# The barley genome: a source of genes for breeders and biotechnologists

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Two of our main goals are to develop molecular markers for use in breeding programmes and to isolate genes for use in transgenic approaches to barley improvement. These approaches are complementary and are described below.

**Molecular marker technology development and deployment** Phenotypic differences between individual accessions have provided the basis for successful plant breeding. However, phenotypic appearance is not always a good indicator of genetic potential. Recently, the ability to detect polymorphism at the DNA level has profoundly changed plant genetic analysis and is poised to impact on barley breeding and plant biotechnology. An important element of this development is the technology for detecting DNA sequence variation. Microsatellites, or simple sequence repeats (SSRs), provide an important intermediate technology for barley breeders, since SSRs are PCR-based, exhibit co-dominant inheritance and are multi-allelic. The high information content of SSRs means that diagnostic markers will have a high probability of detecting polymorphism in germplasm of direct relevance to breeders. For this reason, we have invested considerable resources in the area of microsatellite discovery and mapping (SCRI Ann. Rep. 1996/97, 82-83). To date, 385 functional microsatellites have been identified, and 258 SSRs have been mapped, generating 299 loci (Table 1).

In parallel with the microsatellite marker development and mapping, we have also been active in deploying microsatellites for various projects:

| | Sequences | PCR product | Mapped | Loci |
|---|---|---|---|---|
| Enriched libraries | 792 | 319 | 215 | 254 |
| New EMBL | 41 | 23 | 11 | 11 |
| Hvm/B&H | 47 | 43 | 30 | 32 |
| Wheat | 99 | 20 | 2 | 2 |
| Total | 979 | 405 | 258 | 299 |

**Table 1** Discovery and characterisation of SSRs.

**Genotypic diversity in the cultivated barley genepool** A key factor in this programme is the utilisation of mapped microsatellites to create a genotypic database for barley. The microsatellites are represented by both

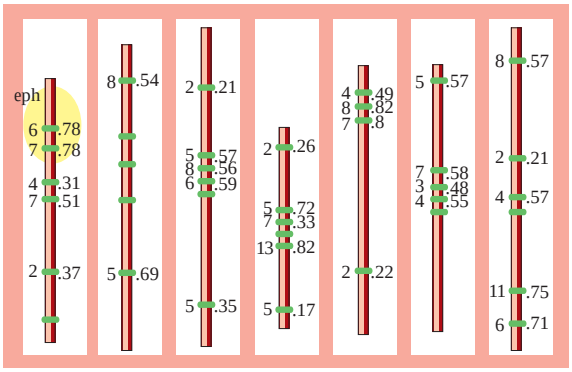


**Figure 1** Allele numbers and diversity indices at chosen SSRs - 67 genotypes.

anonymous SSRs and SSRs that are associated with genes of known function. A sample of 100 cultivars, represented by genotypes produced in the last century, together with modern day cultivars, was genotyped with 30 SSRs spanning the seven barley linkage groups. A total of 168 alleles was observed with on average 5.6 alleles per SSR being detected. The number of alleles and diversity indices for each SSR are given in Figure 1, providing a two-dimensional representation of the pattern of genetic variability present in the barley genepool.

An alternative method of presenting the information on allelic diversity is shown in Figure 2. In this graphical display, the pattern of allelic variability is given for two of the seven linkage groups and 'pinpoints' specific allelic substitutions that have accompanied the introduction of new cultivars. This form of graphical genotyping, when coupled with data on pedigree relationships, provides a means of monitoring the flow of alleles through ancestral lineages and identifying regions of the genome that have been preferentially transferred through selective breeding. This is illustrated for the pedigree of Cooper in Figure 3 where the inheritance of alleles at four SSR loci is

