

Improving Breeding Efficiency in Blackcurrant - the Application of New Selection Techniques in *Ribes*

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Abstract

The deployment of marker-assisted breeding strategies in blackcurrant has started with the use of a PCR-based marker for gall mite resistance. This is now a routine part of the selection process within the breeding programmes at the James Hutton Institute, and further work is in progress to develop similar approaches for other more complex traits. Whilst the need for accurate field- and glasshouse-based phenotyping has never been more vital, the integration of enhanced phenotyping with the development of significant genomics resources offers an important opportunity for the improvement of breeding efficiency in blackcurrant.

INTRODUCTION

The breeding of new cultivars of woody fruit crop species is inevitably a lengthy process, and as running costs increase, especially in terms of field and glasshouse expenditure, there are clear advantages in the development of new selection procedures and more targeted breeding systems for woody crops. A key area of development in berry fruits has been the use of molecular markers for the selection of key traits, particularly for traits that either cannot be evaluated until the plant is mature, eg. fruit quality-related characteristics, or traits that require complex phenotyping procedures, eg. for some pest and disease resistances.

Blackcurrant has been used at the James Hutton Institute (JHI) as a model outbreeding species in a minor crop, where significant investment in marker and linkage map development is now leading to considerable advances in breeding efficiency as marker-assisted selection strategies are employed.

GALL MITE RESISTANCE IN BLACKCURRANT

Gall mite (*Cecidophyopsis ribis* Westw.) remains the most serious pest of blackcurrant (*Ribes nigrum* L.) in Europe, and measures to control *C. ribis* in Europe are now restricted to sulphur sprays, with previously available chemical agents now withdrawn on environmental grounds. The effective application of sulphur requires careful timing, and additionally there are reported toxicity effects on some modern commercial cultivars, such as 'Ben Gairn'. As a result, breeding for resistance to *C. ribis* is a major objective in most blackcurrant breeding programmes (Brennan, 2008), and the programme at the James Hutton Institute (JHI) has focused on the *Ce* resistance gene from gooseberry (*Ribes grossularia* L.) (Knight et al., 1974). Following an extensive backcrossing programme to BC₇ and beyond at the JHI, there are now resistant diploid hybrids with fruit of commercial processing quality in advanced trials. However, the identification of resistant segregants within breeding progenies has significantly delayed

the commercialisation of resistant cultivars, as previously the only available means for phenotyping was through the use of infestation plots containing infector rows of galled bushes and replicated plots of test germplasm planted between them, taking approximately 4 years to produce reliable results. A rapid high throughput screening method was therefore required.

Using the reference 9328 blackcurrant mapping population, from a cross between the mite-susceptible line SCRI S36/1/100 and the resistant EMR B1834-120, a bulk segregant analysis identified AFLP markers linked to gall mite resistance, using a field infestation plot at the JHI (Brennan et al., 2009). From this, one of the AFLPs was converted to a PCR-based marker based on sequence-specific primers which amplified only in resistant individuals. The marker was validated across a range of blackcurrant germplasm with varying genetic backgrounds and resistance status, and was confirmed as a reliable means of identifying resistant germplasm within the breeding progenies at JHI. The PCR-based marker now provides a high-throughput screening system for gall mite resistance, and has been adopted as an integral part of the JHI blackcurrant selection process. One resistant cultivar, 'Ben Finlay', has already been released to commerce, and further releases will be available in the near future as the number of resistant lines in trials increases.

Dormancy-Related Gene Expression

Despite recent fluctuations in the levels of winter chilling experienced in the UK, there is a clear trend towards warmer winters and also changes in the rates of development of blackcurrant, particularly flowering traits (Jones and Brennan, 2009). Blackcurrant is particularly adversely affected by mild winter temperatures, which can lead to insufficient winter chill and poor bud development in the subsequent year in older 'high-chill' cultivars such as 'Ben Lomond'. As warmer winters are likely to become increasingly prevalent, there is a risk of insufficient chilling becoming more widespread in cultivars requiring high levels of winter chilling, so breeding strategies using low-chill parental material for the development of environmentally-resilient cultivars are now required (Snelling and Langford, 2002). To achieve this, breeders require information regarding the genetic control of chilling-dependent dormancy traits and also accurate and high-throughput phenotyping protocols for the identification of the varying levels of chill requirement in the available germplasm (Jones et al., 2011)

