Blackcurrant breeding and genetics

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Introduction Blackcurrant (*Ribes nigrum* L.) is the most widely grown bush fruit in Europe, and is comparatively recent in its domestication from wild accessions.

The blackcurrant breeding programme at SCRI was established in the 1950s, with a remit to provide cultivars suitable for cultivation in northern parts of the UK. Initially, the work covered only the establishment in 1952 of variety trials for both black- and redcurrants, under the aegis of Malcolm Anderson, but results from the trials showed scope for considerable improvement within the existing genetic base, and breeding of blackcurrants was inaugurated in 1956.

The initial objectives of the programme were to produce improved germplasm with cold tolerance, especially in the spring, even and earlier ripening and fungal disease resistance. Initial hybridisations were made between established UK cultivars and cultivars from Canada, Scandinavia and northern Europe, together with a small interspecific programme, and the first commercialised product of the continuing breeding effort, 'Ben Lomond', was released in 1972.

The Industry The blackcurrant crop in the UK currently occupies ca. 1.4 kha. Most is grown on contract to SmithKline Beecham plc (SB) for the production of Ribena™. In the lifetime of the breeding programme at SCRI, the scale of production has changed with an overall increase in farm size and complete mechanisation of all processes involved, including harvesting.

Since 1990, the breeding programme has been entirely funded by the SB Growers' R&D Fund, to produce cultivars meeting their processing and agronomic specifications.

Germplasm The *Ribes* genus consists of ca. 150 species, usually classified into 6 subgenera, distributed mainly in temperate areas of Europe and North America, although species are also found in South America and North Africa. All species are diploid, and an extensive species collection is held at the Institute, with new accessions added on a regular basis. Several species have been used to develop breeding strategies, such as the use of *Ribes dikuschka* and its derivatives for introgression of resistance to Blackcurrant Reversion-Associated Virus (BRAV)[1].

Breeding of new commercially useful cultivars is based on recurrent selection from seedling populations at SCRI, with initial selections in the first (non-fruiting) year based on vegetative characters, followed by selection on fruiting characters and longer term agronomic traits. Trialling of the most promising seedlings is carried out at sites in Norfolk and Gloucestershire (Fig. 2) where commercial large-scale fruit assessments are made. Recommendations for release are made after 3 years' trialling.

Breeding objectives FROST TOLERANCE The objectives of the programme were initially to improve the frost tolerance of the available cultivars and thereby to produce consistency of cropping. This has been done

Figure 1 Blackcurrant hybridisations.

Figure 2 Commercial blackcurrant trials in the West Midlands.

by introducing a late-flowering character into many of the SCRI cultivars, reaching its highest expression in Ben Tirran, released in 1990. Beyond this point, however, further delays in flowering are likely to compromise the eventual yield, and therefore genuine physiological tolerance of freezing temperatures is sought. Further introgression of germplasm from northern latitudes is in progress within the breeding programme to provide the required tolerance. The physiological basis of genotypic differences in frost tolerance, relating to rates of ice movement through tissues, is under investigation in collaboration with J. Carter of the University of Minnesota, and work to examine genotypic differences in the differential expression of cold-induced genes during acclimation in the autumn is in progress, using cDNA-AFLPs.

FRUIT QUALITY Of the various fruit quality components in *Ribes*, sensory attributes have hitherto been neglected in breeding terms, due to the complex nature of their inheritance and the uncertain origin of variation between genotypes. Recent collaborative work with the Hannah Research Institute and BioSS has demonstrated the range of genotypic variation in sensory characters, notably appearance, aroma, flavour, aftertaste and mouthfeel, in juices made from individual genotypes[2]. Flavour appeared to be the most important sensory attribute, and initial studies have suggested that some flavour characteristics are dominant in their inheritance. Overall, however, sensory attributes show highly complex patterns of inheritance and further work is in progress to examine this so that specific breeding strategies can be used.

