

Microarray Detection of Viroids

Detection of viroids

Viroids represent a growing threat to plant health in the UK; however, diagnosis of viroids is not straightforward, and traditional approaches using specific tests and host lists cannot provide a fully effective means of excluding non-indigenous viroids from the UK. This can only be achieved by developing generic, broad-spectrum diagnostics, such as microarrays.

Microarray design

The genome sequence from each of 38 viroid species were downloaded from GenBank and a multiple sequence alignment completed, from which discriminatory 45-mer oligonucleotide probes were designed and spotted onto aldehyde-coated glass slides. In most cases 2 oligos were designed for each viroid.

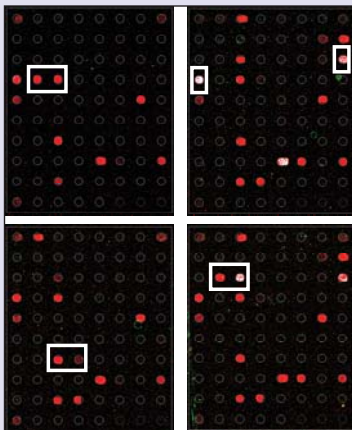


Figure 2. Microarray results for detection of 4 species of Pospiviroid, showing target spots for each species (clockwise from top left: CSVd, PSTVd, TCDVd, TASVd).

Table 2. Summary of results for detection of 4 species of Pospiviroid.

oligo	Viroid			
	CSVd	PSTVd	TASVd	TCDVd
CSVd #1	✓			
CSVd #2	✓			
PSTVd #1		✓		
PSTVd #2		✓		
TASVd #1			✓	
TASVd #2			✓	
TCDVd #1				✓
TCDVd #2		(✓)	(✓)	✓

Discussion

The results obtained using labelled PCR products suggest that the viroid array could be highly effective in discriminating closely related viroids, if a suitably non-specific labeling / amplification strategy can be found. Degenerate primers could be designed for other viroid genera and multiplexed together to give greater coverage. This approach is most likely to be useful for niche applications such as viroid detection, where the number of targets is relatively small but other methods for discriminating closely related species are not available. The fact that some oligos were observed to cross-hybridize could increase the chances of detecting new viroid strains.



Figure 1. PSTVd outbreak in tomato

Table 1. Viroid species represented on the array

Family	Genus	Species
Pospiviroidae	Pospiviroid	9 species
	Hostuviroid	1 species
	Cocaviroid	4 species
	Apscaviroid	9 species
	Coleviroid	4 species
Avsunviroidae	Avsunviroid	1 species
	Pelamoviroid	2 species
Unclassified		8 species

Results

Our typical approach for the detection of viruses in plant material (incorporation of amino-allyl modified nucleotides into cDNA during reverse transcription, followed by post-labeling with Cy dye) resulted in no hybridization of labeled viroid cDNA to the array, despite strong signals for plant control oligos. (Production of cDNA had been confirmed by real-time PCR.) This was likely to be due, at least in part, to the high level of secondary structure characteristic of viroids; however, hybridization was not improved by fragmentation of viroid RNA, or cDNA (after reverse transcription).

As an alternative labeling strategy, pospiviroid primers were used to amplify pospiviroid cDNA by PCR incorporating Cy5-dCTP, and the resulting labelled PCR products were hybridized to arrays. Each of the species tested showed a characteristic pattern of hybridization, which could potentially be used to diagnose an unknown sample.

Unlike cDNA reverse transcribed from viroid RNA, the amplicons produced by PCR were found to hybridize efficiently in spite of their considerable secondary structure (predicted using mfold (<http://www.bioinfo.rpi.edu/applications/mfold/>)). This suggests that although secondary structure is likely to be a major factor affecting the efficiency of hybridization, increasing the amount of labelled probe applied to the array may overcome this.



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Acknowledgement

Dan Thompson (CFIA Canada), Claudia Jansen (PPS, The Netherlands), Rudra Singh (CFIA, Canada), Ricardo Flores (Univ. Valencia, Spain), and Nuria Vila Duran (IVIA, Spain) kindly supplied infected material. Funding for this work was provided by Defra Plant Health Division.



CSL is an Executive Agency of Defra