Molecular markers for agriculturally important traits in barley

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The advent of comprehensive molecular marker maps means that breeders can use DNA sequences directly to select for the presence of particular genetic factors rather than attempting to measure the effect these factors have on the appearance (phenotype) of the character under selection. This technique is called Marker Assisted Selection (MAS). When selection for donor alleles at target loci, where changes are desired, is combined with recipient alleles in the remainder of the genome, problems associated with linkage drag, i.e. the inadvertent selection of unwanted traits, in the introgression of new genes are greatly reduced. This potentially makes great savings in time and cost in a breeding programme, and offers more accurate selection when reliable measurement of phenotype is difficult. The large library of molecular markers available to SCRI is, therefore, a valuable resource in potential MAS applications. Over the past 4-5 years, we have identified markers that can be used to select for a number of key traits in barley.

Barley Yellow Mosaic Virus The Barley Yellow Mosaic Virus complex (BaYMV) is spread by a soil-borne fungus and can cause severe loss in yield and quality in winter barley crops grown in certain parts of the world. Reliable assessment of resistance to the disease can only be done with specialised testing facilities and, even then, is not totally effective. A number of resistant cultivars have been developed in Europe, utilising resistance genes located on the distal portion of the long arm of chromosome 3H. The two main sources are the *rym4* gene derived from Ragusa, which is effective against BaMMV and BaYMV-1 but not BaYMV-2, and the *rym5* gene derived from Mokusekko 3, which is effective against all three races. BaYMV-2 is gradually becoming more prevalent and it is therefore important for breeders to be able to distinguish between the two resistance genes in their selection programmes. A Simple Sequence Repeat (SSR) marker developed by SCRI can not only distinguish between resistant and susceptible genotypes at the locus on 3H but can also distinguish between the two different sources of resistance. This marker is now being used by a number of commercial breeders in their selection programmes and has proved to be very reliable. We have recently identified Single Nucleotide Polymorphisms (SNPs) that can also be

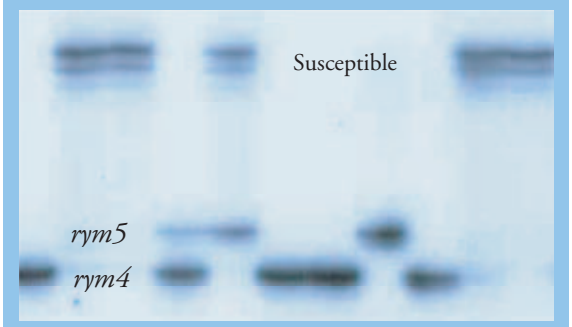
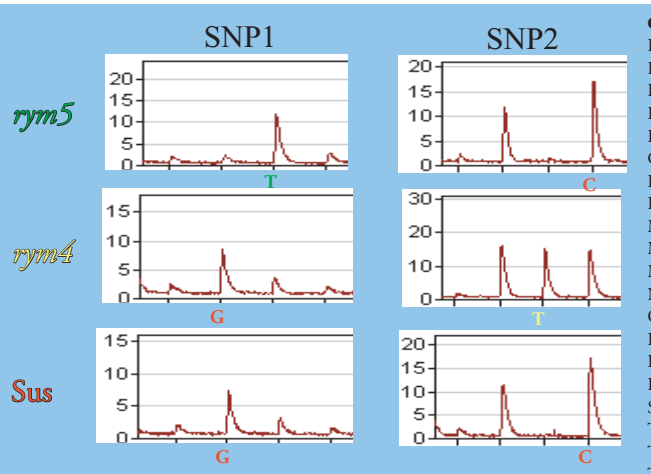


Figure 1 Identification of BaYMV resistance using a molecular marker.



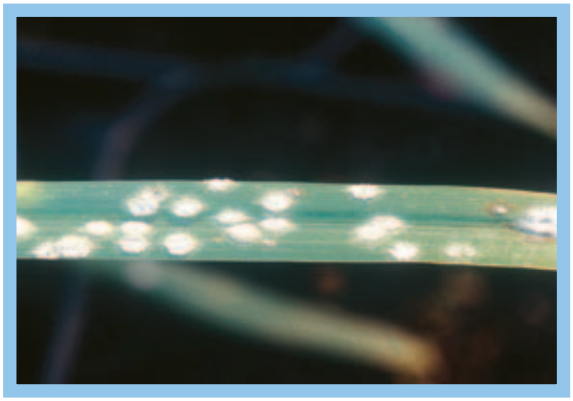
Genotype	SNP1	SNP2	BaYMV
Barke	G	C	Susceptible
Derkado	G	C	Susceptible
Epic	G	T	*rym4*
Firefly	G	T	*rym4*
Franka	G	T	*rym4*
Gaulois	G	T	*rym4*
Haruna Nijo	G	C	Susceptible
Igri	G	C	Susceptible
Mokusekko 3	T	C	*rym5*
Morex	G	C	Susceptible
Muscat	G	T	*rym4*
Nudinka	G	C	Susceptible
Optic	G	C	Susceptible
Plaisant	G	C	Susceptible
Proctor	G	C	Susceptible
Ragusa	G	T	*rym4*
Steptoe	G	C	Susceptible
Tokyo	T	C	*rym5*
Torrent	G	T	*rym4*
Triumph	G	C	Susceptible

Figure 2 Pyrosequencing detection of Single Nucleotide Polymorphisms Identifying Cultivars Resistant or Susceptible to BaYMV.

used to identify the two sources of resistance. Rapid, non-gel based methods, such as Pyrosequencing™ (www.pyrosequencing.com), can easily be applied to detect SNPs. SNPs therefore offer the possibility to screen germplasm on a far greater scale than previous molecular marker systems and have great potential utility in MAS programmes.

