

# Association Mapping of Frost Tolerance QTL in Barley

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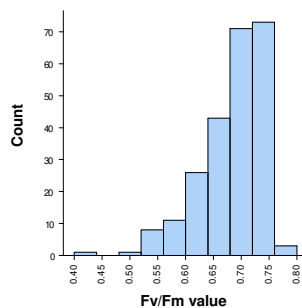
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## BACKGROUND

Barley (*Hordeum vulgare*) is an excellent model system to unravel the genetic bases of frost tolerance, because of the large variation for this trait within the primary gene pool and an ever-expanding set of tools for genome analysis. Within the ERA-PG funded project ExBarDiv (Genomics-assisted analysis and exploitation of barley diversity) three different populations - namely cultivar, landrace and wild (*Hordeum spontaneum*) germplasm collections - have been assembled in order to test the efficiency of an incremental association mapping approach for identifying new useful gene alleles.



**Figure 1.** Distribution of the Fv/Fm values measured on 230 spring barley cultivars, after freezing at -12°C.

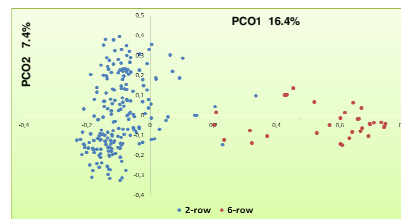


## PLANT MATERIAL - PHENOTYPING

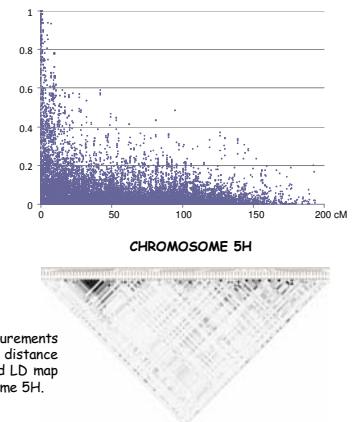
Here we report the evaluation of frost tolerance in 230 spring barley cultivars deriving from across Europe. For each accession, 8 first-leaf stage plants have been cold acclimated for 4 weeks (3°C, 8 h light and 2°C, 16 h dark), then exposed to a freezing stress at a temperature of -12°C. To evaluate the effect of freezing on the functionality of the Photosystem II (PSII) reaction centers, the Fv/Fm physiological parameter has been measured using a Pulse Amplitude-Modulated fluorometer (Rizza et al. 2001, Plant Breeding 120:389-396), after the stress treatment and after a 24 h recovery time. The distribution of the recorded Fv/Fm values is reported in Figure 1.

## PLANT MATERIAL - GENOTYPING

The same germplasm collection has been genotyped with 1536 gene-based SNPs using the Illumina Oligo Pool Arrays marker technology. In order to determine the underlying population structure, Principal Coordinates Analysis has been carried out on a similarity matrix created from 967 SNP marker with Minor Allele Frequency (MAF) >0.10 using a simple matching coefficient. A clear division within the spring barley germplasm due to row number has been observed (Fig. 2). Patterns of Linkage Disequilibrium along barley chromosomes have been evaluated as well, and an example (chromosome 5H) is given in figure 3.



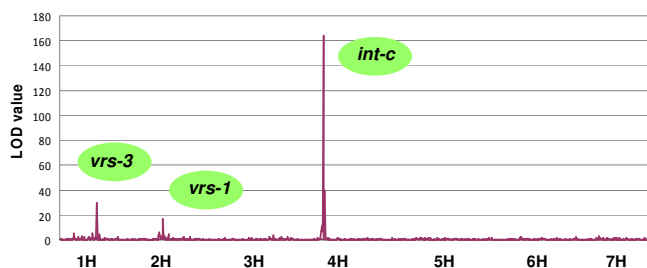
**Figure 2.** PCO Analysis of 230 spring barley cultivars.



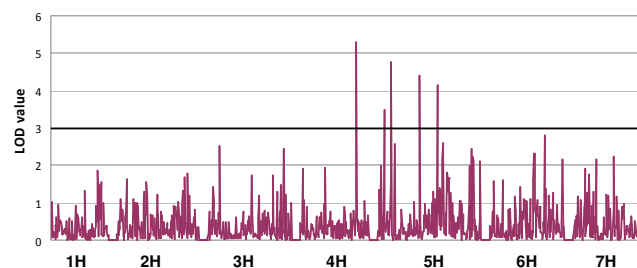
**Figure 3.** Plots of  $r^2$  measurements as a function of genetic distance between loci pairs (up) and LD map (down) of barley chromosome 5H.

## PHENOTYPE/GENOTYPE ASSOCIATION

Association analyses have been carried out by Restricted Maximum Likelihood, fitting a mixed model that included the similarity matrix as covariance term for the random effect, in order to account for the population structure. As shown in Figure 4, the method successfully identified the major genes determining barley ear-row number, namely *int-c* on chromosome 4H, *vrs1* on chromosome 2H and *vrs3* on chromosome 1H. Regarding barley frost tolerance, significant marker/trait associations have been detected on chromosome 4H and 5H (Fig. 5). These QTL, although preliminary, do not co-localize with loci previously related to barley frost tolerance (e.g. *Fr-H1* and *Fr-H2*), and could represent new resistance genes. Sinterity with sequenced genomes such as rice and *Brachypodium* will be exploited to find gene candidates in the regions of interest.



**Figure 4.** Major loci controlling barley row-number, as identified by association analysis.



**Figure 5.** Whole genome association scan for frost tolerance.