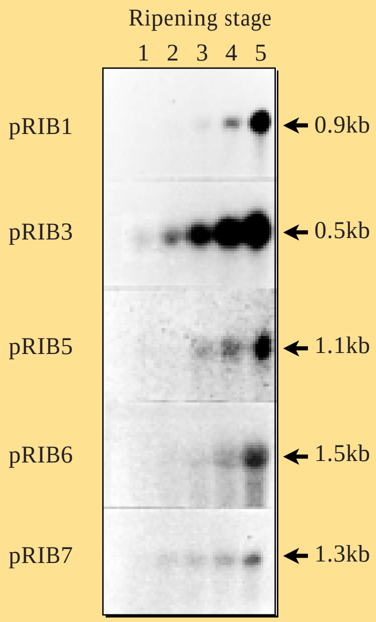

Figure 3 RNA blot analysis of pRIB genes from blackcurrant fruit at five ripening stages (1= green, 2 = green/red, 3 = red/green, 4 = red, 5 = black), showing increasing expression levels as ripening progresses.

Work with M. Woodhead and M. Taylor (CEP Dept.) showed that blackcurrant is a non-climacteric fruit in its ripening. Qualitative and quantitative changes in mRNA populations were found as ripening progressed, leading to the isolation of cDNA clones of five genes showing enhanced steady-state transcription levels in fully ripe fruit[3] (Fig. 3). Genomic clones of some of these genes have been isolated, and two of the genes (RIB1 and RIB7) showed highly fruit-specific expression. The promotors driving the expression of these two genes may therefore be of considerable value in the future manipulation of ripening processes in transgenic fruit.

Ascorbic acid levels represent a major part of the appeal of blackcurrant to consumers and the processing industry. Levels of ascorbate production in blackcurrant fruit are highly variable: most commercial UK cultivars such as Ben Alder contain typically 120 mg/100 ml juice, whereas most Scandinavian cultivars

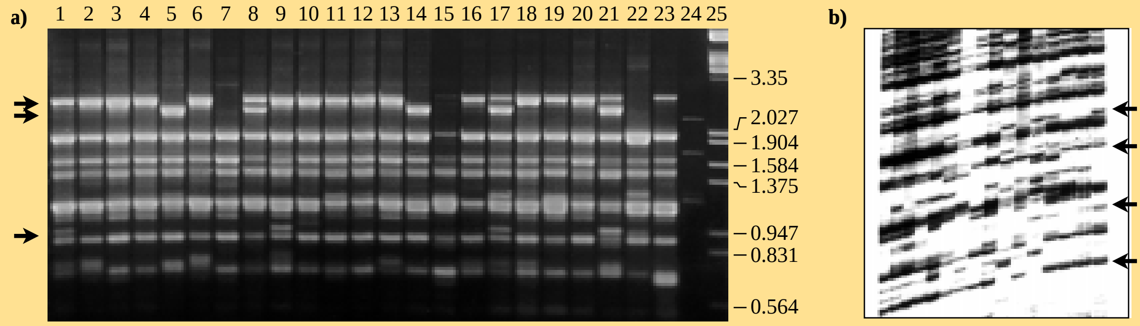

Figure 4 Molecular markers detected in *Ribes* germplasm
a) RAPD markers in *R. nigrum* germplasm b) Anchored microsatellite markers in *Ribes* subgen. *Ribesia* germplasm, DNA polymorphisms are indicated by arrows.

are barely half that value. However, breeding lines within the SCRI programme containing up to 400 mg/100 ml have been identified, and are being used in combination with appropriate parents. Additionally, work to manipulate the biosynthetic pathways for ascorbate within fruit is in progress.

Recently, interest has been expressed by researchers and commerce in the role of antioxidants in a nutritional context. Blackcurrant is high in ascorbic acid, as already mentioned, but it also has a high concentration of other antioxidants, such as anthocyanins. Whilst breeding for high juice colour has long been an objective of the *Ribes* breeding programme, more recent information suggests that (i) the anthocyanins present in blackcurrant (mainly delphinidin- and cyanidin-3-glucosides and -rutinosides) have antioxidant capacities in their own right[4] and (ii) the anthocyanin content of blackcurrant is exceeded only by some genotypes of blueberry (*Vaccinium* spp.).

