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Enzyme-linked immunosorbent assay (ELISA) for the detection of potato viruses A and Y in potato leaves and sprouts

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Abstract

With the enzyme-linked immunosorbent assay (ELISA) potato virus A (PVA) could be detected reliably in potato sprouts, especially when these were young and sappy. The detection of this virus in leaves of glasshouse-grown potato plants was less reliable.

The tobacco veinal necrosis strain of potato virus Y (PVY^N) was readily demonstrated in foliage of glasshouse-grown potato plants using an antiserum to this strain. Plants infected with the common strain (PVY^O) did not react in ELISA with this antiserum. In young sappy sprouts, using the PVY^N antiserum, PVY^N could be detected reliably when samples with PVY^O were excluded, as the reaction of samples infected with the latter virus was intermediate between PVY^N-diseased and PVY-free samples. PVY was also detected in plants inadvertently infected during the experiments.

Introduction

Clonal selection is the basis for Dutch seed-potato growing. Clones must be free from all known viruses and therefore plants are tested. To this end, in the Netherlands the presence of potato virus A (PVA) and potato virus Y (PVY) is determined by the A6-leaf test (De Bokx, 1972), that is also used in tuber indexing of other grades of potato seed. The test is easy to perform and very reliable, but requires glasshouse space for growing test plants and special chambers for incubation of the inoculated A6-leaves. Moreover, results cannot be read until after a 4–7 days' incubation. Therefore it was investigated whether the A6-leaf test could be replaced by an other sensitive test, the enzyme-linked immunosorbent assay (ELISA; Clark and Adams, 1977). Leaves of glasshouse-grown potato plants and sprouts of potato tubers of which dormancy was broken naturally, were submitted to ELISA.

Materials and methods

Antisera to PVA isolated from cv. Lichte Industrie and to PVY, tobacco veinal necrosis strain (PVY^N), isolated from cv. Record were prepared by Maat and Mierzwa (1975) and by De Bokx et al. (1975), respectively.

ELISA (Clark and Adams, 1977) was performed largely as indicated by Maat and De Bokx (1978). To determine optimum concentrations of antiserum γ-globulin and of enzyme conjugates we used extracts from potato leaves. In a second experiment with potato leaves and the PVY antiserum, the γ-globulin fraction was prepared in a different way, using caprylic acid (Steinbuch and Audran, 1969) followed by precipitation with ammonium sulphate.

Potato tubers, harvested from healthy and virus-infected field-grown plants in

Table 1. Potato cultivars tested, numbers of tubers screened and the viruses with which they were infected.

Cultivar	Virus isolates				
	*PLRV-'Bintje'	*PVA-'Lichte Industrie'	*PVY^N-'Gineke'	*PVY^O-'Paul Kruger'	Virus free
Alpha		3		3	2
Bintje	3		3	3	3
Désirée	3	3		3	3
Doré		3	3		2
Eigenheimer		3	3	3	3
Element	3		3		2
Mirka		3	3	3	3
Ostara	3				1
Radosa	3				1
Resy	3	3	3	3	4
Total	18	18	18	18	24

* PLRV = potato leafroll virus; PVA = potato virus A; PVY^N = potato virus Y, tobacco veinal necrosis strain; PVY^O = potato virus Y, common strain.

Tabel 1. Getoetste aardappelrassen, de aantallen getoetste knollen en de virussen waarmee deze besmet waren.

July 1977, were stored at 3°C in a store-house until December. Then from each tuber one eye was planted in a glasshouse at about 20°C, with supplementary illumination. The remaining part of the tuber was stored in the dark at 18–25°C to promote sprouting. From the plantlets grown from the eyes, the youngest completely expanded leaf was tested about 5 weeks after planting. Sprouts were tested 7 and 11 weeks after the tubers were submitted to 18–25°C. The sprouts at 7 weeks were thick and woody, and extracts were prepared with a handpress (De Bokx, 1972). After 4 more weeks new sprouts had formed. From these thin and sappy sprouts sap was prepared with a power-driven crusher. More information on the potato material used is given in Table 1. This material was from the IPO-collection, grown under conditions preventing contamination. Moreover it was checked regularly for the presence of viruses.

Samples were tested in duplo and of the two extinction values obtained, the average is presented in the Figures and Tables.

Results

In tests with the PVA antiserum 1 μg/ml of γ-globulin proved to be the optimum for coating of the plates in combination with ca. 3.5 μg/ml of γ-globulin in the enzyme conjugate. In tests with the PVY antiserum optimum coating was obtained with 0.2–0.5 μg/ml of γ-globulin, depending on the preparation; optimum γ-globulin concentrations of the enzyme conjugate were 0.7–1.75μg/ml.

