

FORWARD AND REVERSE GENETICS ANALYSES OF CULM MORPHOLOGY IN BARLEY (*HORDEUM VULGARE* L.)

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Nowadays and in future climate change scenarios, lodging remains one of the most impactful limiting factors in cereal production, particularly in barley. As an alternative strategy to plant height reduction, strengthening culm architecture has been proposed as a breeding target to enhance lodging resistance. For example, positive relationships between culm diameter and lodging resistance have been reported in different cereal crops. Barley is one of the most widely cultivated cereals and it is a recognized model plant for the *Triticeae* tribe. However, knowledge of genetic and molecular bases correlated with culm morphology is limited.

Addressing this gap, an image analysis protocol has been developed by our group allowing precise measurements of the diameters of barley straw

internodes (Bretani et al., 2022, DOI 10.3389/fpls.2022.926277). Based on data collected in a previous multi environment genome wide association study (GWAS), two double haploid (DH) populations have been developed using parental lines with stable contrasting culm morphology across environments. A total of 381 DH lines from these populations were grown under field conditions in Germany and Estonia and genotyped using the 15k XT Infinium Illumina SNP array (TraitGenetics GmbH). Preliminary GWAS analyses for culm traits detected two Quantitative Trait Nucleotides (QTNs) located near genes already known to regulate plant height and/or flowering time, suggesting an effect of these genes also on culm morphology. Nevertheless, significantly associated QTNs specific for culm traits were identified.

To further explore genetic diversity for culm morphology traits, we are exploiting induced allelic variation from two TILLING populations: *Hor*TILLUS developed in the spring two-row cv. Sebastian, and TILLMore obtained from mutagenesis of the six-row cv. Morex. Taking a reverse genetics approach, the *Hor*TILLUS population was screened in search of mutated alleles in two candidate genes (chr 1H and 4H) selected from genomic regions identified from previous GWAS studies: 2 and 4 lines carrying different alleles for the first and second gene were isolated, respectively. Phenotyping under greenhouse conditions showed that two mutated lines for the gene on chr 4H had reduced diameter of second internodes sampled at dough stage, suggesting a role of the gene in culm development. Results from ongoing phenotyping analyses will also be reported.

As a complementary approach, a forward genetics screen was carried out on the TILLMore population to isolate big culm mutants. Replicated greenhouse experiments confirmed the identification of two lines with increased culm diameter compared to cultivar Morex. Two F2 populations are being developed for each line in order to identify the corresponding genes through a mapping-by-sequencing approach.

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