DEVELOPMENT OF A MONOCLONAL ANTIBODY FOR THE DETECTION OF POTATO MOP-TOP VIRUS

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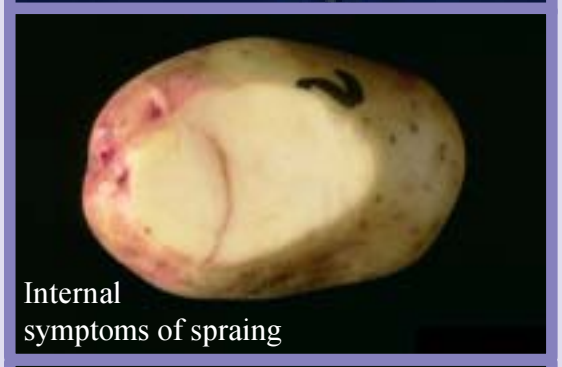
Introduction

Potato mop-top furovirus (PMTV) causes a wide range of symptoms in potato (*Solanum tuberosum)* including blotching and mottling of leaves and internal brown arcs ('spraing') in tubers of some varieties. The virus is transmitted by the soil-borne fungus *Spongospora subterranea* and has been reported in South America, Western Europe and more recently in North America. Diagnosis of the virus is primarily by visual symptoms in plants and tubers which can be confirmed by inoculation onto the diagnostic species *Chenopodium amaranticolor, Nicotiana debneyi, and N. tabacum.*

PMTV Foliage symptoms in potato



Internal symptoms of spraing

External symptoms of spraing

Development

Monoclonal antibody secreting cell lines were generated by the fusion of NSO myeloma cells and splenocytes from Balb/c mice immunised with a purified preparation of PMTV.
Hybridomas were screened by TAS ELISA for positive reactions to PMTV infected plant material. A single cell line which showed specificity to PMTV was cloned by limiting dilution and the antibody from it used to develop a DAS ELISA for PMTV in potato tuber and leaf material. Purified antibody from the cell line was used to trap virus in the DAS ELISA and conjugated to alkaline phosphatase to produce the probe.

Evaluation

An evaluation of the SASA DAS ELISA was made with a commercial assay obtained from Adgen Ltd, Scotland.

Three tubers from each of 10 progenies were tested, 27 of cv Slaney and 3 of cv Cara.

3 cores were taken from each tuber, from the rose end, middle and heel end. Samples were homogenised, processed according to either the SASA or Adgen ELISA protocol and read one hour after substrate addition.

Samples deemed positive if > 2x mean of negative control.

Results at 1h after substrate addition
49 samples +ve with both SASA and Adgen assays
37 samples –ve by both SASA and Adgen assays
3 samples +ve with SASA assay only (borderline +ves- 2.18, 2.19 and 2.48x –ve control)
1 sample +ve by Adgen assay only (borderline +ve-2.23x –ve control)

Although OD values were higher for positives with the Adgen assay, the lower OD value for negative samples meant that the positive/ negative ration for the SASA assay was slightly higher.

Assay system	Mean –ve OD	Mean +ve OD	Mean +/- ratio
Adgen	0.031	0.34	10.9
SASA	0.021	0.28	13.3

Comparison of mean OD values and positive/negative ratios after 1hr substrate incubation

The SASA assay was found to be as sensitive as the Adgen assay for detection of low levels of virus found in tuber samples. To date the SASA assay has successfully detected numerous Scottish, 4 Danish and 3 French PMTV isolates.

A small study on the distribution of PMTV in potato plants by PCR was undertaken at SASA and confirmed that leaflets testing positive by SASA DAS ELISA also gave a positive PCR result. Leaflets testing negative by PCR were also negative using the SASA DAS ELISA.

The SASA Antibody Unit operates a Quality Management system compliant to BS EN ISO 9002 (1994). The PMTV detection reagents along with all our other products are produced within this Quality Management system.

BSI REGISTERED UKAS QUALITY MANAGEMENT 003

CERTIFICATE NUMBER FM 59966