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Detection of squash mosaic virus in seeds of melon (Cucumis melo) by enzyme-linked immunosorbent assay (ELISA)

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Abstract

Squash mosaic virus (SqMV, comovirus) is seed-transmitted in several *Cucurbitaceae*. Therefore, the use of virus-free seed is important to prevent establishment of this virus in the Netherlands and to avoid spread to other countries.

This study was undertaken to develop an enzyme-linked immunosorbent assay (ELISA) for the detection of SqMV in melon seeds. An antiserum was produced to a serotype 1 isolate from melon. Two ELISA variants were investigated viz. an ELISA variant with simultaneous incubation of sample and enzyme conjugate (ELISA 1) and an ELISA variant with successive incubation of sample and enzyme conjugate (ELISA 2). The sensitivity of ELISA was tested by mixing flour of ground infected and non-infected seeds in different proportions. SqMV was detected by both ELISA variants at dilutions of 1 : 160 (1 part of infected flour mixed with 159 parts of non-infected flour) or higher after a substrate incubation period of 4 h. However, ELISA 1 gave relatively higher absorbance values than ELISA 2 for nearly all dilutions. Since ELISA 1 is also faster than ELISA 2, ELISA 1 is advised for routine testing. In these tests, using subsamples of 100 melon seeds SqMV is detected reliably. ELISA 1 is now used in the Netherlands for routine-indexing of melon seed lots for SqMV.

Additional keywords: serology, identification, virus purification.

Introduction

Squash mosaic virus (SqMV) is a comovirus, which has been identified in the Americas, Africa, Israel, Australia, New Zealand, Japan, Iran, the Philippines and Greece (Avgelis and Katis, 1989; Campbell, 1971; Dolores and Valdez, 1988; Izadpanah, 1987; Lockhart et al., 1982). The virus is transmissible by beetles, by mechanical inoculation, and by seeds, and causes serious diseases in *Cucurbitaceae* (Campbell, 1971; Nameth et al., 1986).

Seed transmission has been studied by several authors (Alvarez and Campbell, 1978; Lockhart et al., 1985; Nelson and Knuhtsen, 1973a, 1973b; Nolan and Campbell, 1984; Powell and Schlegel, 1970a). For fruit and, especially, for seed production the use of virus-free seed is of great importance.

The virus has several pathogenically different strains (Campbell, 1971). However, isolates of the virus could be grouped into 2 serological groups. Representatives of these

groups differ in seed transmissibility and to a certain extent, in host range and symptomatology (Knuhtsen and Nelson, 1968; Nelson and Knuhtsen, 1973a, 1973b).

Several methods have been developed to test seeds of *Cucurbitaceae* for SqMV. They include direct testing of parts of individual germinated seeds using ^{125}I-labelled antibodies (Powell and Schlegel, 1970b) or immunodiffusion (Lockhart et al., 1982), or testing of individual seedlings by inoculation to test plants and serological checking (Nelson and Knuhtsen, 1973a). Indexing for SqMV by testing groups of seeds was investigated by Faris-Mukhayyish and Makkouk (1983), and Nolan and Campbell (1984), who applied ELISA, and Lange et al. (1983), who applied ELISA and ISEM (immunosorbent electron microscopy). Faris-Mukhayyish and Makkouk (1983) could easily detect 1 infected squash embryo out of ten, applying the normal double-antibody sandwich ELISA (Clark and Adams, 1977). They obtained more clear results when testing whole germinated embryos as compared to coleoptiles only. Nolan and Campbell (1984) applied ELISA in microtiter plates for testing of whole (or parts of) individual cucurbit seeds and a bead ELISA procedure for testing groups of cucurbit seeds. The enzyme in their ELISA procedures was peroxidase. With the bead ELISA procedure they could detect 1 infected seed in 400, with a 90% probability. In their procedures seeds were soaked overnight in distilled water and then homogenized in extraction buffer. Testing individual seeds and seed parts they found that the presence of antigen in the embryo was independent of its presence in seed coat or papery layer. No embryos judged virus-free by ELISA produced virus-infected plants, but several embryos that contained antigen detectable by ELISA produced virus-free plants.

