



Cost Action 853

Agricultural Biomarkers for Array Technology

**Short term Scientific Mission - Detecting plant
viruses using microarrays**

25th October – 29th October 2004
Central Science Laboratory
Sand Hutton
York, UK.

Scientific Report

Aim

The arrays used for the STSM were for the detection of a range (12) of viruses of petunia; these viruses are usually tested for in the vegetative propagation of petunia. For each of the viruses up to 3 specific oligonucleotides have been designed, in some cases further oligonucleotides designed to give detection at the genus level have also been designed. The aim of the work was to investigate which oligonucleotides spotted on the arrays gave good signals and no cross-hybridisation when tested against a range of viruses in indicator host plants. In addition the experiments were carried out to allow inter-user evaluation of array performance to be assessed.

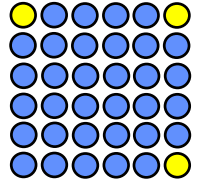
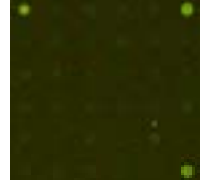
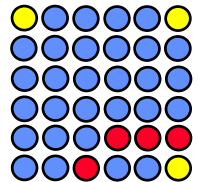

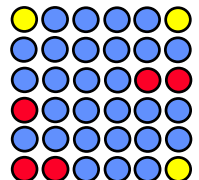

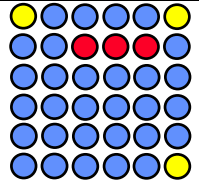
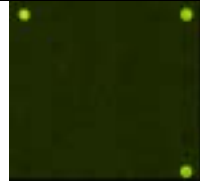
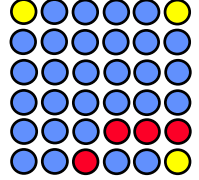

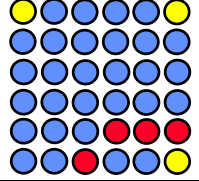
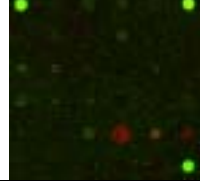
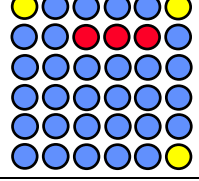

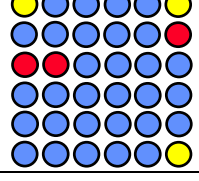

Methods

Each participant (14) carried out 2 array experiments using two plants infected with different viruses. The array experiments were carried out using a dual labelled probe approach where cDNA synthesised from the extracted RNA was labelled using Cy3 for the healthy plant and Cy5 for the infected plant. Thus a comparison could be made between the healthy and infected plants. The methods used throughout the STSM are included in the protocol booklet.

Results and Discussion

The results (table1) show that control spots (conserved region of plant 18S ribosomal gene) were visible on 22 of 24 arrays hybridised, whilst two arrays (41 and 42) did not have any clear spots visible. Of the remaining slides the results were compared to ELISA, where the arrays were scored as +/- depending on the presence of at least one of the correct spots giving a good signal in the Cy5 channel. The results show that the method gave good detection of the following viruses TuMV (9/9 slides from *B napus* and 0/2 from *N glutinosa*), PepMV (2/2 slides), TBSV (3/3), and weak detection of TMV (1/1 slide) and TSWV (1/2 slides) when the fluorescence data was analysed (see figure 1). The remaining slides were probed with cDNA from un-infected plants and all gave good control spots (7/7 slides) only one of these slides gave cross hybridisation with one virus specific oligonucleotide (slide 39).

Table 1: Results showing the images of the slides interpretation of the results and a comparison with results following ELISA testing. The expected results are illustrated, negative spots are blue, control spots are yellow and positive spots are red. Oligonucleotides are identified in columns A-F and rows 1-6.

