

# **STSM Scientific Report**

## **Studies of the molecular methods for diversity studies and marker assisted selection of *Ribes* germplasm**

REFERENCE: Short Term Scientific Mission, COST 863

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Host: **Rex M. Brennan, Scottish Crop Research Institute**

Period: from **14/01/2008** to **31/01/2008** Place: **Scottish Crop Research Institute, Dundee, UK**

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## PURPOSE OF THE VISIT

Purpose of the visit was to gain familiarity with a range of basic genomic techniques, which can be used in *Ribes* germplasm research, including:

- DNA extraction, storage, gel preparation and visualisation.
- The application of a range of SSR markers across a wide selection of *Ribes* germplasm, including representatives of cultivated black- and red-currants, gooseberries and species accessions from the SCRI germplasm collection.
- Using a putative AFLP marker linked to the *Ce* gene conferring resistance to blackcurrant gall mite (*Cecidophyopsis ribis* Westw.), screening of blackcurrant segregants to identify resistant types.
- Linking molecular data to phenotypic information for breeding populations in the SCRI blackcurrant breeding programmes.

## DESCRIPTION OF THE WORK CARRIED OUT DURING THE VISIT

According to the purposes of the visit, the following work was carried out:

1. Check of 8 SCRI breeding lines for gene *Ce*, conferring resistance to blackcurrant gall mite. DNA was extracted using DNease Plant Mini Kit – Mini Protocol. Samples were ground in liquid nitrogen. Then samples were checked on 1.5% agarose gel to be sure of successful DNA extraction. Then diluted (1:10) sample were prepared for PCR and gel electrophoresis, using 2 markers: ERB0102N10 (SSR) as control, which have to amplify on every sample; and GMRes a (AFLP) for *Ce* gene detection.
2. Diversity study of *Ribes* by Fluorescent SSR PCR analysis. 96 accessions of *Ribes* plants, including cultivated black- and red-currants, gooseberries and species, were tested. During my visit, 7 SSR markers (derived at SCRI from genomic DNA of mapping population, and EST (RNA) libraries of blackcurrant leaves and buds) were applied to this material. Analysis included PCR, checks for successful amplification on gel electrophoresis, and amplified locus length measurement by sequencer, done by sequencing service at SCRI.
3. Data analysis of the tested loci (markers). Software used:
  - GeneMapper – for reading and scoring of genotyping (sequencing) results. Results (obtained numbers) then stored in MS Excel. For further data analysis were one-by-one used several free-ware programs
  - GenAlex (free Ad-in for MS Excel) for calculation of Frequency distributions and PCA
  - MSTools (free Ad-in for Ms Excel) for calculations of expected and observed diversity, frequencies etc. Produces table (MSat Ind) for following analysis in MSAT2.
  - MSAT2 (freeware) produces similarity matrix for further production in Phylip3.67
  - Phylip3.67 (freeware) / neighbour produce data for different dendrograms.
  - Dendrograms can be viewed in TreeView X (freeware).
4. Meetings with several scientists of SCRI to get acquainted with specific scientific areas:
  - Genotyping and sequencing

- Microarrays
- Integrated pest and disease management in *Ribes* and *Rubus*.
- Quarantine and healthy nuclear stock collections maintenance for breeding and commercial propagation.

## DESCRIPTION OF THE MAIN RESULTS OBTAINED

The main results are that I can start to run and explain these analyses by myself. And I got quite general information about other work, connected with soft fruit in SCRI, which can be useful in planning further experiments and collaboration.

Following will be the main results from experiments, which were done by me.

1. Black currant gall mite resistance test (gene *Ce* from *R. grossularia*). Two breeding lines were resistant (had the gene), namely 9821-5 and 9873-10. Two more were unclear, so the analysis needs to be repeated with more DNA sample.