Lange et al. (1983) also using a peroxidase-ELISA, could reliably detect 1 infected melon seed in a hundred when mixing infected material with flour of healthy seeds. With ISEM, however, 1 infected seed in 10 000 was detected.

In 1985 SqMV was isolated for the first time in the Netherlands from glasshouse-grown melon plants, that very likely were infected through seed (H.J.M. van Dorst, Glasshouse Crops Research Station, Naaldwijk, personal communication). As a consequence the General Netherlands Inspection Service for Vegetable and Flower Seeds (NAKG) and Dutch seed companies felt the need for a quick and reliable routine test for SqMV in melon seeds. In this paper the preparation of an antiserum and the development of such a test on dry melon seeds are described.

Materials and methods

Virus isolates. The isolate used for purification and antiserum preparation (SqMV-M) was obtained from the Glasshouse Crops Research Station, Naaldwijk, the Netherlands. It originated from a melon plant infected from seed and was provisionally identified with the aid of an antiserum to SqMV prepared by Dr V. Lisa, Torino, Italy.

SqMV serotype 1 (SqMV-Ark; McLeod et al., 1988) was kindly supplied by Dr H.A. Scott, University of Arkansas, Fayetteville, USA. SqMV serotype 2 (SqMV-2), originating from Dr M.R. Nelson, University of Arizona, Tucson, USA, was kindly supplied by Dr B.E.L. Lockhart, University of Minnesota, St. Paul, USA.

Virus propagation. For purification, virus was propagated in cucumber (*Cucumis sativus*) and melon (*Cucumis melo*) grown at c. 20 °C, in an insect-proof glasshouse which was shaded during summer. During winter additional light was given by SON/T

lamps. Systemically infected material was taken.

Virus purification. Leaf material and buffers were chilled to 4 °C before use and all further treatments were done at this temperature. Two hundred grams of leaf material was homogenized in a Waring blender in 400 ml of di-sodium phosphate, 0.1 mol.l^{-1}, adjusted to pH 5 with citric acid, 0.1 mol.l^{-1}, to which 0.25% (w/v) sodium sulphite was added. The homogenate was squeezed through cheese cloth and the pH of the sap adjusted to 5 with citric acid, 0.1 mol.l^{-1}. After incubation for 2 h, the sap was centrifuged for 10 min at 10 400 *g* (All *g*-values are given at R_{max}). To the supernatant polyethylene glycol 8000, up to 6% (w/v), and sodium chloride, up to 0.2 mol.l^{-1}, were added while stirring. Stirring was continued for 30 min. The sediment obtained by centrifugation for 15 min at 10 400 *g* was resuspended in 100 ml of di-sodium phosphate buffer, 0.1 mol.l^{-1}, adjusted to pH 7 with citric acid, 0.1 mol.l^{-1} (buffer, pH 7), and the suspension stirred overnight. After centrifugation for 10 min at 10 400 *g*, the supernatant was centrifuged for 1.5 h at 67 600 *g*, and the sediment resuspended in 12 ml of buffer, pH 7. Stirring for 3 h was followed by centrifugation for 10 min at 7 700 *g*. One ml of supernatant was layered on 36 ml of a sucrose gradient (10-40% (w/v), in buffer, pH 7) and centrifuged for 1.5 h at 103 700 *g* in a Beckman rotor SW27. Virus-containing fractions were collected, diluted c. 1 : 1 with buffer, pH 7, and centrifuged for 3 h at 85 600 *g*. The sediment was resuspended in 20 ml of buffer, pH 7, and 24 g of cesium chloride and buffer, pH 7, were added to make a final volume of 48 ml. The suspension was thoroughly mixed and centrifuged for 22 h at 154 000 *g* in a Beckman rotor SW41Ti, 4 ml per tube with additional liquid paraffin. Virus-containing fractions were collected, dialyzed against buffer, pH 7, and isopycnic gradient centrifugation was repeated. After dialysis the virus was mixed with an equal volume of glycerol and stored at −20 °C until use. To estimate antigen concentration in purified preparations (a mixture of middle and bottom components) the average of the extinction coefficients at 260 nm of these components (6.8 and 8.65, respectively; Campbell, 1971) was taken.

