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GENETIC BASIS OF *Fr-H2* IN BARLEY (*HORDEUM VULGARE* L.): SIGNIFICANT IMPROVMENTS IN THE UNDERSTANDING OF THE "*CBF* NUMBER GAME"

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Freezing temperatures can severely compromise barley yield potential. Freezing tolerance depends on a period of cold acclimation mediated by *CBFs* (C-repeat binding factors), a class of transcriptional factors that modulate the expression of multiple effector genes. In order to investigate the relationship between frost tolerance effect due to the major QTL *Fr-H2* and copy number variation (CNV) for a specific cluster of *CBF* genes, a qPCR approach was used on a contrasting set of barley cultivars. The panel, composed of 41 genotypes with different growth habits, was accurately phenotyped by field survival and Fv/Fm measurements. Data showed that there was a positive correlation between frost tolerance and CNV for the *HvCBF2-HvCBF4* genomic region in the proximal cluster and a lower yet significant negative correlation between CNV for *HvCBF3* in the distal cluster and frost tolerance. These results strongly support the hypothesis that *Fr-H2* effect is due to a sort of"*HvCBF*-number game" rather than to a CNV at a single *HvCBF* gene. In addition to this, a protocol to discriminate the *HvCBF2* forms showed an increased copy number of *HvCBF2A* form in highly resistant facultative and winter genotypes. Resequencing by third generation sequencing technology (PacBio) of *Fr-H2* in the frost tolerant cv. 'Nure' is in progress. A comparison of the region with the structure of the locus in both the susceptible reference genotype 'Morex' and the wheat genomes allowed a detailed characterization of the genic and intergenic space of the proximal subcluster harbouring *HvCBF4* and *HvCBF2*. The obtained results are being validated by detailed expression analysis aimed to define how each gene involved in CNV contribute in terms of *CBF* transcripts accumulation. These results represent a significant step forward the understanding of the genetic bases of frost tolerance in barley.