

DECIPHERING THE ROLE OF GRF4 IN BARLEY AND DURUM WHEAT

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Advanced knowledge of the molecular mechanisms affecting the expression of yield components in cereals is crucial for breeders to improve yield capacity. GRFs (Growth Regulating Factors) are plant-specific transcription factors involved in plant development and stress response. GRF mRNAs are target of miR396 and GRF proteins interact with GIF (GRF Interacting Factor) to regulate the expression of target genes. In rice a mutation leading in the miR396 binding site making *OsGRF4* insensitive to post transcriptional regulation results in an increased seed size and promotes nitrogen assimilation and carbon fixation. In durum wheat, a locus carrying the wheat ortholog of *OsGRF4* from wild emmer has been associated with larger seeds. Using the reverse genetics approach, our project aims to

identify and characterize in barley and durum wheat the genes homologous to *OsGRF4* and assess their functions.

We identified 18 GRF sequences in *H. vulgare* cultivar Golden Promise and 31 in *T. durum* cultivar Svevo, through a phylogenetic analysis we identified the genes orthologous to rice GRF4: *HvGRF4A*, *HvGRF4B* in barley and *TdGRF4A*, *TdGRF4B* in durum wheat. A gene expression analysis across a range of barley tissues has revealed that *HvGRF4A* and *HvGRF4B* are mainly expressed in young reproductive tissues, with a peak in the seeds 0-3 days after pollination, these results find validation in literature data.

To study the function of GRF4A, we developed a set of barley and durum wheat transgenic and edited lines:

- 1) ectopic expression of *HvGRF4A* in barley and durum wheat
- 2) ectopic expression of *HvmiR396* in barley
- 3) ectopic expression of a miR396-insensitive *HvGRF4A* sequence generated through mutation in the mRNA binding site in barley and durum wheat
- 4) CRISPR-Cas9 genome editing in barley to produce *grf4* mutants
- 5) CRISPR-Cas9 construct for homologous recombination to abolish the miR396 regulation in barley.

We have also FIND-IT (Fast Identification of Nucleotide variants by droplet DigITal PCR) barley lines with specific single nucleotide changes in the miR396 binding site.

Molecular and phenotypic analysis of these lines are ongoing. Preliminary data suggests that in barley GRF4A may influence flowering time, anther formation and seed length. Interestingly, these functions are not completely conserved in durum wheat where we observed a further effect on spike architecture.