TOWARDS A RAPID AND RELIABLE DETECTION METHOD FOR IRIS SEVERE MOSAIC VIRUS IN IRIS BULBS

Cornelia I.M. van der Vlugt (1,2), A.F.L.M. Derks (1), Jeanne Dijkstra (2), Rob Goldbach (2).

1. Bulb Research Centre, P.O.Box 85, 2160 AB Lisse, The Netherlands.
2. Agricultural University, Department of Virology, P.O. Box 8045, 6700 EM Wageningen, The Netherlands.

Abstract

Detection of iris severe mosaic virus (ISMV) in ISMV-infected iris bulbs during storage, by either ELISA or electron microscopy has been problematic. We have applied different storage-temperature treatments and a cutting method as possible procedures to enhance ISMV detection in stored iris bulbs cv. Professor Blaauw. While at lifting ISMV could not be detected in the bulbs, gradually the virus became detectable in time when bulbs were stored at about 17°C. Better results were obtained when the cutting method was employed. Using ELISA, a 100 % score was obtained for infected bulbs that were cut 1 month prior to testing. The virus could be detected only in tissue adjacent to the cut surface. These results offer good prospects for the development of a reliable detection method for ISMV in iris bulbs.

1. Introduction

Annually, approximately 700 million iris bulbs are produced in the Netherlands of which 70 % are exported. To improve the quality of the iris-stocks, bulbs have to be indexed for the presence of viruses, a.o. iris severe mosaic virus (ISMV). This virus belongs to the potyvirus group and is serologically distinct from other iris-infecting members of this group, such as iris mild mosaic virus (IMMV), and bean yellow mosaic virus (BYMV). ISMV has been found to occur naturally in bulbous and rhizomatous irises and in *Crocus vernus*. In iris, ISMV causes distinct chlorotic stripes or mosaic patterns (Brunt et al., 1988). It has been difficult to detect ISMV in iris bulbs by enzyme-linked immunosorbent assay (ELISA) or electron microscopy during storage after lifting in late August until planting in October. By contrast, IMMV is readily detected in Dutch bulbous irises during the same period (Van Schadewijk et al., 1988). Stein et al. (1986) have shown improved detection of BYMV in gladiolus corms during storage by cutting them. The latter virus was detected in the cut area dependent on storage time and temperature. In this paper we report on the detection of ISMV by double antibody sandwich (DAS) ELISA and immunosorbent electron microscopy (ISEM) in secondarily infected iris bulbs by different temperature treatments during storage and by cutting the bulbs.

2. Material and methods

2.1 Virus and antiserum

To avoid possible contamination with other iris-infecting viruses, in particular IMMV (Asjes, 1979), ISMV was propagated in *C. vernus* cv. Remembrance, and purified according to Derks and Vink-Van den Abeele (1980). Antiserum was prepared according to Derks et al. (1982), and tested for its specificity. The antibodies did not cross-react in ELISA with other iris-infecting potyviruses.

2.2 Bulb material

In mid June 1986, virus-free iris plants of *Iris* x *hollandica* cv. Professor Blaauw, grown in the field under a gauze, were inoculated with sap from ISMV-infected *C. vernus* cv. Remembrance. In 1987, this inoculated stock was grown again under a gauze in the field and healthy plants were removed. Bulbs from this infected stock and from a virus-free stock were lifted on 28 August and dried for three days. Thereafter, bulbs from both stocks were stored in different lots at 2,5,9,13,17,23 and 30°C. Bulbs varying in weight from 7 to 35 g were equally distributed over the different temperature treatments. On 14 September bulbs stored at 30°C were treated with 500 ppm ethylene for 24 h as a standard procedure.

2.3 Sampling of bulb material

After 0,1,2,3 and 5 months of storage, we collected 10 ISMV-infected and 3 non-infected bulbs from each temperature treatment, and tested them for the presence of ISMV by cutting a sample of 2.0 g from each bulb (denoted A, figure 1). The cut bulbs were immersed in water for 20 min to reduce infection with micro-organisms (mainly *Penicillium* spp.) during prolonged storage. They were then stored at room temperature for 1 month, sampled in portions of 2.0 g each (denoted B, C and D, figure 1) and tested.