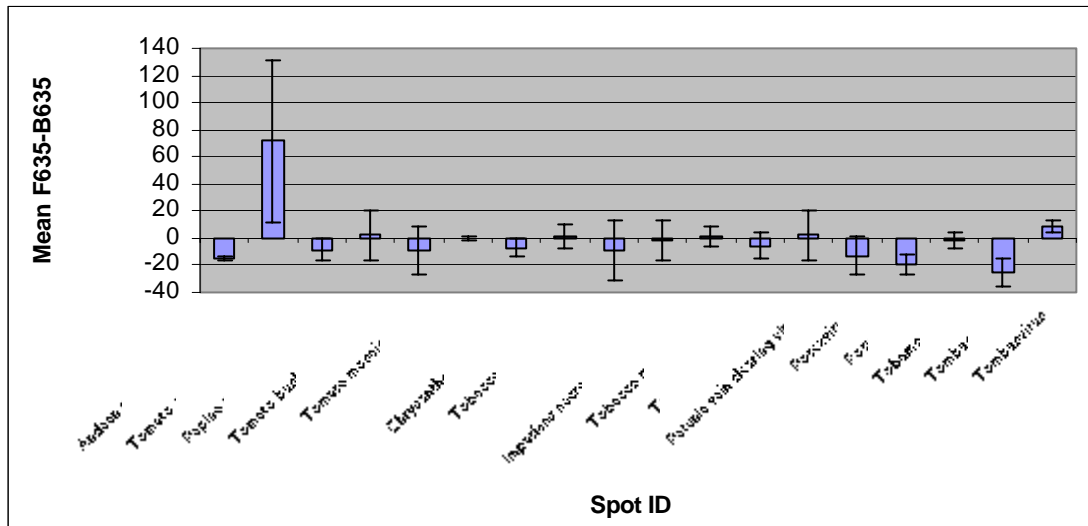
Plant Inoculated with	Slide	Expected result	Array (ratio image Cy3/Cy5)	Array result	ELISA
Healthy <i>Nicotiana rustica</i>	19			-	-
<i>Turnip mosaic virus</i> (TuMV) in <i>Brassica napus</i>	21			+	+
<i>Tomato bushy stunt virus</i> (TBSV) <i>N clevelandii</i>	24			+	+
<i>Tomato spotted wilt virus</i> (TSWV) in <i>N rustica</i>	25			-	+
<i>Turnip mosaic virus</i> (TuMV) in <i>Brassica napus</i>	26			+	+
<i>Turnip mosaic virus</i> (TuMV) in <i>Brassica napus</i>	27			+	+
<i>Tomato spotted wilt virus</i> (TSWV) in <i>N rustica</i>	28			-	+
<i>Impatiens necrotic spot virus</i> (INSV) in <i>N benthamiana</i>	29			-	-

<i>Impatiens necrotic spot virus (INSV) in N benthamiana</i>	30			-	-
<i>Turnip mosaic virus (TuMV) in N Glutinosa</i>	31			-	-
<i>Turnip mosaic virus (TuMV) in N Glutinosa</i>	32			-	-
<i>Pepino mosaic virus (PepMV) in N benthamiana</i>	33			+	+
<i>Pepino mosaic virus (PepMV) in N benthamiana</i>	34			+	+
<i>Turnip mosaic virus (TuMV) in N Glutinosa</i>	35			-	-
<i>Turnip mosaic virus (TuMV) in N Glutinosa</i>	36			-	-
<i>Turnip mosaic virus (TuMV) in Brassica napus</i>	37			+	+
<i>Turnip mosaic virus (TuMV) in Brassica napus</i>	38			+	+

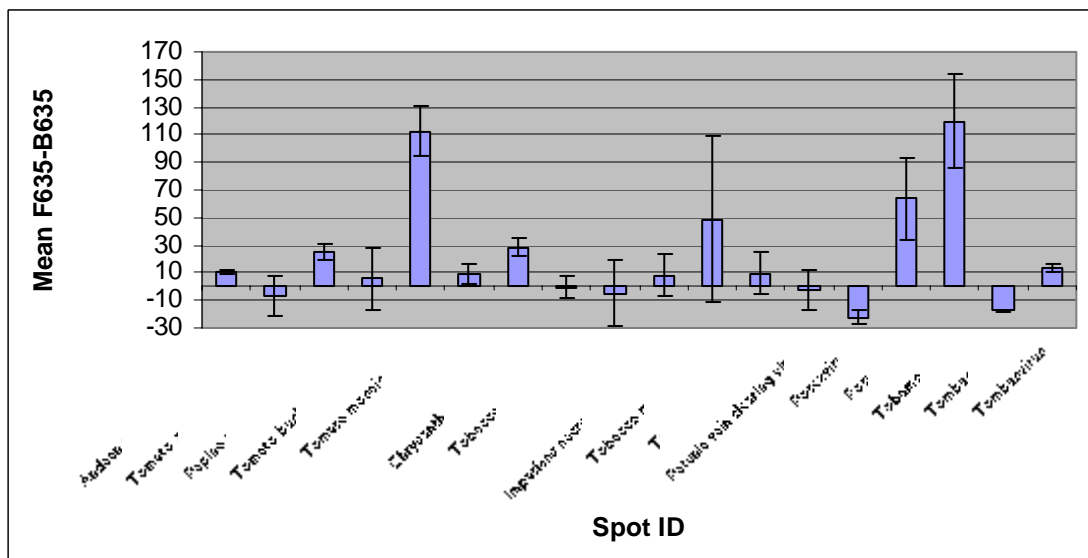
<i>Tomato mosaic virus (ToMV) in N tabacum</i>	39			-	-
<i>Turnip mosaic virus (TuMV) in Brassica napus</i>	40			+	+
<i>Tobacco mosaic virus (TMV) in N tabacum</i>	43			+	+
<i>Turnip mosaic virus (TuMV) in N glutinosa</i>	44			-	-
<i>Turnip mosaic virus (TuMV) in Brassica napus</i>	69			+	+
<i>Tomato bushy stunt virus (TBSV) in Datura stramonium</i>	70			+	+
<i>Turnip mosaic virus (TuMV) in Brassica napus</i>	71			+	+
<i>Turnip mosaic virus (TuMV) in Brassica napus</i>	72			+	+
<i>Tomato bushy stunt virus (TBSV) in Datura stramonium</i>	73			+	+

Figure 1: Histograms showing the spot ID vs. the mean fluorescence at 635nm – local background at 635nm. The histograms are A: for TSWV slide 25 and B: TMV slide 43.

