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Dieffenbachia maculata (Lodd.) G. Don.
Virus attack in Danish cultures, survey and diagnosis

Virusangreb i danske kulturer, kortlægning og diagnosticering

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Summary

Dieffenbachia maculata is one of the more important pot plants in Denmark, and therefore a selection work was started in order to achieve improvement and health of mother plants.

In this respect a survey concerning virus attack in commercially grown *Dieffenbachia* cultures was carried out in 11 specialized nurseries. Further more healthy looking plants were collected and assessed regularly for possible virus symptoms during the whole year.

Dasheen mosaic virus (DMV) was found in 2 of 4500 assessed plants showing chlorotic vein bands and retarded growth. Serological reactions were achieved with DMV-antiserum in immunodiffusion test and virus particles trapped and decorated by immunoelectronmicroscopy. The DMV was transmitted to *Philodendron* varieties by sap and in the non persistent manner by *Myzus persicae*.

In the healthy looking plants tobacco necrosis virus (TNV) was found once in connection with a sporadic vein clearing. In the meantime TNV was not shown again, and back transmission to *Dieffenbachia* failed.

The present results show, that healthy *Dieffenbachia* plants regarded as improved material were found in commercially grown cultures by a careful selection work.

Key words: *Dieffenbachia*, selection, dasheen mosaic virus, diagnosis, survey, tobacco necrosis virus.

Resumé

Dieffenbachia maculata er en af de vigtige potteplantekulturer i Danmark, og i denne forbindelse er der påbegyndt et selektionsarbejde for at fremskaffe et bedre og sundt plantemateriale.

En kortlægning over virusangreb i kommercielle kulturer blev udført i 11 specialgartnerier. Desuden blev indsamlede, sundt udseende planter, symptomregistreret regelmæssigt igennem et helt år.

Dasheen mosaik virus (DMV) blev iagttaget i 2 planter med nervebåndsklorose og hæmmet vækst af i alt 4500 registrerede. Serologisk reaktion er opnået med DMV-antiserum i immunodiffusionstest og viruspartikler fanget og dekoreret ved immunoelektronmikroskopi. DMV blev overført til *Philodendron* arter ved henholdsvis plantesaft og bladlus (*Myzus persicae*) efter kort tids sugning på infektorplanten.

Tobaknekrosevirus (TNV) er en enkelt gang blevet påvist i planter, der periodevis viste nervelysning. TNV kunne imidlertid ikke påvises igen, og tilbageføring af viruset til *Dieffenbachia* mislykkedes.

De opnåede resultater viser, at sunde *Dieffenbachia* planter er fundet i kommercielle kulturer ved en omhyggelig udført selektion.

Nøgleord: *Dieffenbachia,* selektion, dasheenmosaikvirus, kortlægning, diagnosticering, tobaknekrosevirus.

Introduction

Dieffenbachia is one of our more important pot plants, grown for its variegated leaves. The culture includes many varieties, and new mutations in the coloration pattern increase the number of varieties.

Regarding diseases *Dieffenbachia* mainly suffers from attack of the bacteria *Erwinia chrysanthemi,* but even virus infection has been described as a problem for the culture (*Hakkaart & Waterreus,* 1976; *Hill & Wright,* 1980; *Jensen* 1976; *Paludan & Thomsen,* 1976, 1980; *Wisler et al.* 1978; *Zettler et al.* 1970).

In order to improve the quality of the plant material the nurserymen have asked the Danish Nursery Control Commission and its Committee for Pedigree Work to start a selection work so as to find the best and the most genetically stable clones, free from bacteria and virus diseases.

This paper deals with the virus part of the work comprising survey, assessment of symptoms and diagnosis by infection experiments, serology and electronmicroscopy carried out at the Institute of Plant Pathology.

A virus in *Dieffenbachia* was described by *Zettler et al.* in 1970 as dasheen mosaic virus, which infect the members of the family *Araceae.* The virus is transmitted both by aphids in the non persistent manner and by plant sap and belongs to the potyvirus group with flexous filamentous particles about 750 nm long, including cylindrical inclusions (»pinwheel«) in the host. The virus is found mainly in tropical and subtropical regions, but has spread to most parts of the world including countries in Europe such as Holland (*Hakkaart & Waterreus,* 1976), England (*Hill & Wright,* 1980) and Denmark (*Jensen* 1976; *Paludan & Thomsen* 1976, 1980).

The virus symptoms consist of mosaic and distortion and may disappear during certain parts of the growing season.

Methods

Selection

In order to collect plant material, 11 different nurseries all specializing in large scale growing of *Dieffenbachia* were visited. Together with the cultivator the most desirable, true to type and healthy looking plants were selected representing 9 different varieties. These plants were cultivated and propagated for further genetic tests at the Institute of Glasshouse Crops at Årslev.

Survey

During the collection in the nurseries a survey concerning virus infected plants was carried out based on mosaic symptoms in leaves from saleable and mother plants. At each nursery at least 500 *Dieffenbachia* plants were assessed.

Infection experiments

The selected, healthy looking plants as well as plants with virus-like symptoms were assessed for leaf symptoms on the following dates: 16th December 1980, 11th February, 4th March, 9th April, 7th May, 23rd July, 25th August and 28th October 1981. The varieties and number of plants included: 'Candida' (8), 'Camilla' (47), 'Carina' (12) 'Compacta' (56), 'Exotica Perfection' (16), 'Janet' (10), 'Marianne' (24), 'Veerle' (9), and 'no name' (4).