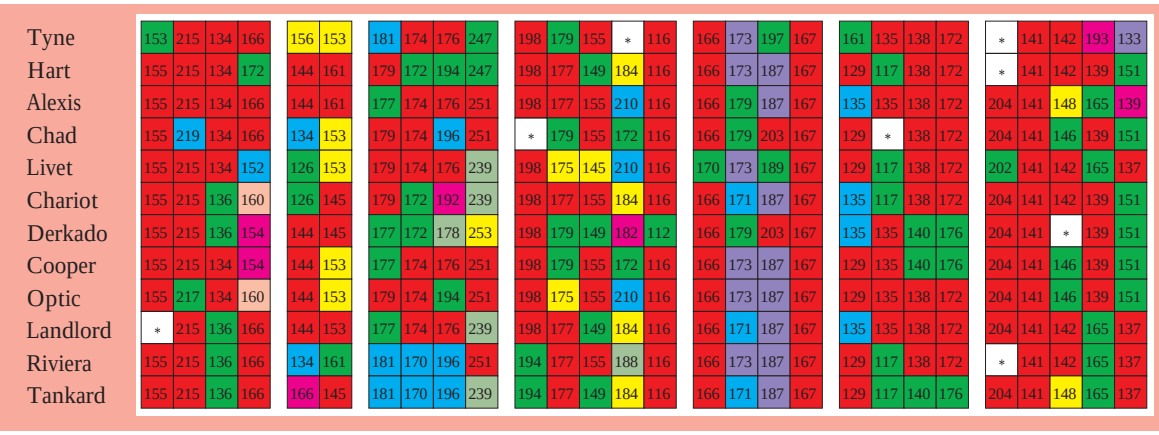


**Figure 2** SSRs in selected modern spring barley cultivars arranged in chromosome order (1H-7H).

shown. Based on this pedigree, it would appear that chromosome 5H of Cooper has been 'inherited' directly from Force. The high discriminatory power of microsatellites allows closely related genotypes to be distinguished, enabling SSRs to be used in delineating complex pedigrees constructed from a narrow genetic base. The relationship between genetic and kinship estimates of relatedness is shown in Figure 4 for the cultivars Cooper and Force. Since Force is one of the 'grandparents' of Cooper, on average one would expect 25% of the genetic information from this cultivar to be inherited by Cooper. The radar plot given in Figure 4 shows the poor correspondence between the two estimates of relatedness. Furthermore, it shows that, based on this data, chromosomes 5, 6 and 7 have been inherited directly from Force rather than receiving contributions from Corniche and Troop. This retrospective analysis of cultivar production will provide new insights into significant 'historical recombination events' and provides an important tool for selection of parents based on genetic rather than phenotypic information.

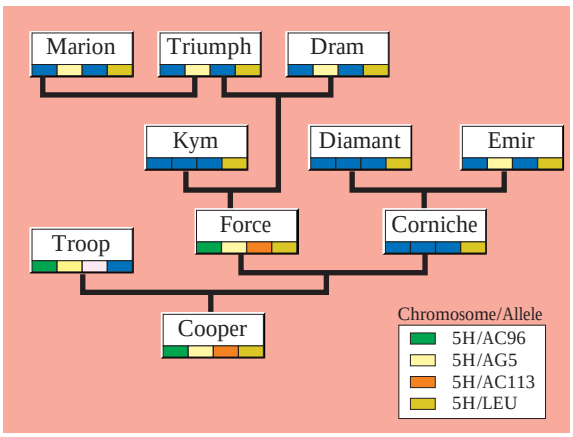


**Figure 3** SSRs in the pedigree of Cooper - origin of chromosome 5H alleles.

**Identifying a microsatellite marker linked to the *Ym4* virus resistance locus in barley** Barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV) are important diseases of winter barley in Europe. The fungal vector *Polymyxa graminis* is responsible for transmission of virus particles to roots of susceptible plants. The soil-borne transmission of the pathogen causes problems for both chemical control of the disease and for the testing of resistant lines for eventual deployment in plant breeding programmes.

Previous studies[1] have localised the recessive resistance gene *Ym4* to the distal region of chromosome 3H.