In ELISA, the extinction values of duplicates of a sample incidentally differed much when testing leaves or the thick, woody sprouts using the PVA antiserum. The results of ELISA, obtained with this antiserum are summarized in Table 2. Extinction values were between 0.19 and 0.55 for 17 out of 18 leaf samples with PVA and only 0.09 for one, and from 0.07–0.17 for 74 out of 75 leaf samples not infected with PVA and

Table 2. Results of ELISA with an antiserum to PVA and potato leaves and sprouts, virus free or infected with PVA, PLRV, PVY^N or PVY^O. For potato cultivars tested see Table 1.

Virus	Number of samples tested	Range of extinction values at 405 nm (0 — 0.1 — 0.2 — 0.4 — 0.8 — 1.6)
PVA	18	-
	18	+
	18	xxxxxxxxxxxxxxxxxxxxxxxxxxxx
PLRV	18	-----
	18	++++++++
	18	xxxxxxxxx
PVY^N	18	----- -
	18	xxxxxx
PVY^O	15^1_2	----------
	17^2	xxxxx
Virus free	24	-------- -
	12_2	++++++++
	17^2	xxxxxxxxx

[1] Leaves of 'Désirée' with PVY^O could not be tested because of death of plants.
[2] Not all tubers sprouted a second time.
---- potato leaf material; ++++ extracts from thick woody sprouts;
xxxx extracts from thin sappy sprouts.

Tabel 2. Resultaten van ELISA met een antiserum tegen het aardappelvirus A en aardappelbladeren en -spruiten, virusvrij of geïnfecteerd met aardappelvirus A, aardappelbladrolvirus of de aardappelvirussen Y^N of Y^O. Voor de getoetste cultivars zie Tabel 1.

Table 3. Results per cultivar of ELISA with an antiserum to PVA and potato leaf material with and without PVA.

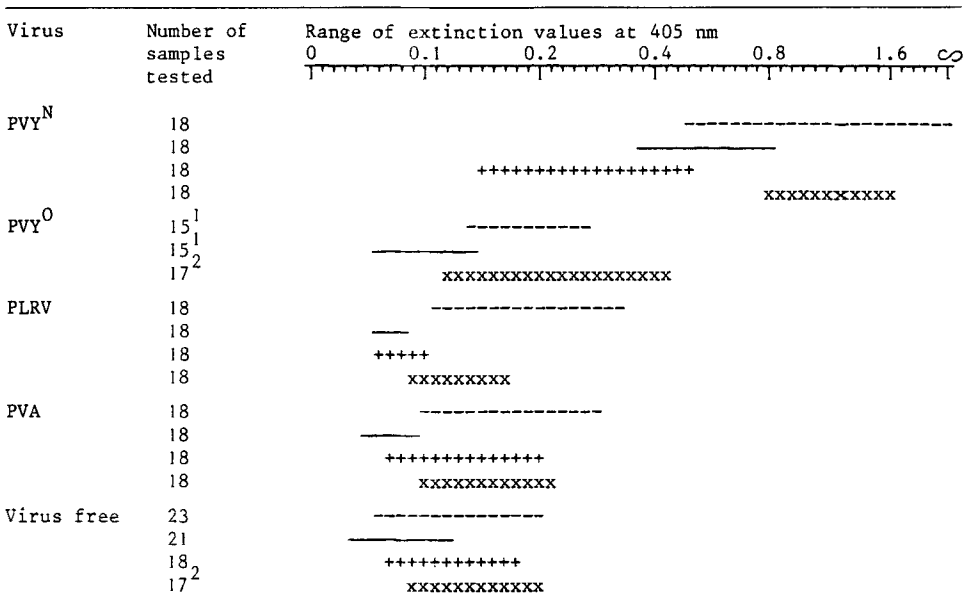
Cultivar	Numbers of samples and ranges of extinction values			
	PVA-free		PVA-infected	
	number	range	number	range
Alpha	4	0.08–0.11	3	0.34–0.55
	1	0.21		
Désirée	7	0.08–0.10	3	0.20–0.23
Doré	5	0.07–0.10	3	0.24–0.27
Eigenheimer	9	0.07–0.11	3	0.19–0.23
Mirka	9	0.07–0.11	3	0.09, 0.28, 0.35
Resy	13	0.09–0.17	3	0.36–0.44
Bintje	12	0.09–0.16		
Element	8	0.09–0.17		
Ostara	4	0.09–0.12		
Radosa	4	0.08–0.13		

