

## TOWARDS PHYSICAL MAPPING AND SEQUENCING THE *FR-H2* (*FROST RESISTANCE-H2*) REGION OF BARLEY CHROMOSOME 5H

PASQUARIELLO M.\*, BARABASCHI D.\*\*\*, TONDELLI A.\*\*\*, SCHULTE D.\*\*\*, STEIN N.\*\*\*, STOCKINGER E.\*\*\*\*, PECCHIONI N.\*, FRANZIA E.\*

\*) Department of Agricultural and Food Sciences, University of Modena and Reggio Emilia, Via Amendola 2, 42100 Reggio Emilia, Italy

\*\*) CRA–Genomic Research Centre, Via San Protaso 302, 29017 Fiorenzuola d’Arda (PC), Italy

\*\*\*) Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Department of Genebank, AG Genome Diversity, Corrensstraße 3, D-06466 Gatersleben, Germany

\*\*\*\*) Department of Horticulture and Crop Science, the Ohio State University/OARDC, 1680 Madison Avenue, Wooster OH 44691

*barley, 5H, Fr-H2, CBF, 454 sequencing*

*Frost resistance-H2* is one of two major quantitative trait loci, located on chromosome 5H, that affect freezing tolerance and winter hardiness of barley. Coincident with *Fr-H2* is a cluster of more than 14 genes encoding *CBF* transcription factors, that are at present the best candidates in barley to explain the effects of frost tolerance given by the QTL. It is not known whether the effect of *Fr-H2* is either the result of a single *CBF* gene, or the combined effect of a subset/all the *CBF* genes, or an effect of other sequences independent from the *CBF* genes. As a first step towards *Fr-H2* physical mapping we have generated a large mapping population derived from the freezing tolerant genotype ‘Nure’, and the freezing susceptible ‘Tremois’, in order to both fine map the *Fr-H2* interval, and to generate recombinants between the different *CBF* genes. Screens for recombinant individuals from F2 populations consisting of 2,849 plants, and their subsequent phenotypic evaluation in F4 lines provided an estimated refined genomic interval of 4.6 cM for *Fr-H2*. Recombinants between seven out of the 14 *CBF* genes under *Fr-H2* have been identified and showed that the *CBF* gene cluster spans 0.81 cM on barley chromosome 5H.

A positional cloning effort of *Fr-H2* has been undertaken. A genomic BAC library of barley (cv. ‘Morex’) was screened with a total of six *CBF* markers mapping in this locus. Using a PCR-based screening strategy the first BAC clone addresses were obtained for all the *CBF* markers assayed. To create anchor points between the genetic map and a ‘future’ physical map of barley, in this region, a high information content fingerprinting (HICF) of the selected BACs has been performed and then the selected BAC clones have been assembled into contigs.

To close the gaps between the assembled clones, additional BACs belonging to the contigs detected, have been screened with further *CBF* markers and a total of three BACs were sequenced and assembled using 454 sequencing. The construction of a single physical contig encompassing the *Fr-H2* region will be our next purpose. This will provide further information on gene content and structural locus organization and thus provide a fundamental resource for detailed comparative analyses of the genomic organization of the locus in other barley cultivars, like ‘Nure’ and ‘Tremois’.