Antisera. For antiserum preparation, a rabbit was injected intravenously twice, with an interval of three days, administering 100 μg of purified virus per injection. This was followed by 4 intramuscular injections of 250 μg of virus each, emulsified in Freund's incomplete adjuvant. They were given at intervals of 2-4 weeks, depending on antiserum titre. Antiserum titres were determined in the agar double-diffusion test (see below). Samples with equal titres were pooled.

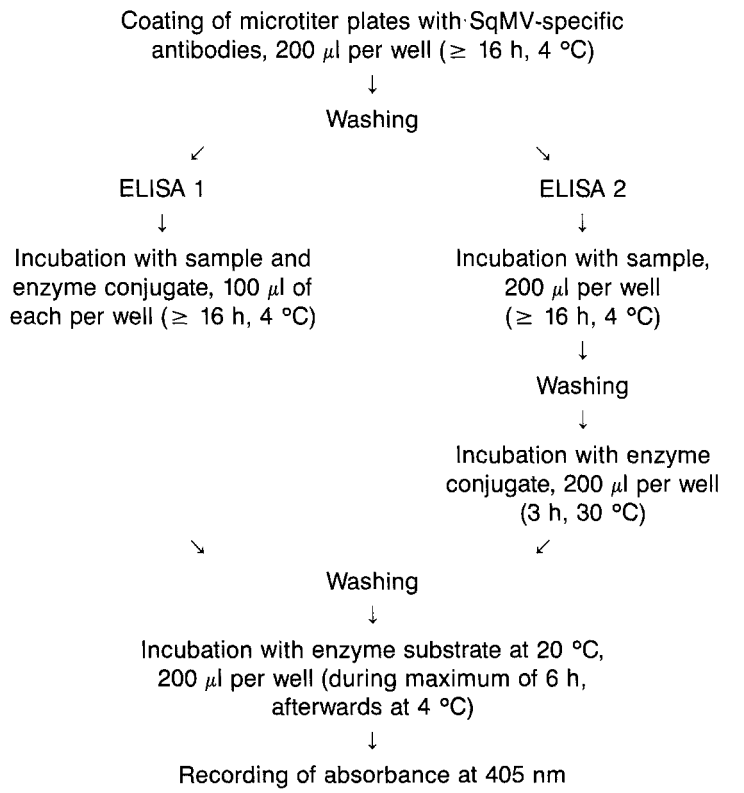
Dr V. Lisa, Torino, Italy, kindly provided an antiserum to SqMV. Antisera to serotypes 1 and 2 of the virus were kindly provided by Dr M.R. Nelson (1H and 2A) and Dr B.E.L. Lockhart (1, Arizona type strain).

Immunodiffusion. Serological agar double-diffusion tests were in 1% agar in saline, containing 0.05% sodium azide (w/v). Wells had a diameter of 3 mm. Distances between centres of antiserum and antigen wells in antiserum titration experiments were 7.1 mm. In spur tests these were 7.5 mm between adjacent antigen wells and 8.4 mm between antigen and antiserum wells. In titration experiments, four-fold serial dilutions of antisera were tested, starting with dilution 1 : 4. They were prepared in tris, 0.1 mol.l^{-1}, adjusted to pH 8 with citric acid, 0.1 mol.l^{-1}, containing 0.05% sodium

azide (w/v). Spur tests were with antiserum dilutions 1 : 4 and 1 : 16.