Tabel 3. Resultaten per cultivar van ELISA met een antiserum tegen het aardappelvirus A en aardappelbladeren met en zonder aardappelvirus A.

one at 0.21. For the thick woody sprouts, extinction values were between 0.29 and 1.4 for 17 out of 18 samples with PVA and 0.17 for one, and from 0.07–0.14 for 30 samples without PVA tested. Best results were obtained with the thin sappy sprouts. Extinction values for 18 samples with PVA varied from 0.29–2.0 and of the 70 PVA-free samples tested from 0.06–0.14. Table 3 gives the results per cultivar of the leaf material tested. One sample of cv. Mirka infected with PVA failed to react in the expected way, and one sample of cv. Alpha, not infected with PVA, showed a rather high extinction value (0.21). For the rest within a given cultivar PVA-free and PVA-infected samples could readily be distinguished.

The results of ELISA, using the PVY^N antiserum are summarized in Table 4. Since in the first experiment with potato leaf material the extinction values obtained with the PVY^N-free samples were rather high, the test was repeated one week later, using other preparations of γ-globulin and of enzyme conjugate. In the first experiment with leaf material, of the 18 samples with PVY^N extinction values ranged from 0.50–∞. The extinction value of one of the plants supposed to be healthy, also fell within this range (not included in Table 4); later on, this plant appeared to be in-

Table 4. Results of ELISA with an antiserum to PVY^N and potato leaves and sprouts, virus free or infected with PVY^N, PVY^O, PVA or PLVR. For potato cultivars tested see Table 1.



[1]Leaves of 'Désirée' with PVY^O could not be tested because of death of plants.
[2]Not all tubers sprouted a second time.
---- potato leaf material, first experiment; —— potato leaf material, second experiment; ++++ extracts from thick woody sprouts; xxxx extracts from thin sappy sprouts.

Tabel 4. Resultaten van ELISA met een antiserum tegen het aardappelvirus Y^N en aardappelbladeren en -spruiten, virusvrij of geïnfecteerd met aardappelvirus Y^N, aardappelvirus Y^O, aardappelbladrol virus of aardappelvirus A. Voor de getoetste cultivars zie Tabel 1.

fected with PVY, both visibly and in the micro-precipitin test. The extinction values for 74 PVY^N-free samples, including those with PVY^O, varied from 0.06 to 0.34. In the second experiment with leaf material these values were from 0.39–0.80 for 18 samples with PVY^N, and from 0.04–0.14 for 72 PVY^N-free samples, including those with PVY^O. In this test three plants, originally virus free, proved to be infected with PVY (not included in Table 4). Their extinction values were 0.23, 0.33 and 0.40. Testing the thick woody sprouts, extinction values were from 0.15–0.52 for 18 samples with PVY^N, and from 0.06–0.20 for 54 PVY^N-free samples tested. Samples with PVY^O were not included in this test. With the thin sappy sprouts the extinction values obtained for the 18 PVY^N-infected samples were from 0.80–1.5, for the 17 PVY^O-infected samples from 0.12–0.45, and for the 53 PVY-free samples tested from 0.09–0.21.

Discussion

Extinction values obtained in ELISA are supposed to be correlated with virus concentration (Clark and Adams, 1977; Casper, 1977). Thus a great variation in extinction values, as shown in Table 2, 3, and 4, can be expected when virus-infected material is tested using the homologous antiserum. However, our results also show widely varying extinction values with non-infected material, or material infected with other viruses. Therefore, it is difficult to distinguish PVA-free from PVA-infected leaf material when results of all cultivars are joined as in Table 2. Close examination of the results (Table 3) suggests that differentiation per cultivar is possible. The high extinction values obtained with two PVA-free samples (e.g. 0.21 with cv. Alpha, Table 3) and indicated separately in Table 2, are, at least partly, due to big differences between the duplicates. This probably indicates experimental errors. Why one leaf sample of cv. Mirka with PVY^N (Table 3) did not react in the expected way is not clear. Better results were obtained with PVA antiserum and potato sprouts, especially with the thin sappy sprouts (Table 2). With the latter, distinction between PVA-free and PVA-infected samples seems very well possible.

In the first experiment with the PVY antiserum and potato leaves extinction values of PVY-free material were very high (0.06–0.34; Table 4). Therefore a second experiment was done one week later. The same antiserum was used, but the γ-globulin was prepared in a different way. It is not clear whether this improved the results, but in this experiment PVY^N-free material could easily be distinguished from PVY^N-infected material. Leaves infected with PVY^O reacted like PVY-free leaf material. This was not true for sprouts. In tests on thin sappy sprouts an almost continuous range of extinction values was obtained, due to the fact that reactions with material infected with PVY^O were much weaker than those with PVY-infected material, but often stronger than those with PVY-free material. Using PVY^N-antiserum, samples with PVY^N could easily be distinguished from the PVY^N- and PVY^O-free ones, although not when thick woody sprouts were tested. The results suggest that in ELISA, when testing for PVY, it will be necessary to use antisera to all PVY strains that can be expected in the material to be tested, or to use a multivalent PVY antiserum.

