

PHYSICAL MAPPING OF THE BARLEY *Fr-H2* (FROST RESISTANCE-H2) LOCUS

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A positional cloning effort of one of the major quantitative trait loci that affects freezing tolerance and winter hardiness of barley - *HvFr-H2* - where the QTL is linked to the physical sequence of the genome via the fingerprinting of large insert clones, has been undertaken. The *CBF* genes are the best candidates in barley to explain the effects of frost tolerance given by the QTL *Fr-H2*. Determining whether the effect of *HvFr-H2*, is the result of a single *CBF* gene, the combined effect of a subset (or all) of the *CBFs*, or independent by the *CBF* genes remains to be determined.

To address this issue a genomic BAC library of barley (cv. Morex) comprising 313,344 BAC clones was screened with a total of six *HvCBF* markers, out of the 14 *CBF* genes mapping in this locus. A four-step PCR-based screening protocol was used employing DNA of BAC pools. Using that strategy the first BAC clone addresses were obtained for all *CBF* markers assayed. To create anchor points between the genetic map and a ‘future’ physical map of barley, in this region, the fingerprinting (HICF- high information content fingerprinting) of the selected BACs has been performed. After HICF analysis, the selected BAC clones have been assembled into contigs based on the overlapping bands shared by the individual BACs, using FPC V8.5 software. To close the gaps between the assembled clones, additional BACs belonging to the contigs detected, have been screened with the previous six *CBF* markers, additional *CBFs* and other markers deriving from rice-barley synteny investigation. Moreover BAC-end sequencing has also been undertaken for direct linking of assembled contigs. The establishment of a contig between flanking markers is the first aim to clarify the genomic structure of *Fr-H2* QTL region. This will also provide a fundamental resource for detailed comparative analysis of the genomic organization of the locus in other barley cultivars, like cultivar ‘Nure’ and ‘Dicktoo’.