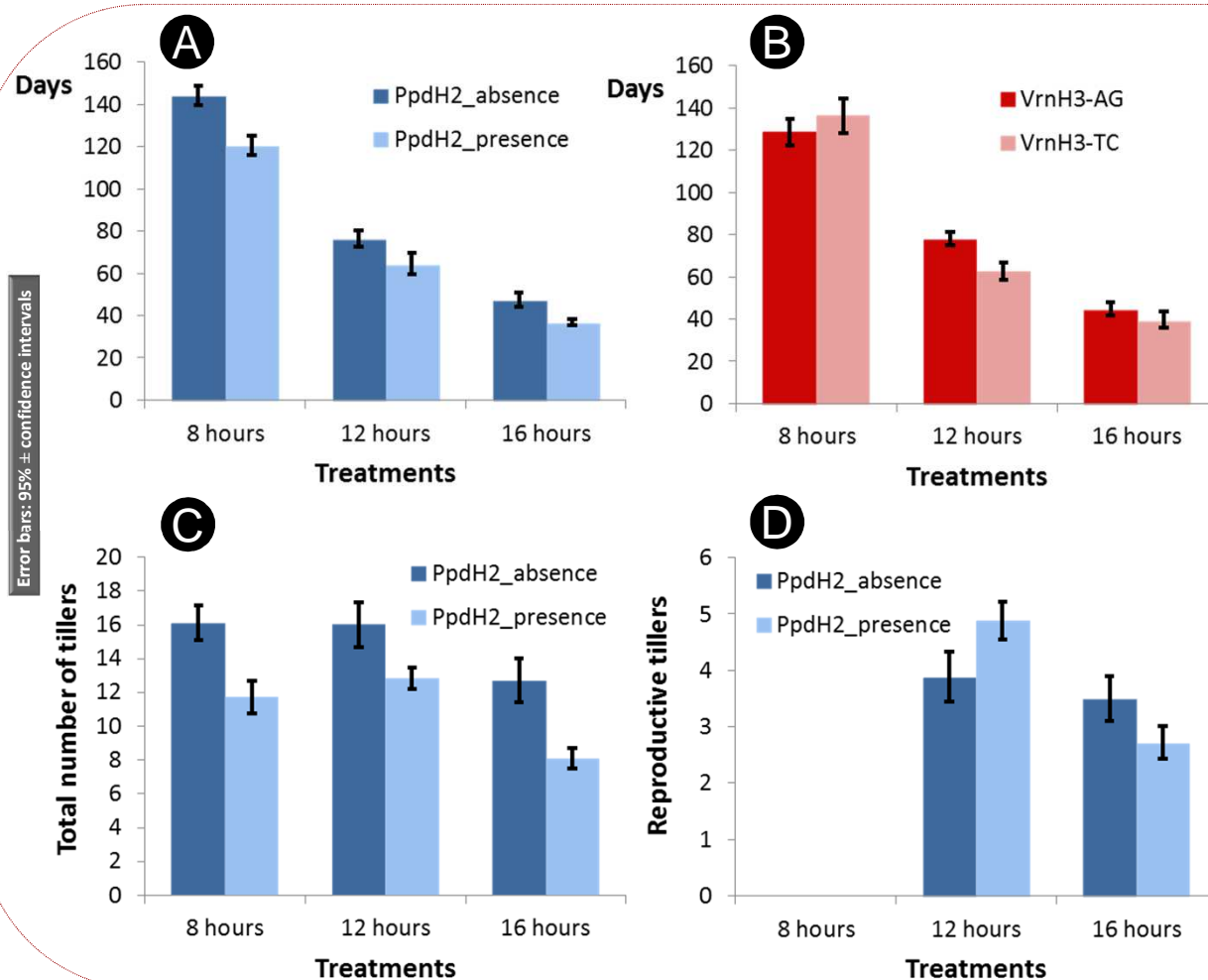


3 treatments: 8, 12 16 h daylength

12 RILs: 4 haplotypes *VrnH3/PpdH2* (winter)
3 RILs/haplotype
3 reps/RIL.treatment

Growth chambers, pots

Traits: date of first internode visible
date of awns visible
tillers
leaves in main stem
reproductive tillers
plant height



