Oligonucleotide-based microarray detection of plant viruses

- M. Sip[a*], D. Bystricka[a,b,c], O. Lenz[b,c], I. Mraz[b], L. Piherova[d], S. Kmoch[d],
-
- [a] Department of Medical Physics and Biophysics, Faculty of Health and Social Studies, University of South Bohemia, Dukelska 3, 370 01 Ceske Budejovice, Czech Republic
- [b] Institute of Plant Molecular Biology, Academy of Sciences of the Czech Republic, Branisovska 31,
- 370 05 Ceske Budejovice, Czech Republic
- [c] Faculty of Biological Sciences, University of South Bohemia, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic
- [d] Institute of Inherited Metabolic Diseases, 1st Faculty of Medicine, Charles University, Ke Karlovu 2,
- 128 53 Prague 2, Czech Republic

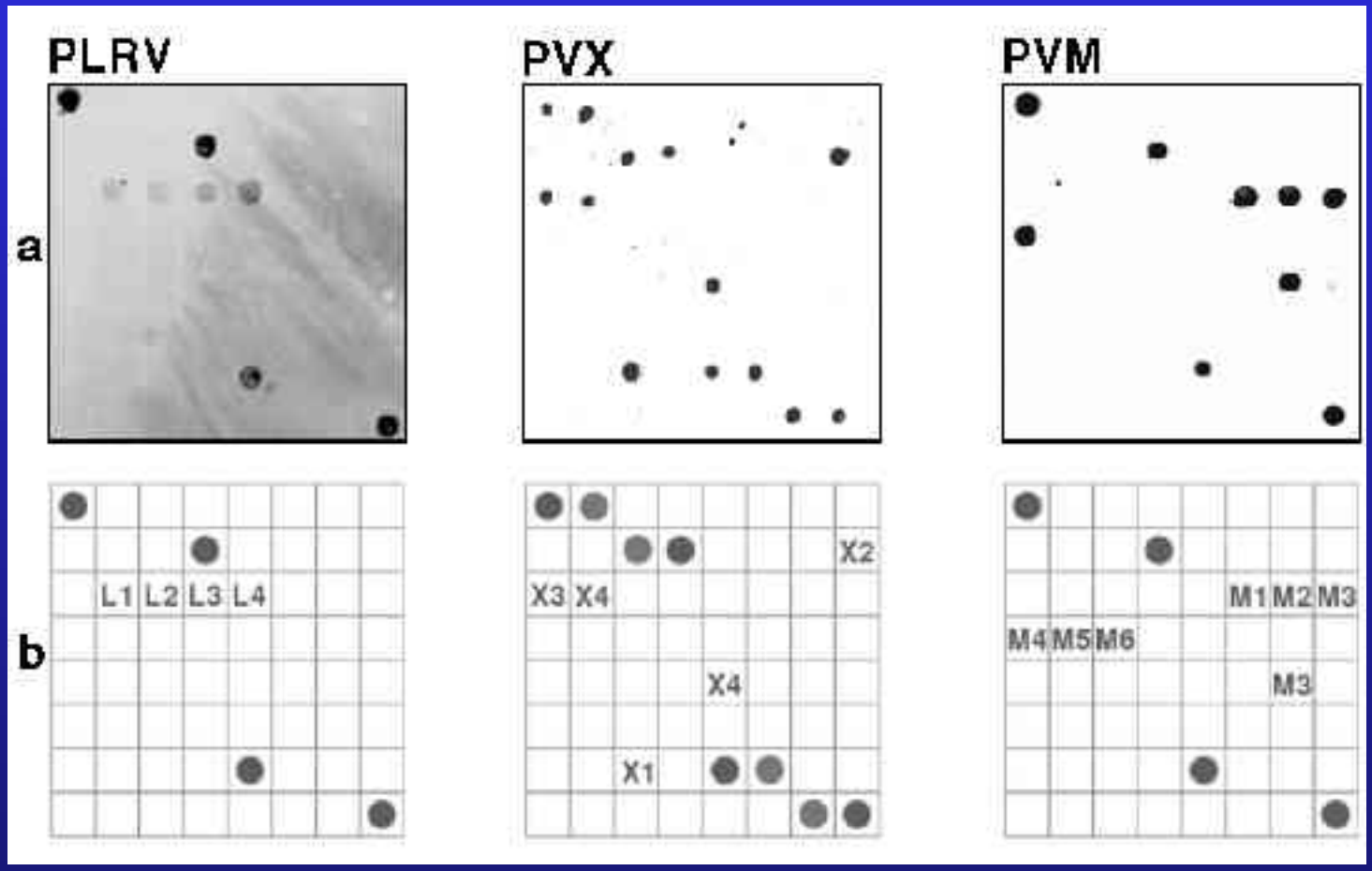
Short oligonucleotide approach

- short synthetic single stranded oligomers (40 nt)
- length 40 nt , melting temperature 60 - 65°C, deltaG < -65 kcal/mol (Vector NTI Suite, InforMax)
- simultaneous detection of several potato viruses (PVA, PVS, PVM, PVX, PVY and PLRV)
- designed to distinguish between the main PVY and PVS strains
- at least 4 probes for each of virus, according to results obtained from BLAST