Genetic analysis of Quantitative Trait Loci (QTL) and genes associated with dormancy-related processes were examined by Brennan et al. (2008) and Hedley et al. (2010). Putative QTL linked to key developmental traits, including time of budbreak, were identified on the blackcurrant linkage map by Brennan et al. (2008), and further work looked to identify some of the key genes involved in budbreak. This will enable diversity within the *Ribes* germplasm to be assessed, across the considerable variation in chilling requirement within the genetic base for blackcurrant (Lantin, 1973; Rose and Cameron, 2009), and eventually markers linked to the genes and QTL controlling budbreak will be used to identify useful new genotypes within the breeding programmes.

Patterns of gene expression in blackcurrant leaf buds and key differential changes in these profiles around budbreak were examined using microarrays developed from sequential samples of blackcurrant buds throughout the winter/spring period (Hedley et al., 2010). From this, candidate genes associated with budbreak were mapped onto a blackcurrant genetic linkage map. Three genes, encoding for a calmodulin-binding protein, β -tubulin and acetyl CoA-carboxylase respectively, were found to co-localise with budbreak QTL previously identified on the blackcurrant genetic linkage map by Brennan et al. (2008), and work is now ongoing to identify and validate markers linked to these genes, that can be deployed in the characterisation of diverse blackcurrant germplasm.

The current unpredictability of weather events in most areas of Northern Europe means that a range of blackcurrant cultivars with varying chilling requirements will be desirable for most growing regions. Spring frost damage at flowering has been observed

on blackcurrants in many northern areas, notably Scandinavia, exacerbated by early budbreak, so this must be considered by breeders for the future.

Next-Generation Sequencing (NGS) in Blackcurrant

With the recent advances in sequencing technologies, markers can now be generated on an unprecedented scale. The use of NGS technologies for marker development is a highly cost-effective means of identifying SNP and SSR markers in minor crop species with unsequenced genomes, both in terms of the numbers of markers identified and in their biological informativeness (Parchman et al., 2010; Russell et al., 2011). This approach was used in blackcurrant to generate over 7000 novel SNP and 3000 SSR markers from which a high-density genetic linkage map was constructed (Russell et al., 2011). The map, which represents a considerable advance from the initial blackcurrant linkage map (Brennan et al., 2008), is now being used to identify genes responsible for important traits in blackcurrant, and putative QTL for some quality and processing traits have been located on the map, building on previous work on mapping of quality traits based on the original blackcurrant linkage map (Brennan et al., 2008; Hackett et al., 2010).

Using informatics analysis of the NGS data has been used to select the most robust and informative SNPs to design a multiplex high-throughput 384-SNP detection assay based on the Illumina BeadXpress platform, and this is currently being used to examine polymorphism in *Ribes* germplasm, thereby enhancing the development of marker-assisted breeding strategies.

Future Prospects

The use of marker-assisted selection for gall mite resistance in blackcurrant represents a major advance in breeding efficiency, whereby 4 years of field screening for a limited range of genotypes is now replaced by a few weeks' laboratory-based screening of whole progenies. Gall mite resistance is controlled by a single gene, and many of the remaining traits of commercial interest in blackcurrant, based around fruit quality and developmental characters, are controlled by several or many genes. Ongoing work in this area at the James Hutton Institute is centred on the new SNP-based linkage map for blackcurrant, together with ongoing work at JHI to design arrays based on fruit quality development. The ultimate aim is to deploy marker-assisted selection of segregating seedlings at an early stage, based on markers for all of the main traits and aligned with the commercial demands of the processing and other industry sectors. Through the use of markers accurately linked to desirable phenotypes, the numbers of plants in the field can be reduced by removal of those with completely unacceptable genotypes, thereby reducing programme costs and increasing breeding efficiency.

The blackcurrant work at JHI is an example of significant genomic resources developed for a minor crop, and deployed to provide clear benefits to both breeders and end-users. However, there is a vital need for improved phenotyping methods in order to effectively connect genotypes assessed using the methods outlined above with the phenotypes that provide added value to the blackcurrant industry worldwide.

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