Rhynchosporium Rhynchosporium (Leaf Blotch or Scald) is becoming more prevalent in UK spring barley but the resistance of currently recommended cultivars is, with the exception of Pewter, poor. Some sources of resistance are available but a specialised disease nursery is required to select resistant types from amongst the progeny of crosses. Results from the UK Cereal Pathogen Virulence Survey show that the cultivar Digger, which was on the UK Recommended List from 1986 to 1991, has consistently good levels of resistance. The SCRI cultivar, Livet, also had a good level of resistance to scald and inherited a resistance gene from Digger. From studies of a mapping population, we have identified an SSR marker that is closely linked to this resistance gene and have been able to trace the allele back to Osiris and forward to Pewter. We have used this marker to correctly predict the resistance of a number of other cultivars and it therefore can be used in MAS schemes to detect lines carrying a particular gene for resistance to scald.

Powdery Mildew Powdery mildew is the most prevalent disease of spring barley in Europe. Breeders have incorporated the *mlo* resistance gene into a number of cultivars and it has provided effective resistance for over 20years. Selection for resistance in the field is generally effective in most European countries but incidence of the disease can occasionally be low, causing problems in identifying resistant lines. A number of breeders now utilise Doubled Haploidy to develop populations of inbred lines, in which selection is more effective than in products of a pedigree programme. Doubled Haploid plants are initially multiplied in the glasshouse before field assessments but, in crosses between resistant and susceptible lines, half the plants will be susceptible to powdery mildew. Early identifi-



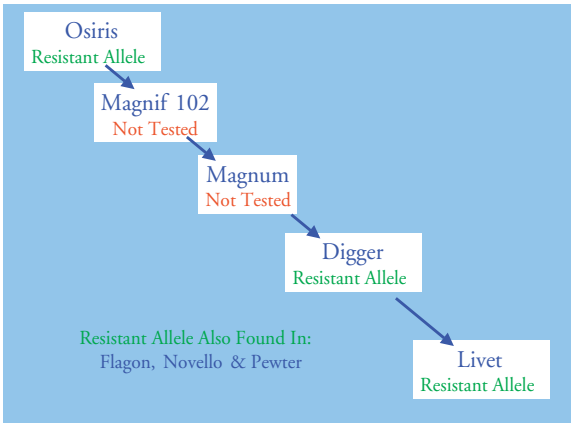


Figure 3 Use of molecular markers to trace source of scald resistance in Livet.

cation of such plants would save time in culturing and glasshouse facilities and is possible through MAS being applied to leaf tissue being sampled at the stage of green plant isolation. A barley SSR marker can identify the *mlo11* allele possessed by cultivars carrying this resistance and is therefore ideal. There are two main sources of *mlo11* resistance and evidence from the UK Cereal Pathogen Virulence Survey suggests that the L100 source, found in the cultivars Apex and Riviera, can show infection levels that were higher than expected. This contrasted with the L92 source, found in Atem and its derivatives. The SCRI marker can distinguish between them and is therefore of additional significance to breeders.

Epiheterodendrin Epiheterodndrin (EPH) is a cyanogenic glucoside produced during germination. Enzyme activity during mashing and fermentation releases a breakdown product that can, under certain conditions, react with ethanol in copper stills to form ethyl carbamate during whisky production. In cases where this is a problem, the industry has utilised cultivars that have inherited an allele at the *eph* locus that appears to block formation of epiheterodendrin. In an HGCA and LINK funded project, SCRI identified SSRs that are linked to *eph* and can be used to select the non-producers. Whilst the linkage is close, a number of recombinants have been found, so the markers may not be diagnostic in all cases. They can still be used in MAS programmes, however, provided one knows the parental phenotypes, i.e. can identify one of them as a non-producer of EPH, and can also distinguish the parents genotypically by using the SSRs. In the longer term, we plan to identify the gene and develop a direct gene marker that will be diagnostic for non-producers. This could be adopted by plant breeders to ensure that there were sufficient cultivars that did not produce EPH to meet all the requirements of the distilling industry.

Further Developments As we acquire more knowledge about the barley genome, we will be able to identify more markers of value in selecting for economically important characters, particularly those relevant to stable yield and quality. Genomics initiatives such as the BBSRC Investigating Gene Function are expected to yield large amounts of potentially valuable information. The challenge is to connect such data to biologically relevant questions and then deploy it in a targeted manner to improve the end-user value of barley.

For further information on the above markers, contact: Jonathan Snape at Mylnefield Research Services, Invergowrie, Dundee DD2 5DA, UK.

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