ELISA. Preparation of gamma-globulins (IgG) from antisera and conjugation with alkaline phosphatase was as described by Tóbiás et al. (1982). In the tests, coating IgG was used at 1 $\mu g.ml^{-1}$ and enzyme conjugate at final IgG concentrations of 0.25 $\mu g.ml^{-1}$. For seed testing ELISA was applied according to Flegg and Clark (1979), hereafter referred to as ELISA 1, with some modifications (Fig. 1), or according to Clark and Adams (1977), hereafter referred to as ELISA 2, with some modifications (Fig. 1). For seed testing phosphate-buffered saline (PBS; di-sodium/mono-potassium phosphate, 0.05 $mol.l^{-1}$, pH 7.4, containing 0.8% sodium chloride (w/v), 0.05% sodium azide (w/v)) with 0.15% Tween 20 (v/v) was used as extraction and conjugate buffer. When testing melon cotyledons or melon seedlings, 0.2% egg albumin (Sigma, grade II; w/v) was added to the extraction and conjugate buffer. Incubation with enzyme substrate (0.75 $mg.ml^{-1}$ p-nitrophenyl phosphate in 10% diethanolamine buffer (v/v), pH 9.8) took place under reduced light conditions at c. 20 °C. In between steps, plates were washed with tap water, using a washing device, which was followed by rinsing with 0.05% Tween 20 (v/v) in distilled water.

Fig. 1. Flow diagram of ELISA variants to test the presence of squash mosaic virus in melon seeds.



Results were recorded at 405 nm using a Titertek Multiskan photometer. Unless otherwise stated, results were considered positive if the average absorbance value of a sample was higher than the average absorbance value of the non-infected control sample plus three times the standard deviation of this control.

For testing the strain specificity of the antiserum, ELISA 2 was used. Conditions were as described for seed testing except that the extraction and conjugate buffer contained 0.1% Tween 20 (v/v) and 2% polyvinylpyrrolidone (w/v). When testing cucumber cotyledons, 0.1% egg albumin (Sigma, grade II; w/v) was added to the conjugate buffer. Incubation of enzyme conjugate was overnight at 4 °C. Washing in between steps was only with tap water.

Production of infected seeds. Virus-infected seeds were produced by inoculating cotyledons of 17 days old seedlings (first true leaf not yet fully developed) of honeydew melon (*Cucumis melo* L. var. *inodorus* Naud.) with infected cucumber-leaf extract. Plants were grown in a glasshouse set at 12 h at 23 °C (day) and 12 h at 20 °C (night). A minimum of 12 h light per day was given. If necessary, artificial light was added to normal day light. The seeds of each melon were harvested separately 90-120 days after inoculation of the plants. After harvesting, seeds were washed with tap water and dried during 18-24 h at 35 °C. In this paper, these seeds are referred to as one seed lot.

Preparation of extracts from individual seeds, seed parts and seed lots. Commercial seed lots of various origins were tested using 20 batches of 100 seeds per seed lot. Batches of 100 dry seeds were ground in a large coffee grinder with exchangeable grinder parts (Braun MX32/400 W). Extracts were prepared by thoroughly mixing c. 0.2 g of melon-seed flour with c. 0.8 ml of extraction buffer (the total volume was made up to 1 ml). The homogenates were left for 10 min at room temperature before use in ELISA.

Individual seeds or seed parts were squeezed with a glass rod in a final volume of 1 ml of extraction buffer and further treated as were seed lots. Unless otherwise stated, these extracts were considered undiluted extracts.

The sensitivity of ELISA in detecting SqMV in individual seeds and seed lots. Individual seeds were split and separated into seed coat, including the papery layer (nomenclature in accordance with Corner, 1976), and embryo (cotyledons, plumule, radicle, etc.). Subsequently, one half-embryo of each seed was tested in ELISA 1. To distinguish different levels of infection, each half-embryo was arbitrarily classified on the basis of absorbance values after 30 min of substrate incubation (Table 1). The remaining half-embryos of each seed were pooled per class, ground and diluted by mixing with flour of non-infected seeds (w/w), thus imitating mixing infected with non-infected seeds. Each dilution was tested in ELISA 1 and 2 in two replicates *in duplo.*

Results

Antiserum preparation. Two weeks after the first intramuscular injection the antiserum titre was 256. After one more intramuscular injection it became 1024.