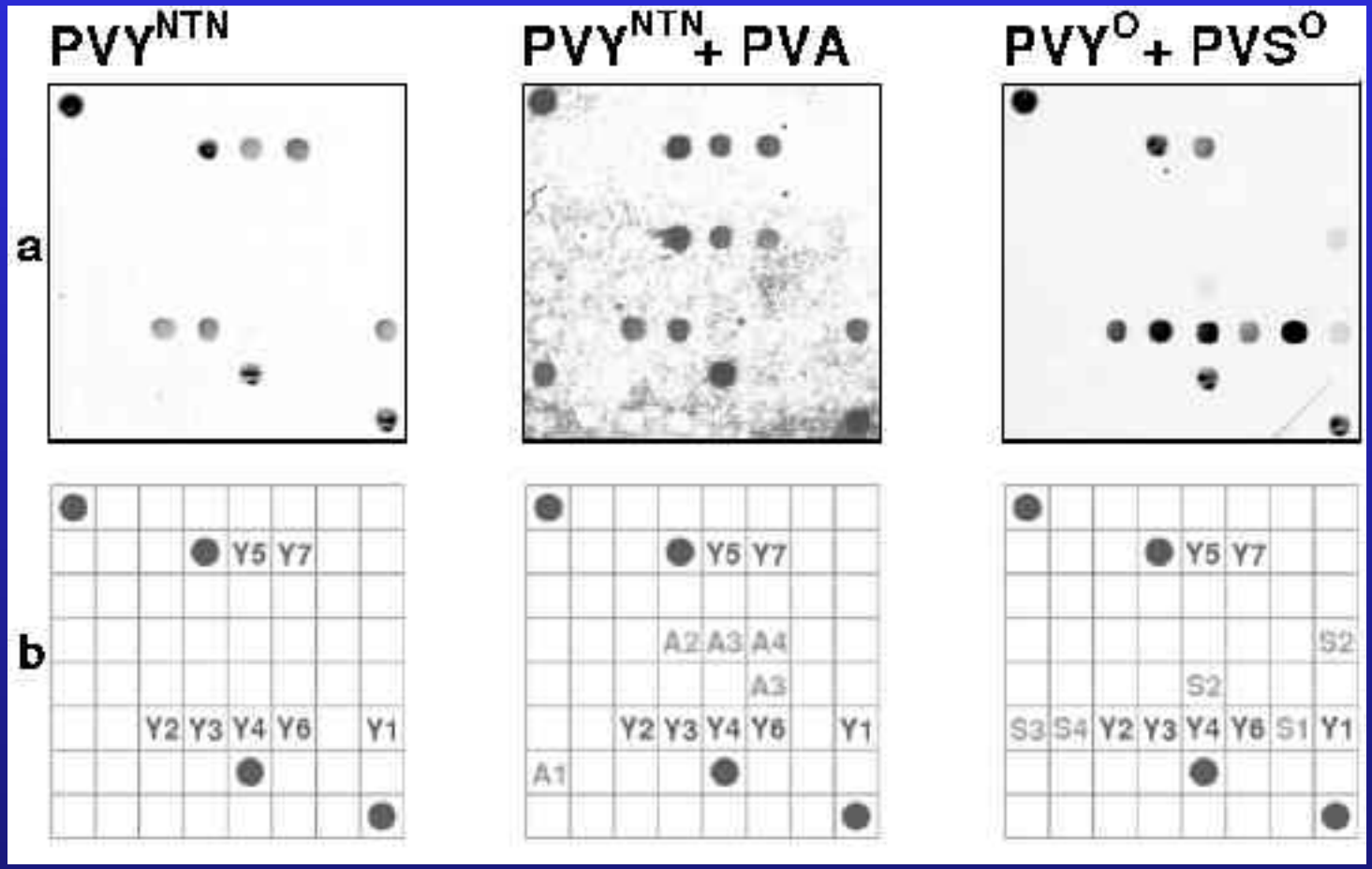
Probes

symbol	sequence (from 5‘ to 3‘)			gene
L1	AAGACGCAGAAGAGGAGGCAATCGCCGCTCAAGAAGAACT	CP		PLRV
L2	GAGGACGAGGCTCAAGCGAGACATTCGTGTTTACAAAGGA	CP		PLRV
L3	CAACACCCACTCCAACTCCCCAGAAGCACGAGCGATTTAT	CP		PLRV
L4	TGTGTAACTAACGCCCCACTGTGCTCCCTTAAATCCAAAC	RNA-pol.		PLRV
M1	ACTCGGGGTGTCGCTTGCTCTAGTTGTTTGGCTGCTTATA	TGB2 prot.	*PVM-O	
M2	GCTACTTGGTCAACCTTGGGCTATCGTCGTCCTGCTAGTA	TGB2 prot.	PVM	
M3	GCGCCTTCTGCATTGTGCTGTATTTGATCTCTCAGGGACA	TGB3 prot.	*PVM-O	
M4	GAGATAACCTGGTGGAACATTTGGGAAAACAGTTACGCCT	RNA-pol.		*PVM-O
M5	AACCTTGGGCTTTAGTGATCCTCCTGATTGTGCTTATTTG	RNA-pol.		*PVM-I
M6	AGCGGCAACTGGATTAGAAAGTGGGAAGGGCAGCAAACAG	RNA-pol		*PVM-I
Y1	GCAGTGACTATGTCTGGATTTAGTTACTTGGGTGATGCTG	3-UTR		PVY
Y2	CATACGACATAGGAGAAACTGAGATGCCAACTGTGATGAA	CP		PVY
Y3	CGGAGTTTGGGTTATGATGGATGGGAATGAACAAGTTGAG	CP		PVY
Y4	TTGCTGGGTGGTAAAGTGTGTGTTGACGACTTCAATAATC	Nla-Pro		*PVY-O
Y5	CATGAAAGCTGCGGTCGGAGCCATGTATGGTGGAAAAAAG	Nlb		PVY
Y6	TCATGCAAAGCTGTCTACGATTGTATAAAGGCTTACTCGG	Nlb		*PVY-O
Y7	AGAGTAAAGGTGCAACTGTACTAAATTTGAAACACTTACTC	CP		*PVY-NTN
S1	AAAGTAGTGACTTTTATTCGGGGTTGGAGTAACTGAGGTG	11K prot.	PVS	
S2	GGTTATACTGAAGGTCGTAATTATGTGCGCAAGCGTGAGC	CP		PVS-A
S3	CGCTTGGTGTATCTCTTGCTGCTGTAGTTGCCTTATTCAC	12K prot.	*PVS-A	
S4	AAGCTCGAATATACAGTCTCACAGCAAGAATGCCGCCTAA	5-CP		*PVS-A
A1	CTGGTATGATGGCGTTATGGCAAGTTATGAGTTAGAGGAA	CP		PVA