It remains a matter of concern that little information on the inheritance of fruit quality components or on the genotype-environment interactions affecting fruit quality is currently available for any of the berry fruits.

PEST RESISTANCE The development of new cultivars with resistance to gall mite (*Cecidophyopsis ribis*), and to BRAV which is transmitted by the mite, remains a high priority within the programme. Uncertainty regarding the future use of acaricides on toxicological and environmental grounds could potentially leave the industry without effective means of controlling this damaging pest. Resistance breeding for *C. ribis* is still based mainly on the dominant *Ce* gene from *Ribes grossularia*, and commercially acceptable types are now in commercial trials. Further screening of *Ribes* species to identify other sources of resistance is in progress, although collaborative work with the Agricultural Research Centre, Jokioinen, Finland has demonstrated that *Ce* is effective against other *Cecidophyopsis* mites, as well as Finnish strains of *C. ribis*.

The identification of BRAV as the causal agent of blackcurrant reversion has made the development of a rapid detection assay possible[5]; this will then enable the mode of resistance to be accurately assessed by screening of graft-inoculated progenies segregating for resistance. Initial work produced unclear results, and the use of rapid assays will enable further work to be done and the range of BRAV-resistant cultivars available to the industry to be increased.

Marker-assisted selection The development of molecular markers affords considerable opportunities for breeders, particularly those working with highly heterozygous perennial crops, to both characterise the available germplasm at the DNA level, and to indirectly select for agronomic traits faster and more accurately than otherwise possible. In the specific context of the *Ribes* breeding programme, we have so far characterised the germplasm using RAPDs in the *Eucoreosma* subgenus[6] (Fig. 4a). AFLPs and ISSRs (anchored microsatellites) were used for the *Grossularia* and *Ribesia* subgenera[7] (Fig 4b). Work to develop AFLP markers for the gall mite resistance gene *Ce* is also well-advanced, with test progenies in field infestation plots providing the required susceptible and resistant bulks from which the analysis can be made. The use of a molecular marker for mite resistance will obviate the present 3-year screening of mature plants to identify resistant genotypes, and enable screening of seedlings to be carried out prior to planting.

Further use of markers to map important multigenic traits, such as fruit quality characteristics and freezing tolerance, as well as resistance genes including those controlling resistance to BRAV, is also under investigation using mapping populations established in the field.

Transgenic blackcurrants Protocols for the *Agrobacterium*-mediated transformation of blackcurrant have been optimised in collaboration with S. Millam (Crop Genetics Dept.), and plants transformed with a range of genes controlling various aspects of fruit quality are currently being assessed.

Cultivars The first cultivars from the SB-funded breeding programme are now in commerce; Ben Hope and Ben Gairn, both released in 1997, provide alternative strategies for pest and disease management, since Ben Hope demonstrates a high degree of resistance to gall mite, while Ben Gairn is resistant to BRAV. Another three, presently un-named, seedlings have been approved for propagation and release by SB, and a further 20 seedlings are currently in commercial trials with SB, including several gall mite-resistant genotypes.

Future advances Blackcurrant breeding at SCRI is strongly and uniquely placed to make large advances in the future, as the conventional breeding is assisted by a range of enabling technologies.

It is envisaged that there will be further releases of gall mite-resistant cultivars, in order to provide the industry with a firm basis for successful integrated pest management in the future. Also, further BRAV-resistant seedlings will be developed as the means of identification of resistant segregants becomes easier. Marker-assisted selection is being developed to identify desirable genotypes within the programme at a much earlier stage, particularly for characters that have hitherto required long selection periods. Also, there is likely to be increased use in the future of interspecific hybridisation to provide genes controlling desirable resistance and quality characters.

The use of molecular studies relating to fruit ripening and increased quality makes large improvements in fruit quality components, and hence commercial desirability, possible, especially through the use of transgenic methods.

Acknowledements

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