Serological identification. The results of testing crude extracts from squash or cucumber, or purified virus (the latter only with SqMV-M) with 5 antisera to SqMV

Table 1. Classification of half-embryos on the basis of absorbance values at 405 nm after 30 min of substrate incubation in ELISA 1.

Classes	Absorbance values[1]	n^2
1	$0.1 < A_{405nm} \leq 0.5$	155
2	$0.5 < A_{405nm} \leq 1.0$	192
3	$1.0 < A_{405nm} \leq 1.5$	181
4	$1.5 < A_{405nm}$	54

[1] Non-infected control: $A_{405nm} = 0.050 \pm 0.006$ (n = 12). Buffer control: $A_{405nm} = 0.052 \pm 0.005$ (n = 12).
[2] Number of half-embryos tested.

Table 2. Titres in agar double-diffusion tests of five squash mosaic virus antisera tested against three virus isolates.

Antigen	Antisera					
	SqMV-M	SqMV-1Ar	SqMV-1H	SqMV-2A	SqMV-It	normal serum
SqMV-M	1024	4096	256	256	4096	—
SqMV-Ark	1024	4096	1024	256	4096	—
SqMV-2	1024	4096	1024	256	4096	—
Non-infected material	—	—	—	—	—	—

— = no reaction.

by immunodiffusion are summarized in Table 2. They show that, except for the antiserum to SqMV-1H, titres to the three antigens tested were equal for the individual antisera.

When placing SqMV-M and SqMV-Ark in adjacent wells, precipitation lines, obtained with the five antisera mentioned in Table 2, coalesced completely. Placing SqMV-M and SqMV-2 or SqMV-Ark and SqMV-2 in adjacent wells, precipitation lines obtained with the antiserum to SqMV-2 or with the Italian antiserum also coalesced completely. The same was true when SqMV-Ark and SqMV-2 were tested with the antiserum to SqMV-M. However, spur formation occurred with SqMV-M when SqMV-M and SqMV-2 were placed in adjacent wells opposite wells with the antiserum to SqMV-M and with the serotype 1 antisera and also with SqMV-Ark when SqMV-Ark and SqMV-2 were placed in adjacent wells opposite wells with the serotype 1 antisera mentioned in Table 2.

In ELISA 2, applying successive incubation of sample and enzyme conjugate, and testing crude extracts, 20 times diluted in extraction buffer, absorbance values after 30 min of substrate incubation were 1.31, 1.80, and 1.53 for SqMV-M, SqMV-Ark, and SqMV-2, respectively, when *Chenopodium quinoa* was the host, and 0.33, 1.68 and 1.53 with cucumber. Absorbance values with non-infected materials in this experiment were below 0.02.

Germination and infection of the self-grown seed lot. The percentage of germination of the self-grown seed lot was 83%. For this seed lot, the percentage of infected seedlings after germination varied from 82-96% between replicates (determined by ELISA); 100% of the seeds were infected with SqMV (either seed coat or embryo).

Preparation of extracts from individual seeds, seed parts and seed lots. Preliminary experiments had shown that grinding of dry seeds needed less space and time than soaking of seeds in buffer or germinating seeds prior to extract preparation. Therefore, grinding of dry seeds was used throughout this study. Small-seeded varieties of melon were easier to grind than relatively large-seeded melon varieties.

The sensitivity of ELISA in detecting SqMV in individual seeds and seed lots. Dilution ranges of extracts of 132 individual half-embryos prepared with buffer were tested in ELISA 1 and ELISA 2. The results are summarized in Table 3. They show that for testing individual seeds diluted in buffer, ELISA 1 gives higher absorbance values than ELISA 2 at dilutions up to 1 : 80 - 1 : 320. Analysis of variance showed interaction between dilutions and ELISA variants ($P < 0.001$), which indicates that relative differences between ELISA 1 and 2 are not the same at all dilutions and that both ELISA variants have different dilution end-points.