2.4 ELISA

ELISA was performed according to Clark and Adams (1977), with some minor modifications. Wells of ELISA plates (Petra plastics type F) were filled with 200 µl fluid in the following procedure. Coating of the plates was with 1 µg gammaglobulin per ml and the incubation time was 1 - 3 days at 6°C. The plates were washed several times with deionized water and finally once with 0.05 % Tween 20 in water. For preparation of the antigen samples, 2.0 g of bulb material was ground in 10 ml of 66 mM Na-phosphate buffer, pH 8.3, containing 130 mM NaCl, 3 mM KCl, 3 mM NaN_3. The starch and other coarse material was allowed to sediment for 0.5 - 2.0 h and the liquid was collected. Plates filled with the latter were incubated overnight at 6°C and washed again. The gammaglobulins were conjugated according to Derks et al. (1988). The suspension obtained was diluted with a 47 mM Na-K phosphate buffer, pH 7.4, containing 130 mM NaCl, 3 mM KCl, 3 mM NaN_3, 0.05 % Tween 20, 0.4 % normal horse serum. Plates with diluted conjugated gammaglobulin suspension were incubated at 37°C for 3 h, and washed. Incubation with the substrate (p-nitrophenyl phosphate at a concentration of 0.5 mg/ml) was at 37°C for 1 h. The absorbance values at 405 nm were measured with a Titertek Multiskan or a Dynatech MR 600 spectrophotometer.

Absorbance values of ISMV-free controls ranged from 0.00 to 0.09. Values above 0.15 were considered positive as they could be correlated with the presence of virus particles as observed in ISEM.

2.5 ISEM

This procedure was based on the methods of Derrick and Brlansky (1976) and Milne and Luisoni (1977) with some minor modifications. For trapping, grids with a pioloform-F carbon film were incubated with antiserum 1:20 diluted with 70 mM phosphate buffer, pH 7.0, at 37°C for 30 min, and washed in the same buffer for 5 min at room temperature. The grids were then floated for 1 h at room temperature on a droplet of antigen sample as prepared for ELISA, washed and subsequently floated on a droplet of the diluted antiserum at 37°C for 30 min. Thereafter, the grids were successively rinsed in buffer and in demineralized water, and then floated on a droplet of 2 % uranyl acetate for about 2 min. Excess staining fluid was absorbed by filter paper. The grids were then air-dried and examined with a Philips 201 electron microscope.

3. Results

3.1 Effect of the storage-temperature treatment on the detectability of ISMV by ELISA

Immediately after lifting and drying, no ISMV could be detected in iris bulbs (figure 2). After storage for 1 month, there was a slight increase in the mean ELISA values at different temperatures, especially at 17°C (figure 3). At this temperature 3 out of 10 bulbs were found to be positive. Bulbs stored at 17°C for 2 and 3 months showed gradually increasing A405 values (data not shown) and a larger number of bulbs became positive. After a storage period of 5 months at 17°C, ELISA values were quite high (figure 4) and all bulbs were rated as positive (lowest A405 value 0.56). At 13 and 23°C also there was an increase, but less pronounced than that at 17°C (figure 4). At other temperatures the number of positive bulbs was very low or even zero.

3.2 Effect of cutting on the detectability of ISMV by ELISA

3.2.1 Detection of ISMV in sample B of cut iris bulbs.

Sample B (figure 1) of cut bulbs, stored for another month at room temperature after cutting, gave high readings and therefore a good detection of the ISMV in infected bulbs (figures 2 and 5): the cut bulbs gave a 100 % score already after 1 month of storage, in contrast to the uncut bulbs giving only a 30 % score when stored at 17°C, as seen earlier. Similar results were obtained with bulbs stored for 2 and 3 months prior to cutting (data not shown). However, when bulbs were stored longer (for 5 months, figure 6), the effect became less pronounced.

3.2.2. Detection of ISMV in different samples of cut iris bulbs.

Figure 7 shows the ELISA values of bulbs stored at 30°C for 1 month prior to the first sampling. After storing these cut bulbs for 1 month at room temperature, the highest A405 values were

obtained with sample B, those with samples C and D were invariably lower. With D samples of small bulbs (S, about 7-16 g), ELISA values were quite high but lower than those with B and C, whereas the values of D samples of large bulbs (L, about 17-35 g) did not exceed the level of uninfected bulbs. This shows a decrease in ELISA values with increasing distance from the site of cutting. To demonstrate that the high ELISA values with B and C resulted from a combined effect of cutting and subsequent storage, and not from the position of the samples in the bulb, uncut infected bulbs were divided into 7 - 12 parts and each part was tested separately. All slices of one bulb were found to have comparable A405 values (data not shown).

4. Discussion

Iris bulbs are stored at different temperatures depending on their use. Optimal storage conditions used for commercial greenhouse forcing are a sequence of 1 month at 30°C, 2 months at 9°C and 2 weeks at 17°C. The 17°C period is known to be optimal for good flower production (Beyer and Slootweg, 1963). Storage at 17°C (for 2-3 months) is also applied for planting material (Tips, 1970). In our experiments, each batch of bulbs was stored at one temperature only. We found that in infected bulbs stored at 17°C for several months, ISMV became gradually detectable. The improved detectability may be correlated with certain physiological activities brought about by the 17°C treatment. Our results clearly show that cutting and subsequent storage improved the detection of the virus to a much greater extent than a mere temperature treatment. After storage for 1 month the virus was readily detected in tissue adjacent to the site of cutting, but not in that farther away from it. Stein et al. (1986) observed a similar phenomenon with BYMV in gladiolus corms. Apparently, physiological activity induced by wounding stimulates processes leading to better detection of these viruses. From our results it can be concluded that the prospects are favourable for the development of a test for early detection of ISMV in iris bulbs. One should, however, bear in mind that the data presented here were from experiments with secondarily infected bulbs only.