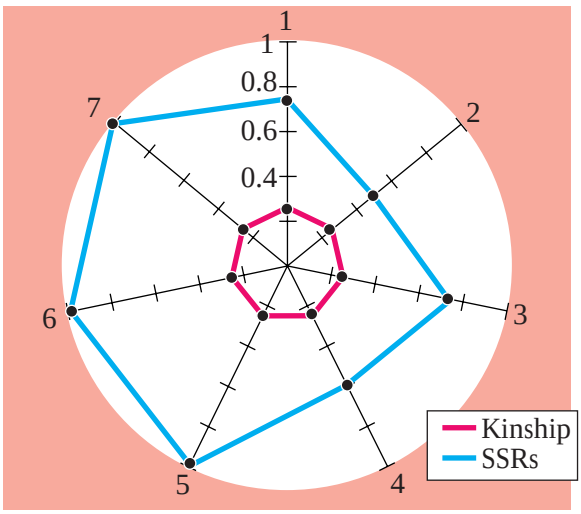


**Figure 4** Radar plots of genetic (SSRs) and pedigree similarity (kinship) for chromosomes 1H-7H.

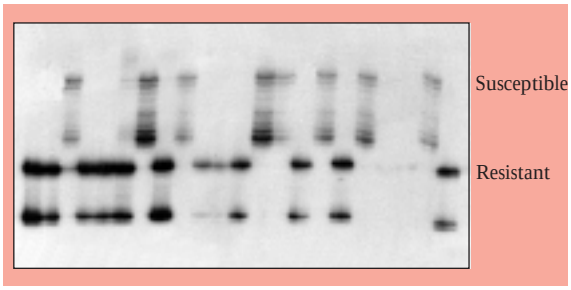


**Figure 5** A co-dominant, PCR-based assay for Barley Yellow Mosaic Virus (BaYMV) and Barley Mild Mosaic Virus (BaMMV).

The microsatellite MAC029 has been shown to be linked (0.9 cM) to *Ym4* in a doubled haploid population (Igri x Franka) segregating for response to BaYMV. Germplasm surveys have confirmed the value of this microsatellite in discriminating between resistant and susceptible genotypes. Furthermore, the allelic differences between the two groups can be resolved on agarose gels (Fig. 5) providing an ideal co-dominant, diagnostic marker for use in barley breeding programmes.

## Gene isolation in barley

The genome of barley is made up of 5.3 x $19^9$ bp. If one assumes that barley possesses 50,000 genes, then on average one would expect one gene every 100 kb. Thus, gene isolation in species such as barley is not trivial. However, recent advances made initially in biomedical science provide fast routes to gene isolation. The approach is simply to sequence the ends of cDNA clones to produce expressed sequence tags (ESTs).

Advantages of this approach include direct access to coding sequences, information on tissue specificity and abundance of different mRNAs in various tissues sampled, and identification of putative function by homology to genes in databases (bacterial, yeast etc). Disadvantages include the cost, the difficulty of identifying low abundance messages and redundancy. In order to evaluate the potential of ESTs in barley, we have initiated a project to isolate 1,000 genes from 2-day malted barley. The strategy being pursued is shown in Figure 6 and, to date, about 70% of the sequences generated exhibit significant homology to known function genes in databases. Approximately 5% of the ESTs also contain short SSRs, providing one potential route to transcript mapping.

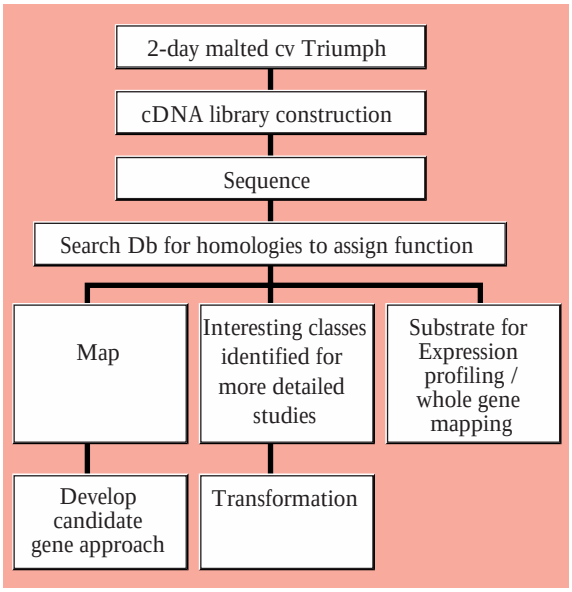


**Figure 6** Strategy for development and exploitation of ESTs.

## Conclusions and future directions

Gene discovery programmes will be less demanding in the future and greater emphasis will need to be placed on understanding the relationship between gene sequence and function. We will therefore need to develop (or have access to) barley transformation and create transcript-based linkage maps. For the latter, the potential of whole genome radiation-hybrid mapping will be explored. These biotechnological programmes will be complemented by large-scale genotyping efforts to expand our microsatellite databases for barley.

## Reference

[1] Graner, A. & Bauer, E. (1993). *Theoretical & Applied Genetics* 86, 689-693.