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INVESTIGATING THE ROLE OF THE GRF4 GENE IN BARLEY AND DURUM WHEAT

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The expansion of human population in the next 50 years will require to double the production of cereals and will put a strong pressure on agricultural systems. Actions are needed, new breeding strategies and knowledge on genes related to yield increase are fundamental. Our group is focusing on studying genes that can be manipulated to achieve a better yield potential and one of the candidate genes belongs to the Growth-Regulating Factor (*GRF*) family. GRFs are plant specific transcription factors sharing two highly conserved domains named QLQ and WRC; these proteins play vital roles in plant development and stress response. GRFs proteins interact with their cofactors: GIF (GRF Interacting Factor) to further bind the DNA and to regulate the expression of target genes. Previous studies showed that *GRF4* in O. sativa controls seed size and promotes nitrogen assimilation and carbon fixation. The balance between the GRF4 and DELLA proteins controls plant growth and metabolism of C and N; for this reason, genetic variation of GRF4 (and orthologues) might become a major target for breeders in enhancing crop yield and nutrient-use efficiency. The expression of *GRF4* occurs at different levels comprising the N availability and the activity of miR396. Focusing on barley and durum wheat, we have identified 18 *GRF* sequences in *H. vulgare* cultivar Golden Promise and 31 in T. durum; we are currently interested in the functional characterization of the *HvGRF4* and *TdGRF4* genes. To this aim, we set up different experimental plans through genome editing, ectopic expression and fine mapping approaches using RIL lines. Exploiting the CRISPR-Cas9

technology we produced *grf4* mutants in *Hordeum vulgare* Golden Promise genetic background and we transformed barley plants with a construct for the homologous recombination to abolish the miR396 regulation. Furthermore, when we ectopically expressed the *GRF4* gene in barley plants we observed an increase in seed length and a delay in flowering time. Phenotyping and molecular data will be presented.