Dilution ranges of infected flour were prepared with non-infected flour and tested in ELISA 1 and 2. Table 4 shows the minimum sensitivity of both ELISA variants in detecting SqMV in flour of melon seeds for each of the classes of Table 1. Generally, the sensitivity increased for both ELISA variants when the substrate incubation period was increased from 1 to 4 h. A substrate incubation period of 24 h did not increase the sensitivity as compared to 4 h. The results show that after 4 h one infected embryo

Table 3. Comparison of ELISA 1 and ELISA 2 in testing dilutions of half-embryos in buffer.

Dilution	Absorbance values (A_{405nm})[1]	
	ELISA 1	ELISA 2
1 : 1	0.540	0.294
1 : 10	0.303	0.163
1 : 20	0.232	0.145
1 : 40	0.171	0.103
1 : 80	0.122	0.078
1 : 160	0.091	0.066
1 : 320	0.075	0.061
1 : 640	0.066	0.058
1 : 1280	0.060	0.056
1 : 2560	0.059	0.056
1 : 5120	0.057	0.056
1 : 10240	0.057	0.060
buffer	0.057	0.056

[1] Values represent means of absorbance values of 132 seeds after 30 min of substrate incubation; standard error of differences of means = 0.018.

Table 4. The minimum sensitivity of detecting squash mosaic virus in embryos of melon seeds by two ELISA variants.

Classes	ELISA 1[1]: substrate incubation period (h)			ELISA 2[1]: substrate incubation period (h)		
	1	4	24	1	4	24
1	1 : 80	1 : 320	1 : 320	1 : 40	1 : 320	1 : 320
2	1 : 160	1 : 320	1 : 640	1 : 160	1 : 640	1 : 640
3	1 : 320	1 : 640	1 : 640	1 : 160	1 : 640	1 : 640
4	1 : 160	1 : 640	1 : 640	1 : 160	1 : 160	1 : 160

[1] Results show the highest dilution of seed flour of which the corresponding absorbance value differed significantly from the non-infected control; per dilution only the lowest absorbance value out of 4 has been used for this table.

could be detected amid 320 embryos. Especially after 1 h, class 1 was not detected up to the same dilution as the other classes for both ELISA variants. Embryos belonging to class 4 were often not detected up to the same level as class 3. To compare the relative performance of ELISA 1 and 2 absorbance values of all dilutions were divided by the corresponding absorbance values of the non-infected controls (background values). Since the ratios cannot be reliably calculated when absorbance values exceed the value of 2.00, ratios were calculated after a substrate incubation period of 1 h only. The calculated ratios are summarized in Table 5. ELISA 1 gave significantly higher ratios

Table 5. Comparison of two ELISA variants at different dilutions of infected seed flour after 1 h of substrate incubation.

Dilution	A_{405nm} dilution / A_{405nm} non-infected control[1]							
	ELISA 1 : class				ELISA 2 : class			
	1	2	3	4	1	2	3	4
1 : 5	9.8	11.0	8.1	9.1	4.3	3.2	4.1	5.3
1 : 10	11.2	16.7	11.5	20.1	4.0	4.2	3.1	6.0
1 : 20	6.2	9.5	19.9	13.2	3.0	2.5	2.9	4.8
1 : 40	5.2	3.9	11.2	5.8	2.4	1.7	4.9	2.6
1 : 80	1.5	3.2	4.6	5.1	1.2	1.9	1.6	3.0
1 : 160	1.1	1.5	7.7	2.0	1.1	1.7	1.6	1.3
1 : 320	1.1	1.4	2.2	1.1	1.1	1.3	1.3	1.0
1 : 640	0.9	0.9	1.3	1.0	1.0	1.0	1.2	1.0
1 : 1280	0.9	1.1	1.1	1.0	1.1	1.1	0.9	1.0

