## Drought tolerance in Mediterranean barley: An association genetics approach

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The Mediterranean region from the Maghreb to the Near East is characterised by strong water deficits and often suffers from strong drought events.

The region has one of the fastest growing food deficit records in the world and could face catastrophe if their remaining natural resources are not properly managed and conserved. This makes drought management and mitigation a high priority in these countries. Barley is one of the most reliable crops in the Mediterranean region, grown in a wide range of conditions, particularly in marginal environments, often at the fringes of deserts and steppes, receiving modest or no inputs, by resource poor farmers. In order to sustain significant genetic gains for drought areas, a deep understanding of the basic underlying physiological mechanisms of adaptation to drought, resulting in improved water use



Figure 1 Inferred population structure based on 192 individuals and 50 markers using STRUCTURE (Pritchard *et al.,* 2000a). Each individual is represented by a line partitioned in k coloured segments that represent the individual's estimated membership fractions in K cluster.

efficiency, is required. Conventional breeding and high yielding varieties have had virtually no success in the poorest sites in this area. But this lack of success has had a positive effect in preserving biodiversity because in these environments all the barley grown is landraces, which have evolved directly from the wild progenitor in hostile environments, providing a rich reservoir of genes for adaptation and survival to the harsh natural environment (for a review of barley landraces see Grando et al. 2001). By tapping into this natural diversity and combining these with a range of analytical approaches, we have identified QTLs that are genuinely associated with yield in stressed environments.

As part of an EU INCO-MED funded project enititled 'Mapping Adaptation of Barley to Drought Environments (MABDE), we have compiled a database of extensive genotypic and phenotypic measurements and developed appropriate statistical methods for assessing marker-trait associations to understand the genetic and physiological dynamics underlying barley domestication and intensive breeding carried out in the last century in relation to adaptation to drought. A diverse collection of 192 barley genotypes (DBG) (83 landraces, 43 old cultivars and 66 new cultivars) selected to represent key agro-ecological regions from all around the Mediterranean Basin together with relevant genotypes from the rest of Europe has been assembled. A particularly important feature of this collection is the inclusion of the Central and Northern European types as this reflects the breeding development of barley in which germplasm flowed from the Fertile Crescent to ultimately give rise to contemporary cultivars in these regions. Population structure and genome-wide linkage disequilibrium (LD) were investigated using 52 nuclear microsatellite and 1131 DArT® markers. Both clustering and coordinate analyses clearly subdivided the sample into five distinct groups clustering around key ancestors and regions of origin of the germplasm, revealing that individuals closely correspond to the geographic regions in which they were grown (Figure 1). In highly structured samples like the DBG, accounting for population structure in association mapping studies is essential and the persistence of LD that was detected meant that association mapping could be used to locate QTLs. The



population was phenotyped and yield data collected from 28 field trials grown across seven countries, using sites with contrasting water status in each (either by irrigation or utilising past history), over 2 years. The yields observed for individual genotypes ranged from 10t/ha to complete failure of individual genotypes to produce any seed whatsoever due to the stress. The multi-environment QTLs detected in the DBG, were found most frequently in bins 4, 6, 6 and 7 on barley chromosomes



Figure 2 Barley bin map showing significant associations with yield.

3H, 4H, 5H and 7H respectively (Figure 2) and with the exception of bin 4 on chromosome 3H, can be substantiated by results from other relevant studies in barley. One of the encouraging findings in the present study is the detection of significant genetic variation for yield in the eight severely stressed environments where the mean yield was less than 2t/ha, and one of the most consistent genomic regions was in bin 7 on chromosome 7H, where four out of the five significant associations came from the Jordanian sites with mean yield ranging from 0.3 to 1.2 t/ha.

This association mapping population is genetically and physiologically diverse, strongly structured based on the pedigrees providing contrasting levels of population stratification. Current genome coverage, together with observed LD decay values, should not present problems for attempting a whole genome scan and makes the population an ideal QTL mapping resource for the Mediterranean environmental conditions provided that population structure is effectively and appropriately controlled within the association analysis.