Development of Recombinant Chromosome Substitution Lines - a barley resource

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The goal of SCRI's barley research programme is to identify the genes controlling commercially relevant characters such as yield and quality. This is currently a stepwise process in which regions of the genome controlling the characters are localised through relating expression of a target character (phenotype) to the genetic constitution of individuals (genotype). The process is called QTL mapping, but the results are fairly imprecise, with it being impossible to decisively refine a QTL to an interval of less than 30 cM, about a third of a barley chromosome arm. This means that association of QTL with candidate genes will also be imprecise and will therefore hinder the exploitation of genomics programmes such as the barley transcriptome resource being developed at SCRI. Another problem associated with QTL analysis is that the first generation mapping procedure, simple interval mapping (SIM), which is implemented by software such as MAPMAKER/QTL, can give misleading results. The development of techniques to account for variation in other parts of the genome, composite interval mapping (CIM), which is implemented by software such as MapQTL, MQTL, QTL Cartographer and PLABQTL, has led to some improvement in QTL location. This is illustrated by mapping plot yield and heading date data from a trial of random lines from the cross Derkado x B83-12/21/5, for which a genetic map had been constructed, carried out at SCRI in 1999 (Fig. 1). For heading date, what appeared to be one QTL has been resolved into three separate QTLs, two alleles from Derkado delaying heading date and one shortening it. The two QTLs delaying heading date are over 30 cM apart from the results of composite interval mapping and the QTL shortening heading date is also nearly 30 cM from the next nearest heading date QTL. Despite these three QTLs being spread out over a distance greater than half a barley chromosome arm, there was no indication of more than one QTL from simple interval mapping. When yield is considered, the situation is even worse for there was no indication of any significant yield QTLs on the chromosome from simple interval mapping, yet composite interval mapping revealed two QTLs some 20 cM apart. As these QTLs were linked in repulsion in the parents, i.e. one parent possessed the increasing allele at one locus and the other parent possessed the increasing allele at the other locus, simple interval mapping was not able to detect any effect in this region. Composite interval mapping has clearly helped to improve the identification of QTLs affecting key traits in barley.

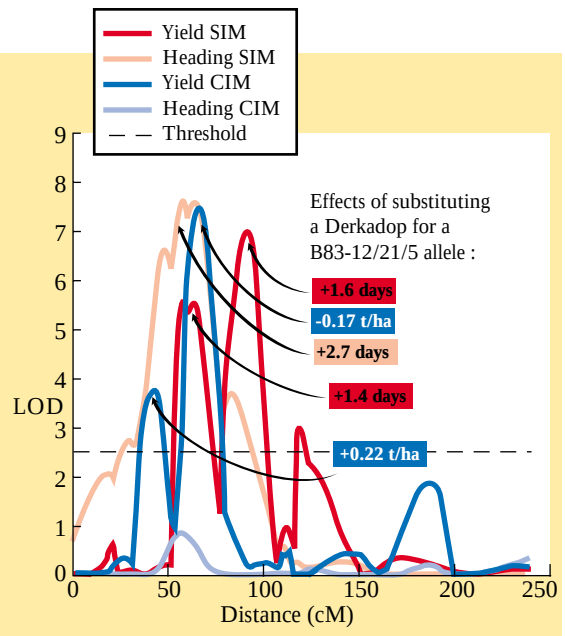

Figure 1 Derkado x B83-12/21/5 Chromosome 7H Scans 1999.

The problem with QTL identification by composite interval mapping is, however, that one is unsure whether the effects detected are real or due to over parameterisation. It is also unclear whether, as in the case of the two variates shown in Figure 1, there are two closely linked QTLs, one affecting heading date and the other yield, or just one QTL with a pleiotropic effect. Another problem is that the effects associated with particular QTLs can also be overestimated. One way to solve this problem is to design alternative populations in which to estimate QTL effects. Paterson *et al.* (1990)[1] proposed the development of a series of isolines in which small segments of a donor genome are introgressed into a common recipient to give a library of lines which covers the whole donor genome. Such isolines are called Recombinant Chromosome Substitution Lines (RCSLs), which are depicted graphically in Figure 2. RCSLs