2. *Ribes* diversity study by fluorescent SSR PCR analysis. Total study is targeted to test if some of about 35 of the developed black currant markers can be transferable also in the other *Ribes* species diversity studies. As markers were derived from the related black currant mapping population, derived from two parents, they worked well (was amplified and polymorphic) in blackcurrants, though with 3 exclusions (for example, only one marker amplified in Ben More), which were excluded from the further analysis. For the rest 41, these 7 markers were polymorphic (Table 1 & 2) and separated the test population very well. In total, 2 – 9 alleles were found, with expected heterozygosity (Hz) levels quite high (0.451 – 0.736), which proves their polymorphism. However, the considerably reduced observed Hz for erb3-J14b means that this marker does not separate alleles quite well, because it is expected that blackcurrants, being an outbreeder, is highly heterozygous.

Some of the tested markers (see Table 1) can be useful for diversity analysis of the other *Ribes* species as well, though their amplification and polymorphism was, as expected, was not as good as in blackcurrants. Therefore in further complete diversity analysis only those accessions were included, which had amplification with at least 6 markers; in total 61 accessions of 41 cultivated blackcurrants, 1 gooseberry, 7 breeding lines of blackcurrant, 1 red currant, and 11 wild species. The analysis segregated the test population well, with clear differences both among single accessions of same group (blackcurrant) and between groups (Figure 1).

Conclusions. Though this assay is still in progress and I analysed only 7 markers out of more than 35, we can conclude that developed markers will be useful of genetic diversity analysis of blackcurrants. Cross-species transferability is still questionable, but some markers show good amplification and polymorphism and can be possibly useful for diversity analysis of other *Ribes* species.

Table 1. Used SSR marker description

No.	SSR name (abr.)	Sequence	Linkage Group	Observed size range	Motif	Polymorphism			
						Blackcurrants	Redcurrants	Gooseberries	Wild
1	e4-J13 R	F TTC CCA AAC ACC ATT ACA TTA CA R AGC CGA TGA TGG TGG TTT AG	8	231-287	(TC)8 (CAT)6 (CTT)5	well amplified, most have band at 242 bp (artefact?), this ignored	most amplified, little polymorphism	most amplified, little polymorphism	most amplified, polymorphic, some have 4 bands
2	e1-F04 R	F TCC GGA AGT GAA TAT GTG TTC TT R TCT CCG ACG AAC CTC TCT GT	9	149-171	(AAG)8	Well amplified, less polymorphism	most amplified, little polymorphism	most amplified, little polymorphism	Amplified, polymorphism like BC
3	g1-D11 R	F GAA GAC GAC AAA GCC TCC R AAT CGA ATG GAA TCG TCC	6	236-246	(TC)10	Only 2 alleles	good amplification, but little polymorphism (2 alleles)	good amplification, but little polymorphism (2 alleles)	good amplification, but little polymorphism (2 alleles)
4	g2-J11 R	F GAA CCA AAC CGA TCG AAG AA R GCC GAC ACT ATG GTA AGG GA	7	168-284	(CT)15	Poor amplification to some samples, most samples homozygous	Many not amplified	Only 1 amplified	About 50% not amplified
5	erb1-M15 R	F TCT AAA ACT GAT CTT TCT CTC ACT GC R TGG TTT AGA GAA AAG TTT TGC GTA G	9	145-162	(AG)7	Good polymorphism	Most not amplified, the rest homogenous	Only 1 amplified	Most amplified, good polymorphism
6	erb3-J14 R	F GGT TCG TTA ATC TCC CAC CA R TTT TTA GTA ACA AAT TGC ATT CTC A	9	144-171	(GA)9	Good polymorphism, though many homozygote	Only 1 amplified	Only 1 amplified	Many amplified, multiallelic to R.lac
7	e3-B02 R	F AAG ACG AAG ACG ACG ACG AT R CTG ATC TTT GCC GAA TGG TT	5	145-178	(GAA)7	Most amplified, little polymorphism	all amplified, polymorph, multialleles exist	All amplified, some polymorphism	all amplified, polymorph, multialleles exist

