

MENDELIZING BARLEY VRN-H1/FR-H1 AND FR-H2 QUANTITATIVE TRAIT LOCI IN ALTERNATIVE BACKGROUNDS

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Freezing temperatures is one of the major limitations among the abiotic stress in cereals. In Triticeae tribe, several genetic studies have revealed the phenotypic variation for frost tolerance (FT) is attributed to two QTLs located in the long arm of homoeologous group 5 chromosome: Frost Resistance 1 (FR-1) and Frost Resistance 2 (FR-2). Besides these QTLs, the genotype background might also play a role in the phenotype. Considering barley as a model specie for the Triticeae tribe, in order to study the impact of the FR-H1 (VRN-H1), FR-H2 (cluster of 13 CBFs) locus and the genotype background, a series of experiments in controlled environment (Votsch chamber) has been carried out to test the freezing resistance of the QTL-NILs (Nure or Tremois x DH/RIL), Nure (winter genotype, resistance FT) and Tremois (spring genotype, susceptible FT). Temperature stress ranges from -10°C to -12°C. Plants at seedling stage (1-2 leaves) were firstly acclimated at 3/1°C (200 uE, 8/16H light/dark regime) for 4 weeks. Frost resistance score was measured using chlorophyll fluorescence analysis (Fv/Fm parameter). A marker-assisted backcross scheme was used to develop four QTL-Near Isogenic Lines aiming at separating FR-H1 and FR-H2 genomic regions in different alternative backgrounds. PCR-based molecular markers for HvCBF3 (Fr-H2) and HvBM5 (Vrn-H1/Fr-H1) were used to select plant with the desired allelic state, and after at least BC4 generation, heterozygous plants multiple selfing generations were obtained. At early selfing generations, the genotypic status of the lines was tested using polymorphic

SSR markers scattered throughout the barley genome. Moreover, a Cod42 Nu-Fr-H2 in Tremois background and Cod44 Tr-Fr-H2 in Nure were genotyped with barley 50K chip (Trait genetics). The result of the freezing tests showed that putatively the Fr-H2 (CBF cluster) appears to have a greater effect compared to FR-H1 (VRN-H1). Moreover, the data showed a difference between Nure and the QTL-NILs with Nure background with at least one allele from the spring Tremois. It can be hypothesized that the spring allelic state at the locus negatively affected the phenotype even in a winter background. All the treatments showed that the role of the background is secondary compared to the two FR locus. To confirm these hypotheses, gene expression analysis will be carried out to evaluate VRN-H1, the impact of the whole cluster and each CBF gene on the FT phenotype. Additionally, further investigation will be assessed on open field trials to evaluate recombinants genotype coming from the cross between Nure (winter genotype, resistance FT) and Pamina (facultative genotype, highly resistance FT).