A2	TATTCTTGGCTATGCTTGTGAATGTGGATGAGTCCGATGC	HC-Pro		PVA
A3	CTGAAACCATCGTGAAGAAGATACACACAGTGGGTAAACA	P1 prot.		PVA
A4	AAGGTAAGAAGAAAGAAGGAGAAGGCAACAGTGGCAAAGC	CP		PVA
X1	CAACCGCTGAGGCTGTTGTCACTCTACCACCACCATAACT	3-CP		PVX
X2	ATACTTTGGACAACACCACAAGGAACTCATACCAGGCACT	TGB1		PVX
X3	GAAGGTGATAGCCATTGACGAGGAGTCCGAGACAACACTG	MP		PVX

Detection of PLRV, PVX and PVM in a single infection.



Detection of PVY^{NTN} and determination of PVY^{NTN} and PVY^{O} in mixed infections.



Summary of test results

- The chip detects PVA, PVS, PVM, PVX, PVY And PLRV in single and mixed inffections
- distinguishes between PVY^{NTN} and PVY^{O} .

To be proven (only some combinations tested):

- PVS^{A} and PVS^{O}
- PVM Idaho and rest of PVM sequences

Published in:

- **Bystricka D., Lenz O., Mraz I., Piherova L. Kmoch S. and Sip M.:**

  DNA Microarray: Parallel Detection of Potato Viruses, Journal of Virological Methods, 2005.

Collaborations

- University of South Bohemia
  Department of Health Physics and Biophysics, České Budějovice, CZ
  - Miroslav Šíp
  - Dáša Bystřická
- Institute of Plant Molecular Biology, České Budejovice
  - Ivan Mráz
  - Ondřej Lenz
- Institute of Inherited Metabolic Diseases , Prague
  - Stanislav Kmoch
  - Lenka Piherová
- Potato Research Institute, Havlíčkův Brod
  - Petr Dědič