[1] A_{405nm} dilution / A_{405nm} non-infected control = ratio of absorbance values of the dilution divided by the absorbance values of the corresponding non-infected control; each value represents the lowest of 4 values per dilution. Absorbance value of non-infected control in ELISA 1: 0.050 ± 0.005 (n = 10), absorbance value of non-infected control in ELISA 2: 0.046 ± 0.003 (n = 10).

than ELISA 2 at nearly all dilutions ($P < 0.00003$; Wilcoxon matched-pairs signed-rank test according to Siegel, 1956). For ELISA 1 it was striking that the ratio for the 1 : 5 dilution was sometimes lower than the ratio for the 1 : 10 dilution.

Discussion

Serological identification. The results of serological titration experiments (Table 2) do not allow distinction between serological groups. Results of spur tests, however, indicate that SqMV-M, like SqMV-Ark, belongs to the serotype 1 group of SqMV. This is supported by the findings of Nelson and Knuhtsen (1973a, 1973b) that, unlike serotype 2 isolates, serotype 1 isolates are seed-transmitted in *Cucumis* spp.

In ELISA, applying successive incubation of sample and enzyme conjugate, the SqMV-M antiserum reacted well with the three isolates tested. However, when cucumber was the host, the homologous reaction was weaker than the heterologous reactions. This indicates that in cucumber the concentration of SqMV-M was lower than the concentration of the other isolates and that both serotypes may be detected in infected plants by ELISA using the SqMV-M antiserum. Seeds with SqMV serotype 2 were not available for testing.

Sample treatment. Especially for relatively large seeds like pea and melon seeds, it is advisable to test dry seeds first. Positive samples may be confirmed by testing seedlings (Van Vuurde et al., 1988). Instead of using a growing-on test, ISEM may be used to verify doubtful ELISA reactions. For SqMV a sensitive ISEM method has already been described (Lange et al., 1983).

Testing germinated seeds will give an estimation of the percentage of seeds with seed-transmitted virus (seed transmission is defined here as the percentage of infected seedlings which arise from infected seeds). Testing dry, ground seeds results in an estimation of the percentage of seeds containing seed-borne virus (transmitted and non-transmitted virus). SqMV can be present in localized parts in the embryo (Nolan and Campbell, 1984). As a result of this phenomenon it is possible that no seed-transmission will occur. However, it still indicates that the seed lot originates from infected plants and thus is suspect.

The extraction buffer used in this study is also used at the Netherlands Government Seed Testing Station for the detection of pea seed-borne mosaic virus in pea seeds. Absorbance values of non-infected seed samples were generally very low. Therefore the standard addition of sugars to the extraction buffer as suggested by other research workers (Nolan and Campbell, 1984) was not necessary. However, for some commercial seed lots background reactions in ELISA were sometimes diminished by adding 5% (w/v) mannose and glucosamine or 0.2% (w/v) sodium diethyldithiocarbamate.

The sensitivity of ELISA in detecting SqMV in individual seeds and seed lots. To determine the size of a sample or a subsample, the sensitivity of detection of ELISA must be known beforehand. The sensitivity of ELISA 1 and 2 in detecting SqMV in individual seeds were compared to check whether there was a 'hook effect' in ELISA 1 (Reinhardt and Richter, 1988). The 'hook effect' implies that the absorbance increases with increasing antigen dilution up to a maximum, after which absorbance decreases again. In general, the 'hook effect' was not found in this study (Table 3). This is probably

due to the fact that seeds and seed parts were already diluted c. 5 times after squeezing.