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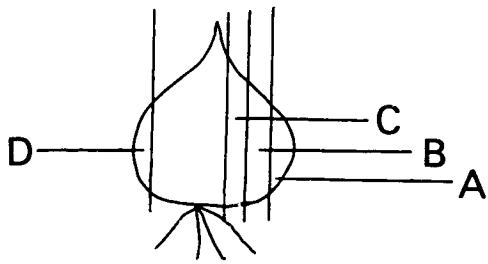

Figure 1. Scheme of an iris bulb showing samples tested for the presence of ISMV. Sample A was cut off at different testing dates and the samples B,C and D after storing the cut bulbs at room temperature for 1 month.

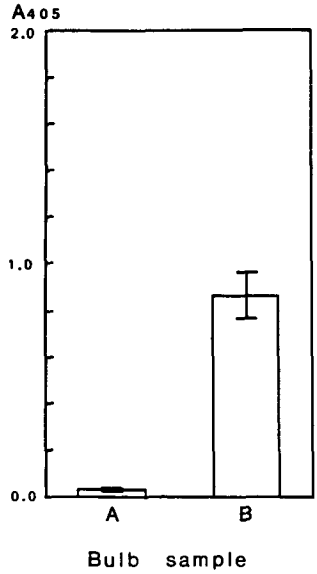

Figure 2. Detection of ISMV by ELISA in samples A from infected bulbs, immediately after lifting and in B after storing the respective cut bulbs at room temperature for 1 month. Mean absorbance values at 405 nm (A405) with standard errors (n=10) are indicated.

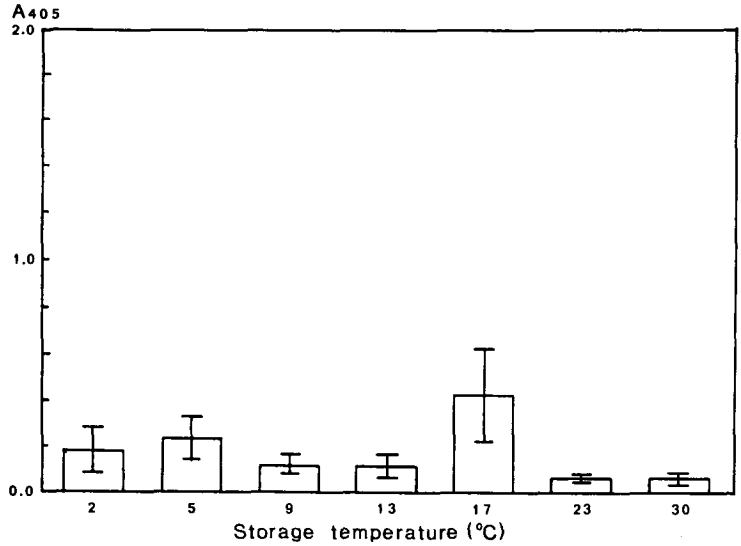

Figure 3. Detection of ISMV by ELISA in samples A from infected bulbs stored at different temperatures for 1 month. Mean absorbance values at 405 nm (A405) with standard errors (n=10) are indicated.

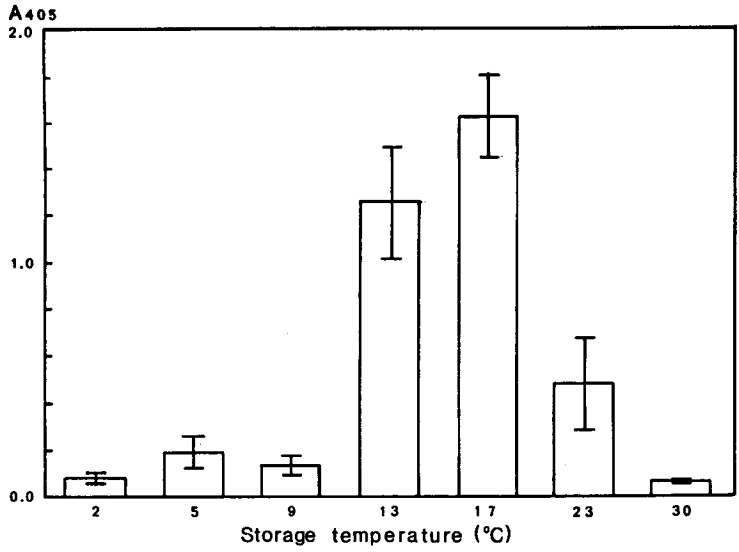

Figure 4. Detection of ISMV by ELISA in samples A from infected bulbs stored at different temperatures for 5 months. Mean absorbance values at 405 nm (A405) with standard errors (n=10) are indicated.