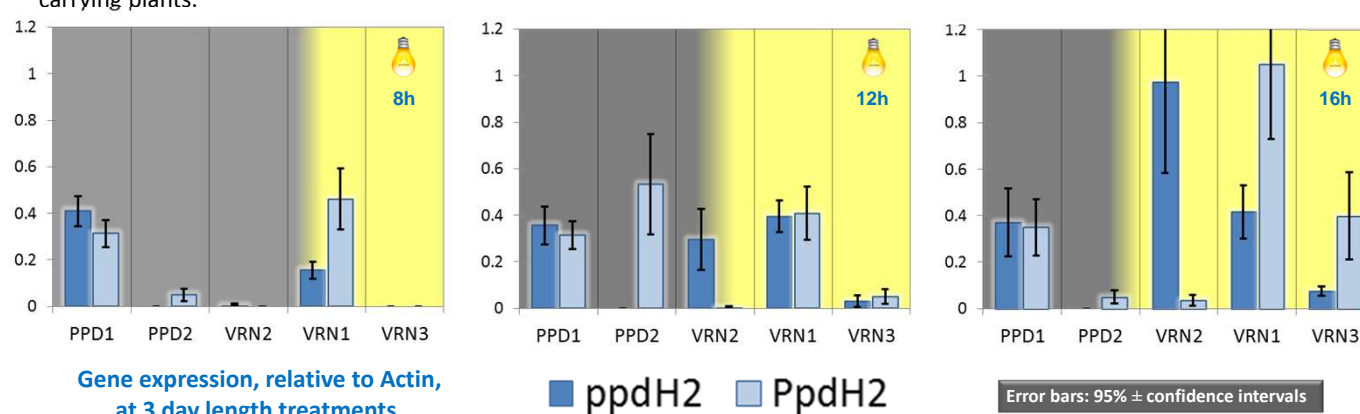
RESULTS, phenotyping. After complete (or close to complete) vernalization, some effects were:

- A** The presence of *PpdH2* induces earliness across all photoperiod treatments measured as days to awn appearance
- B** The effect of *VrnH3* alleles on earliness is only evident at medium (12h) and long (16h) days
- C** There is a large effect of *PpdH2* on total number of tillers produced per plant, consistent across all photoperiod treatments
- D** The effect of *PpdH2* on the number of reproductive tillers, however, presents a strong and significant interaction with day length, at 12h and 16h. No spikes were produced at 8h.

PpdH2 (including a minor but significant interaction with *VrnH3*, not shown) presented a marked effect on plant architecture. The most favorable allele depended on day length. This effect should be validated in the field, in commercial stands.

RESULTS, gene expression.

- At 8 hours, *VrnH2* (VRN2 in the figure) was not expressed, as expected; at 12h, day length was enough to induce *VrnH2* expression. *VrnH1* (VRN) and *VrnH3* (VRN3) expression were the most related with plant development
- *PpdH2* (PPD2 in the figure) was expressed at all day lengths, with a peak at 12h.
- At 12h and 16h, there was an antagonistic relationship between *VrnH2* and *PpdH2* (PPD2 in the figure) expression, even more conspicuous than between *VrnH1* and *VrnH2*. VRN2 expression was detected almost exclusively in *ppdH2* (absence allele) carrying plants.



COMMENTS, FURTHER WORK

The presence of an active allele of *PpdH2* in a winter genetic background causes important changes in the development of barley, with further implications on plant architecture.

The phenotypic effects of *PpdH2* are accompanied by an effect on *VrnH2* expression, consistent with developmental delay.

The adaptive consequences, positive or negative, of the presence of *PpdH2* has to be validated under field conditions, with appropriate plant materials (on this respect, please, see neighbour poster on population bulks, Igartua et al.).