A



B



A number of further observations regarding cross hybridisation and poorly hybridising spots can be made following examination of the results.

- In most cases the Cy3 and Cy5 labelling was not even; 13 slides (19, 21, 24, 25, 26, 27, 29, 30, 32, 34, 36, 44, 72) gave a higher Cy3 fluorescence, 3 slides (69, 70, 71) gave a higher Cy5 fluorescence whilst the remaining 10 slides (28, 31, 33, 35, 37, 38, 39, 40, 40, 43, 73) were acceptable. The unevenly labelled slides could be re-scanned with an un-even laser power/PMT voltage to give Cy3/Cy5 ratios closer to 1 for the control spots. In addition some spots are saturated (white) and the slides should be re-scanned at a lower laser power/PMT voltage.
- Oligonucleotide B4 designed to be specific for TSWV cross-hybridises with TuMV to a greater or lesser degree on each slide tested.
- Oligonucleotide A2 designed to be specific for TMV cross-hybridises with PepMV.
- On slides 37-39 the oligonucleotide designed to be potexvirus genus specific (F5) cross reacts with TuMV and ToMV infected plants. This reaction is not consistent in any other slides, it is possible this batch of plants were cross contaminated at a low level with a potexvirus (e.g. PepMV which has been shown to hybridise very strongly to this oligonucleotide).
- On slide 43 the most strongly hybridising spots belong to TMV and also the closely related (and in this region of sequence identical) ToMV.
- The oligonucleotide designed to be specific for the genus *Potyvirus* (F3) did not hybridise to the potyvirus TuMV.
- The oligonucleotide designed to be specific for TBSV (C5, C6 and D1) did not hybridise to TBSV, this may be an isolate specific problem since the oligonucleotide designed to be specific for the genus *Tombusvirus* did hybridise.

Organisation and attendance

In total 23 people took part in the STSM. Fourteen attendees from nine member states took part in the laboratory exercise. There were three invited expert speakers and three local expert speakers. In addition three members of staff from CSL and two of the speakers demonstrated during the practical sessions.

Participants	Role	Country
Marilyne Duffraisse	Participant	France
Ingrid Franke-Whittle	Participant	Austria
Polona Kogovsek	Participant	Slovenia
Hana Krecic	Participant	Slovenia
Alex Loy	Expert speaker	Austria
Barbara Martinez Bono	Participant	Spain
Sabine Mayrhofer	Participant	Austria
Francoise Monéger	Participant	France
Karel Petrzik	Participant	Czech Republic
Marusa Pompe-Novak	Expert speaker & demonstrator	Slovenia
Almira Ramanaviciene	Participant	Lithuania
Arunas Ramanavicius	Participant	Lithuania
Wojciech Sledz	Participant	Poland
Nancy Stralis-Pavese	Participant	Austria
Deyong Zhang	Participant	Germany
Santiago Fernandez Gonzalez	Participant	Denmark
Juerg Frey	Expert speaker	Switzerland
Neil Boonham	Expert speaker & demonstrator	UK
Ian Barker	Expert speaker	UK
Chris Danks	Expert speaker	UK
Jenny Tomlinson	Demonstrator	UK
Kathy Walsh	Demonstrator	UK
Gina Donovan	Demonstrator	UK

Feedback

At the end of the week feedback sheets were handed out to the participants to enable them to rate the quality of the STSM. The following is a summary of the feedback received in fixed categories. The scoring was on a scale of 1-5, where 1 was poor quality and 5 was excellent quality.

	Average
How did you find the course overall?	4.8
How useful did you find the content of the practical sessions?	4.8
How do you rate the quality of the practical demonstrations?	4.9
How do you rate the facilities?	4.8
How do you rate the quality of the lectures?	4.3
How do you rate the quality of the handouts?	4.6

Other comments

- It would have been useful to carry out the primer design and hybridisation conditions by ourselves.
- Very well organised and run workshop, well prepared and very well catered for different levels.
- I would like to know more about the use of microarrays in general expression experiments and the different statistical programs one can use to analyse the results.
- It would be great to have access to all the powerpoint presentations, for example as PDF files on the web site.
- Real-time PCR covers a large range in the molecular microbiology techniques and should be included in the lab session.
- Very good learning through the course, good discussion possibilities and information, good organisation.