Because of the great variation in extinction values, the use of virus-free and virus-infected standards will be needed. The antisera used in our experiments react with normal plant material in the micro-precipitin test (titer 4). With more specific anti-

sera, ELISA may also be more specific.

So far, only a limited number of samples were tested. With increasing numbers, variation of extinction values may also increase. Yet, our results suggest that ELISA may be a reliable test for PVA in sprouts, and to a certain extent in leaves, as well as for PVY^N in both leaves and sprouts. PVY^N may also be detected in glasshouse-grown plants with primary infections. PVY^O was not, or not reliably, detected with the PVY^N antiserum. Whether the viruses can also be detected in field-grown plants, and in freshly harvested dormant tubers is under investigation.

Samenvatting

'Enzyme-linked immunosorbent assay' (ELISA) voor het aantonen van de aardappelvirussen A en Y in aardappelbladeren en -spruiten

De basis voor de Nederlandse pootgoedteelt is de stamselectie. Planten, behorend tot deze stammen, moeten virusvrij zijn en worden daarom hierop getoetst. In Nederland wordt hierbij en ook bij de keuring van latere stadia in de pootgoedteelt, besmetting met de aardappelvirussen A en Y (PVA en PVY) met de A6-bladtoets aangetoond. Deze is gemakkelijk uit te voeren en zeer betrouwbaar. Er moeten echter toetsplanten worden opgekweekt, wat kasruimte vraagt. Bovendien zijn voor de incubatie van de geïnoculeerde A6-bladeren klimaatkamers nodig en duurt het 4–7 dagen voor de resultaten bekend zijn. Daarom werd een andere gevoelige toetsmethode, de 'enzyme-linked immunosorbent assay' (ELISA) beproefd. Hiervoor werden oude, niet geheel specifieke antisera gebruikt, bereid tegen PVA geïsoleerd uit cv. Lichte Industrie en tegen PVY^N geïsoleerd uit cv. Record.

Toetsingen werden uitgevoerd met blad van in de kas opgekweekte virusvrije en secundair besmette aardappelplanten en met spruiten van in het donker bewaarde aardappelknollen. Tabel 1 geeft een overzicht van de getoetste rassen, de aantallen knollen, die ter kieming werden weggelegd en van de virussen waarmee ze besmet waren. Van elke knol werd bovendien één oog in de kas uitgeplant. De resultaten van de toetsingen, weergegeven in de Tabellen 2 en 3 laten zien dat PVA goed kan worden aangetoond in aardappelspruiten, vooral wanneer deze jong en sappig zijn, doch minder goed in bladmateriaal. Dit laatste moet hoofdzakelijk worden toegeschreven aan het feit dat één bladmonster van cv. Mirka met PVA te zwak reageerde, en één bladmonster van cv. Alpha zonder PVA een erg hoge extinctiewaarde gaf (Tabel 3). Dit laatste kan waarschijnlijk aan een experimentele fout worden toegeschreven. Tabel 4 geeft de resultaten met PVY. In de eerste proef met bladmateriaal gaven ook de PVY^N-vrije monsters hoge extinctiewaarden. Daarom werd deze proef een week later herhaald, waarbij een ander serummonster werd gebruikt. In deze tweede proef kon besmetting met het PVY^N zeer goed worden onderkend. Planten met PVY^O reageerden echter als de PVY-vrije. Dit betekent dat voor het aantonen van deze stam een antiserum tegen PVY^O moet worden gebruikt, of een mengserum tegen alle PVY stammen die in het materiaal voor kunnen komen. Ditzelfde is ook nodig wanneer spruiten worden getoetst. Jonge sappige spruiten met PVY^N waren goed te onderscheiden van spruiten waarin beide PVY stammen ontbraken, maar monsters met PVY^O gaven slechts gedeeltelijk een sterkere reactie te zien dan de PVY-vrije. Het aardappelvirus Y werd met de toets ook aangetoond in oorspronkelijk gezonde

planten, die gedurende de proef besmet geraakten. Of genoemde virussen ook kunnen worden aangetoond in planten te velde en in in kiemrust verkerende, vers gerooide knollen wordt onderzocht.

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