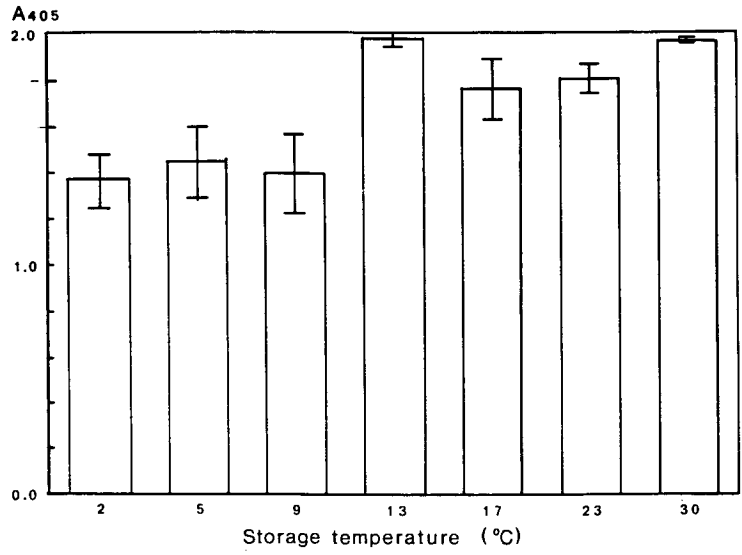

Figure 5. Detection of ISMV by ELISA in samples B from infected bulbs stored at different temperatures for 1 month. Mean absorbance values at 405 nm (A405) with standard errors (n=10) are indicated.

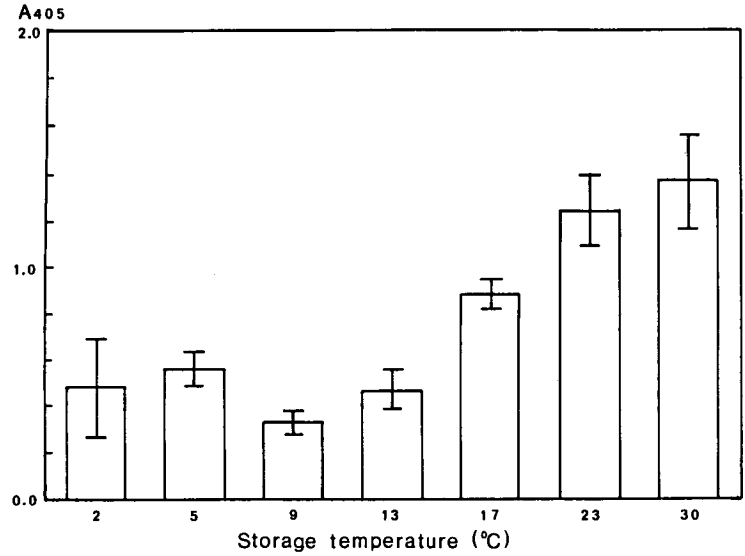

Figure 6. Detection of ISMV by ELISA in samples B from infected bulbs stored at different temperatures for 5 months. Mean absorbance values at 405 nm (A405) with standard errors (n=10) are indicated.

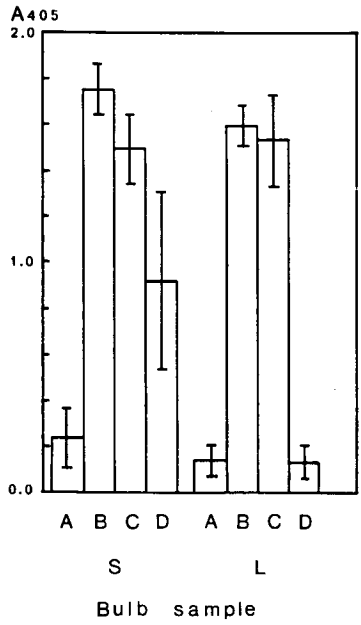

Figure 7.
Detection of ISMV by ELISA in samples A,B,C and D from small (S) and large (L) infected bulbs.
Mean absorbance values at 405 nm (A405) with standard errors of 4 (S) and 6 (L) samples are indicated.
Sample A was taken from bulbs stored for 1 month at 30°C and the cut bulbs were stored at room temperature for another month, whereafter samples B,C and D were tested (see figure 1)