SSR development In *Ribes* and *Rubus*

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Introduction

Rubus and *Ribes* represent two of the most important temperate small fruit genera, containing crop species such as red raspberry (*Rubus idaeus* L.) (Fig 1) and blackcurrant (*Ribes nigrum* L.) (Fig. 2). Breeding programmes for these species have hitherto relied 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 germplasm, and also in the assessment of diversity in the available germplasm, especially wild accessions, is now beginning to be exploited at SCRI. The focus of this effort is in the development of SSR markers, which have clear advantages in identifying polymorphism in outbreeding diploid species such as *Ribes* and *Rubus;* although other markers have been developed in these species, eg. AFLPs (Brennan, 2002a*)* the combination of high discriminatory power, reproducibility, ease of genotyping and codominant nature now make SSRs the marker system of choice (White and Powell, 1997).

Figure 1

Ribes

Figure 2

The genus *Ribes* consists of *ca*. 160 species of highly heterozygous diploid woody shrubs, distributed throughout northern temperate regions of Europe and North America, with species also reported from Asia, South America and northwest Africa (Brennan, 1996). Blackcurrant (*Ribes nigrum* L.) is the most important species in commercial terms.

SSRs were identified using a modification of the membrane enrichment method described by Edwards *et al*. (1998) and Hale *et al*. (2001). Following library enrichment, 60.4% of the clones sequenced contained microsatellites. Primers were designed to 56 clones, and of these 11 are presented in Table 1 (Brennan *et al*., 2002b). The most common motif represented within the 11 SSRs is $(GA)_n$, although more complex SSRs were also found (Table 1). Searches of non-redundant nucleotide databases showed that ten sequences were similar to known sequences (Table 1). Only 2 of the primer pairs amplified in all *Ribes* germplasm tested, whereas eleven primer pairs amplified in germplasm representing cultivated blackcurrant cultivars from various environments including the UK, Scandinavia, France and Russia.

Allele number varied from 2 (RJL-8) to 18 (RJL-5), with an average of 9.1 per microsatellite. Similarly, the level of diversity ranged from 0.18 to 0.91 in the most complex motif.

Examples of SSRs showing polymorphism across arange of *Ribes nigrum* germplasm

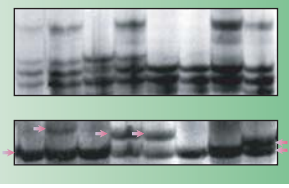

Figure 3

Table 1

Microsatellite locus	Repeat motif	Expected product size (bp)	No. of alleles	Ho	Sequence homology (none if blank)	Amplification R. nigrum (20cvs)	R. nigrum (2cvs)	Redcurrant (1cv)	Landscape (2sp.)
RJL-1	(TCA)8	167	3	0.246	ACO22517 Human chr. 19 (100%)	+	-	+	+
RJL-2	(GA)11	207	16	0.63		+	-	+	+
RJL-3	(GA)14	136	6	0.749	APO02448 Human chr. 11 genomic DNA (96%)	+	-	+	+
RJL-4	(AGA)4	198	4	0.184	(AJ300524-2 *Populus euramericana* dehydrin (96%)	+	-	+	+/-
RJL-5	(ACTTC)2(GA)24	278	18	0.908	ACO84621-1 *Caenorhabditis* cosmid G44014 (100%)	+	-	+	+
RJL-6	(GA)12(TTCA)3(GA)6	291	13	0.646	AL160413 Human DNA, chr. 20 (100%)	+	-	+	+
RJL-7	(GA)17	200	16	0.894	BF631247 *Hordeum* cDNA relating to drought stress (100%)	+	-	+	+
RJL-8	(GA)16	193	2	0.511	AF274665. *Ribes aureum* 26S ribosomal gene (99%)	+	-	-	+/-
RJL-9	(GA)12	205	3	0.575	BG598711 *Solanum tuberosum* cDNA (96%)	+	-	+	+
RJL-10	(GA)15	202	9	0.754	BF023784 *Onchocerca* larval cDNA (96%)	+	-	+	+/-
RJL-11	(GA)11	215	10	0.694	BAB20761. UV-damaged DNA binding protein, Oryza (95%)	+	-	+	+

\+ = Single amplification product
\- = No amplification product
+/- = Difference in amplification between genotypes

Rubus

Commercial production of red raspberry (*Rubus idaeus*) in Scotland is concentrated in the Tayside region, accounting for 80% of the Scottish total. Modern cultivars, including those bred at SCRI, exhibit high levels of genetic similarity (Graham & McNicol 1995). In addition, wild raspberry populations occur in clumps separated by distances of 100 m to 10 km depending on the extent to which land usage has removed suitable habitats. A number of these populations exist within and at increasing distances from the main areas of raspberry cultivation.

The development of SSRs in *Rubus* is focused on the creation of a genetic linkage map, together with the identification of SSRs linked to important traits for deployment by breeders. The interaction of wild and cultivated raspberry is also under investigation (Graham *et al*., 2002).

Microsatellite loci were isolated by screening a *Pst*I size selected genomic library with $AC_{(13)}$ and $AG_{(13)}$. Positive clones were sequenced and primer pairs designed to the sequences flanking identified SSRs. One primer of each pair was fluorescently labelled to facilitate PCR product identification on an automated DNA sequencer. The SSR primer pairs (Fig 4) are now being used to more accurately define the levels of gene flow within and between wild and cultivated raspberries, following earlier studies using RAPDs (Graham *et al*., 1997). SSR markers are also being developed in a red raspberry progeny segregating for resistance to raspberry root rot, caused by *Phytophthora fragariae* var. *rubi*, in order to produce appropriate marker-assisted breeding strategies.

Different alleles from wild raspberry populations

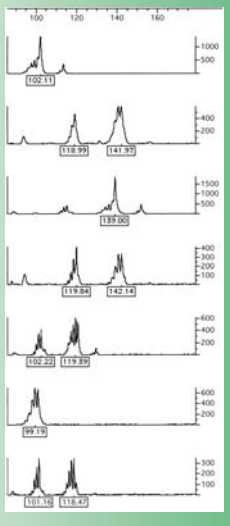

Figure 4

Conclusion

The microsatellites shown here provide a starting point for the production of genetic linkage maps of *Ribes* and *Rubus* (currently in progress), and it is also intended that they will be utilised across a range of conservation, genetic and breeding issues.

Acknowledgements

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