Table 2. Marker polymorphism on cultivated blackcurrants

No.	Loci (SSR)	No. of individuals scored	No. of alleles	Diversity	
				Expected heterozygosity	Observed heterozygosity
1	erb3-J14b	40	8	0.736	0.300
2	e4-J13	41	8	0.710	0.756
3	e1-FO4	41	5	0.633	0.610
4	erb1-M15	41	6	0.691	0.902
5	g1-D11	36	2	0.451	0.667
6	g2-J11	40	9	0.575	0.300
7	e3-BO2	38	3	0.585	0.763
	Mean	41 (total)	5.86	0.626	0.614

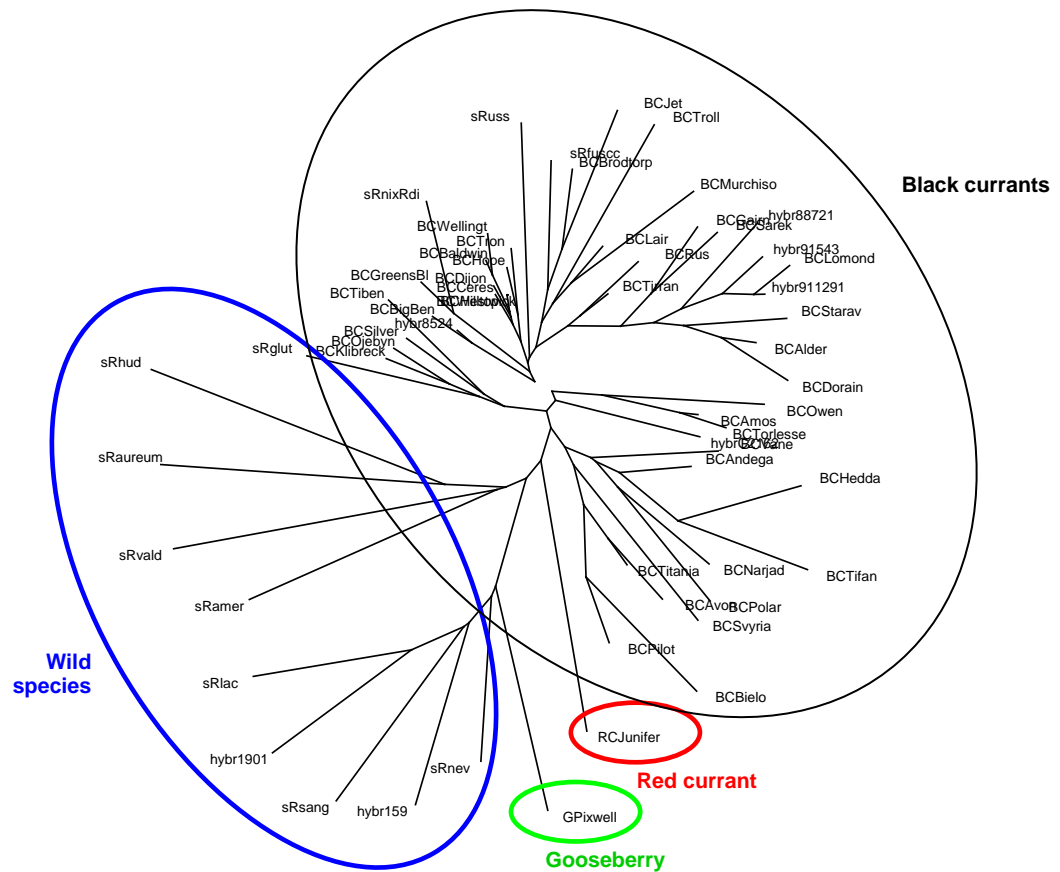


Figure 1. Segregation of tested *Ribes* collection by 7 SSR markers

## FUTURE COLLABORATION

We propose future collaboration between our institutes though there have no specific agreements made during this visit. In particular, work to widen the genetic base through inter-specific hybrids for blackcurrant breeding is a potential area for collaboration. As I participated in a part of ongoing research, I will not publish these data, and it was not proposed also in the purposes of the visit.

31.01.2008.

Kaspars Kampuss