The sensitivity of ELISA in testing seed lots was tested by mixing infected with non-infected flour, thereby imitating mixing infected and non-infected seeds (without seed coat). The weak embryonic tissue of the seed was used because these parts were easier to homogenize and to handle in dilutions. The percentage of seed transmission is very likely to be determined by the percentage of infected embryos. However, possible mechanical inoculation of seedlings from virus in seed coat and papery endotesta should not be excluded completely (Alvarez and Campbell, 1978; Nolan and Campbell, 1984). After a substrate incubation period of 4 h, with ELISA 1 and ELISA 2 in all cases one infected seed can be detected in a sample of 100 seeds (the size of one subsample in routine seed health testing for SqMV). When mixing infected with non-infected seeds (with seed coat) a sensitivity of at least 1 : 100 was also obtained for both ELISA variants (results not shown). In both ELISA variants seeds of class 4 could be less far diluted than those of classes 2 and 3 (Table 4). The reason for this is not clear. In ELISA 1 ratios for the 1 : 10 dilution were higher than for the 1 : 5 dilution. In ELISA 2 this effect was less or not present although the same extracts were tested as in ELISA 1 (Table 5). A full explanation for this effect has not been found yet. ELISA 1, in which sample and conjugate are incubated simultaneously, gave higher differences between absorbance values of infected and non-infected extracts than ELISA 2, also at relatively high dilutions (low antigen concentrations). ELISA 1 seems therefore more suited than ELISA 2 to detect relatively low antigen concentrations as already concluded by Van Vuurde and Maat (1985) for lettuce mosaic virus in lettuce seeds and pea early-browning virus in pea seeds. In addition to the better sensitivity, ELISA 1 is more suited for routine usage because of its higher efficiency (one incubation step less than in ELISA 2).

Routine testing of seed lots. For routine-indexing of melon seed lots on SqMV-infection, 20 subsamples of 100 seeds are presently tested. The division of a sample into subsamples makes it possible to give an estimated percentage of infected seeds (Van Vuurde and Maat, 1983). Various commercial melon-seed lots (e.g. cantaloupe, honeydew melon and other varieties) originating from different parts of the world (China, France, Israel, the Netherlands, Taiwan, Thailand, UK, USA) have already been tested using the ELISA 1 method. By using this method it is possible to find infected seed lots, which will result in preventing SqMV from establishing in the Netherlands.

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Samenvatting

Detectie van het pompoenemozaïekvirus in zaden van meloen (Cucumis melo) door middel van ELISA

Het pompoenemozaïekvirus gaat over met het zaad van verscheidene *Cucurbitaceae*. Het gebruik van virusvrij zaad is belangrijk om te voorkomen dat het virus zijn intrede doet in Nederland en zich naar andere landen verspreidt.

Een antiserum werd geproduceerd tegen een serotype 1 isolaat van meloen. Met behulp van dit antiserum werd een ELISA ontwikkeld om pompoenemozaïekvirus in zaden van meloen aan te tonen. Twee varianten van ELISA werden vergeleken, namelijk een variant waarbij monster en enzymconjugaat gelijktijdig geïncubeerd werden (ELISA 1) en een variant waarbij monster en enzymconjugaat na elkaar geïncubeerd werden (ELISA 2). De gevoeligheid van de ELISA varianten werd uitgetoetst door meel van zieke zaden in verschillende verhoudingen te mengen met meel van gezonde zaden. Het pompoenemozaïekvirus werd met beide ELISA varianten aangetoond in verdunningen van 1 : 160 (1 deel meel van zieke zaden gemengd met 159 delen meel van gezonde zaden) of hoger na 4 uur incubatie met substraat. ELISA 1 gaf doorgaans hogere extinctiewaarden dan ELISA 2 voor bijna alle verdunningen. Omdat ELISA 1 ook nog sneller is dan ELISA 2, wordt ELISA 1 aanbevolen voor routinematig gebruik. Wanneer voor routinematig gebruik 100 meloenezaden per submonster getoetst worden, kan het pompoenemozaïekvirus betrouwbaar worden aangetoond. In Nederland worden momenteel per zaadpartij 20 submonsters van 100 zaden getoetst.

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