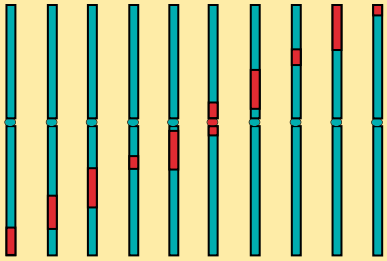

Figure 2 Set of ten Barley Recombinant Chromosome Substitution Lines with over lapping segments of donor genome (red) introgressed into a common recipient background (green).

enable fine mapping of the genome and will solve many of the problems identified above. In *Brassica oleracea*, phenotypic and genotypic analysis of RCSLs has not only confirmed the location of QTLs revealed by mapping studies but has also revealed extra loci[2]. At SCRI, we are developing a series of barley RCSLs to address some key questions in deployment of molecular markers for gene mapping:

1. Can we reveal more QTLs controlling economically important traits?

2. Will the use of RCSLs improve the estimation of effects of individual loci?

3. Are closely linked QTLs affecting different characters really two different loci or due to errors in the estimation of one pleiotropic locus?

4. If there are two loci closely linked in repulsion, can the linkage be broken to produce a marked improvement in phenotype?

We are also posing more fundamental questions through appropriate choice of donor parents and recipient. We have chosen the recently recommended spring barley Chime as the recipient parent as it represents the best available balance between malting for brewing and distilling, yield, and agronomic suitability. One donor parent is Regina, representing a diverse cultivated barley from the winter gene-pool, which has been shown to be quite distinct from the spring. The RCSLs from this donor will therefore be used to investigate the differences between the two gene-pools. Two other donor parents are a Syrian land-race (supplied by S. Ceccarrelli, ICARDA) and an *Hordeum spontaneum* accession from Israel (supplied by E. Nevo, Hebrew University). Previous research at SCRI has revealed the narrowing of the genetic base that has occurred in developing current North-West European spring barley germplasm and these last two donors have been chosen to examine some further questions:

1. Is there useful variation associated with the loci involved in domestication that can be exploited through marker-assisted introgression?

2. Do novel alleles in the donor genotypes at loci associated with economically important QTLs represent novel QTL alleles, some of which may be beneficial?

From Figure 3, it can be seen that not only are there considerable difference between the donor and recipient genotypes, there are also whole regions where the Syrian land-race and the *Hordeum spontaneum* accession possess novel marker alleles. We will therefore be able to use this material to answer these important questions that are central to the targeted use of exotic material in germplasm development. Our initial goal is to develop 'coarse focus' sets of lines with large portions of genome introgressed from each donor, using our library of SSR markers to monitor the process. We then propose to construct 'fine focus' sets of lines from the appropriate 'coarse focus' lines to study closely a target area of the barley genome. We will make these sets of 'coarse' and 'fine focus' lines available to the wider barley research community for further study and development.

References

[1] Paterson, A. H., Devema, J.W., Lanini, B. & Tanksley, S.D. (1990). *Genetics* 124, 735-742.

[2] Rae, A. M., Howell, E.C. & Kearsey, M. J. (1999)[1] *Heredity* 83, 586-596.

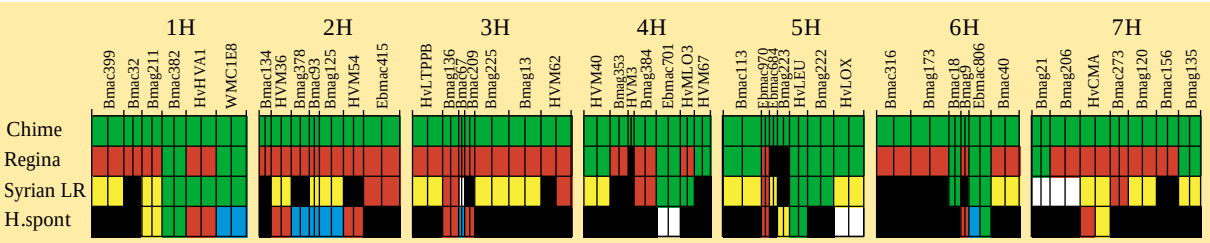

Figure 3 Graphical genotypes of recipient and donor genotypes. Black indicates novel allele. White indicates missing value.