The development of molecular markers for use in a *Ribes* breeding programme

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Introduction

The breeding of commercial cultivars of *Ribes nigrum* has relied to date on conventional breeding methods, notably recurrent selection of elite phenotypes and backcrossing programmes. However, the potential offered by the use of molecular markers linked to traits of interest in the available *Ribes* germplasm provides a firm basis for an increase in breeding efficiency and targeting through earlier selection of desirable genetic combinations.

Initial work has developed ISSR and AFLP protocols for *Ribes* (Lanham & Brennan, 1998; 1999), and AFLP markers linked to pest resistance genes have been identified. However, present work is aimed at the development of microsatellite (SSR) markers as a more informative and ultimately useful system for *Ribes*.

AFLP markers linked to gall mite resistance

Gall mite *(Cecidophyopsis ribis)* is most serious pest of blackcurrant, and breeding for resistance is one of the main objectives of the SCRI breeding programme (Fig. 1)

A single dominant gene (*Ce*) for resistance has been introgressed from *Ribes grossularia* cv. Green Ocean, followed by extensive backcrossing programme

The resistant cv. Ben Hope was released in 1997 (Fig. 2) and has been widely planted commercially

Screening of *R. nigrum* seedling populations segregating for resistance relies on field infestation plots, which are expensive and not entirely reliable (Fig. 3)

AFLP analysis was carried out on a population of *R. nigrum* segregating for gall mite resistance, and also with the parental genotypes of this and a further 3 populations, plus the original resistance source (*R. grossularia* cv. Green Ocean). In the latter analysis, 5 AFLP bands were found that were present in the resistant parents but absent from the susceptible parents (Fig. 4). Results were compared to data from a field infestation plot after 3 years' screening, and 2 of these AFLP bands showed linkage to the *Ce* locus at 11.8 and 19.1 cM respectively (Table 1).

These markers, particularly E1:M7-1, have great potential utility in marker-assisted selection of gall mite-resistant blackcurrant hybrids within the SCRI/Glaxo SmithKline *Ribes* breeding programme, and deployment strategies are under development. Further work to create single-locus markers from these AFLP markers is currently in progress.

Fig. 1 SEM of *C. ribis* within infested bud of blackcurrant

Fig. 2 Cv . Ben Hope (mite-resistant)

Fig. 3 Field infestation plot, showing test progenies between infestor rows

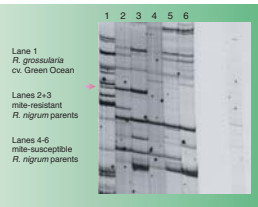


Fig. 4 AFLP marker linked to gall mite resistance controlled by gene *Ce*.

Table 1
Recombination frequencies, θ, standard error of θ , s.e., associated LOD scores and map distance of AFLP markers linked to the *Ce* gene

Paired loci	θ (s.e.)	LOD	Map distance cM
Ce - E1:M7-1	0.1261 (± 0.059)	14.5062	11.8
Ce - E6-M2-1	0.1371 (± 0.061)	13.7724	19.1

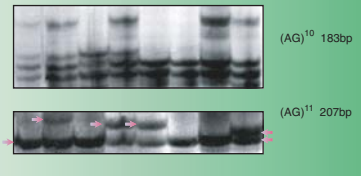
SSR markers

Emphasis within the *Ribes* research programme at SCRI has moved to SSRs, since the latter are highly polymorphic, multi-allelic and codominant, and hence more informative for use within a marker-assisted selection strategy for *Ribes* breeding.

The SSRs developed so far are currently being tested across a range of *Ribes* germplasm encompassing all major subgroups within the genus (Fig. 5). Further studies to find SSRs linked to various traits are planned; these traits include single-gene disease/pest resistances and also QTLs for fruit quality components.

Enriched genomic libraries were developed from the blackcurrant cv. Ben Alder using the method of Hale *et al.* (2001), and enrichment of over 60% was achieved. From the libraries ca. 300 clones were selected so far and sequenced. A number of microsatellites were identified (Table 2), with various repeat sequences including di-, tri- and multiple nucleotide sequences.

Fig. 5 Examples of SSRs showing polymorphism across arange of *Ribes nigrum* germplasm



Further screening across germplasm from other *Ribes* subgenera is currently in progress to test the wider utility of these markers

Table 2
Repeat sequences and expected product size for a range of *Ribes* SSR loci

Locus	Repeat type	Expected product size(bp)
AO1	$(AG)_{11}$	207
AO4	$(CAC)_4(CAG)_4$	174
BO1	$(GA)_{24}(GA)_6$	191
BO2	$(GA)_7$	176
DO3	$(CTT)_4(GA)_9$	208
DO5	$(AG)_{16}{}^*(GA)_{18}$	284
EO5	$(AG)_{12}$	198
FO1	$(GGC)_4(GA)_{25}$	275
FO2	$(AG)_{10}$	292
GO6	$(AG)_8(TCG)_4$	231
HO3	$(ATC)_{11}(CTT)_5$	135
JO3	$(AG)_5(AC)_7(GA)_5(ATC)_4$	297
KO4	$(AG)_{12}(TTCA)_3(AG)_6$	291
LO4	$(GA)_{13}(ACCTC)_3(CAC)_4$	274
MO1	$(TCA)_5(CAT)_{18}$	207
MO2	$(AG)_8$	183
MO3	$(CA)_{13}$	243
MO5	$(AG)_{17}(AC)_8$	153
MO6	$(GTC)_4(GA)_9$	208
NO1	$(GA)_{14}$	136
NO4	$(GA)_{16}$	117
NO5	$(GA)_{16}$	123
OO2	$(AG)_{13}$	133
O04	$(CAT)_4(TCT)_4(CAT)_5(CAT)_5$	275
P01	$(GA)_{13}(AG)_{14}{}^*$	245
P03	$(AG)_{21}$	298
P04	$(CCA)_4$	150
P05	$(ATTTC)_2(GA)_{24}$	278

References

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