Dieffenbachia plants suspected of virus infection were also tested by sap and dry inoculation, using carborundum 700 to *Chenopodium quinoa, Philodendron selloum, P. bipinnatifidum* and *P. scandens.* Transmission by *Myzus persicae* with

long and short time feeding was also carried out using the 2 last mentioned *Philodendron* varieties.

Serology

Virus transmitted to *Philodendron selloum* was diagnosed by the serological double immunodiffusion method (Ouchterlony-test) using antiserum against dasheen mosaic virus (DMV) kindly provided by *F. W. Zettler* in Florida. The test was conducted in 0.8% agar gel containing 0.2% sodium dodecyl sulphate (SDS), 0.1% sodium azid and 0.7% sodium chloride. The antigen was homogenized in 1 part water and afterwards added 1 part SDS 0.3% solution.

Electronmicroscopy

Immuno electronmicroscopy (ISEM) was used to identify the DMV, which only rarely could be detected by ordinary leaf-dip electronmicroscopy. ISEM *(Milne & Luisoni,* 1975) was done with DMV-antiserum. The decoration was performed with a mixture of dasheen mosaic virus and potato virus Y (PVY) to demonstrate the coating of the DMV compared to another serologically, unrelated virus of the same group (potyvirus).

Ultramicrotomy

Material of a DMV infected *Dieffenbachia* was cut in pieces of 10 × 1 mm fixed in Karnowsky fixative (*Karnowsky,* 1965) for 2 hours, impregnated in saturated uranyl acetate for 1 hour, dehydraded in a serie of ethanol postfixed in osmiumtetroxide (*Caulfield,* 1957) 2% 17 hours at 4°C and finally embedded in Spurr resin (*Spurr,* 1969). The sectioning took place on a LKB ultramicrotome and the sections were stained in lead citrate (*Reynolds,* 1963) and uranylacetate mixed with methanol:ethanol and water (1:1:v/v/v) (*Hooper & Weise,* 1972).

Results

Symptom assessment in Dieffenbachia cultures

In 11 commercially grown *Dieffenbachia* cultures, virus symptoms were assessed in 2 of 4500 plants. The plants (*Dieffenbachia maculata* (Dm) no. 7 and 8) were found in a nursery among the mother plants showing retarded growth, deformed leaves and conspicuous chlorotic vein bands typical of infection of dasheen mosaic virus. The leaf symptoms were visible during the whole year, when grown at the Institute of Plant Pathology.

Symptom assessment in selected, healthy looking plants

Leaf symptoms typical of the DMV have never been assessed in any of the selected plants during the whole year. Meanwhile at the symptom assessment in March 64 of 179 plants, irrespective of the varieties, showed weak vein clearing in the tip of the youngest leaves, most visible along the green coloured edge (Dm 11, 12). These symptoms were not seen later on in the original plants, though they turned up again more clearly at December (62 plants) and May (13 plants) in the first developed leaf after propagation but for a short period only (Dm 13, 14).

Diagnosis

Dieffenbachia plants from the survey showing chlorotic vein bands (Fig. 1) and selected plants periodically showing weak vein clearing (Fig. 2) have been diagnosed by indicator plants, serology and electronmicroscopy. Results from the infection experiments are shown in Table 1.

Dm 7 and Dm 8 isolates

Typical DMV symptoms as vein clearing, vein bands and deformed leaves (Fig. 3) were achieved in the *Philodendron* varieties inoculated with the virus isolates Dm 7 and 8 from *Dieffenbachia.* Appearently the Dm 7 is less infectious than Dm 8, causing weaker and less persistent symptoms mostly as a vein clearing.

Virus isolate Dm 8 from *Philodendron* varieties reacted positively with DMV-antiserum in double immunodiffusion test, as well as DMV-antigen received from *F. W. Zettler,* while healthy sap gave a negative result.

The ISEM-trapping and -decoration showed clearly the successful coating of DMV particles from infected *Philodendron* (Dm 8) in contrast to the undecorated PVY-particles (Fig. 4).

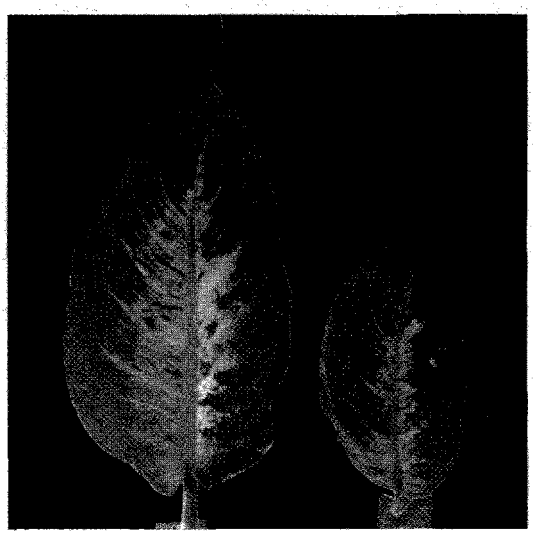

Fig. 1. *Dieffenbachia maculata* naturally infected with dasheen mosaic virus showing chlorotic spotting and vein bands.

Fig. 2. *Dieffenbachia* showing periodic vein clearing in first developed leaf after propagation, the cause being unknown.

Table 1. Infection experiments with *Dieffenbachia* showing leaf symptoms

Transmission method and indicator plants	Dm no.	Reaction in no. of plants of total inoculated: Control	Chlorotic vein bands 7	Chlorotic vein bands 8	Vein clearing 11, 12, 13, 14
Sap inoculation					
Chenopodium amaranticolor		0/3	–	–	0/4
Chenopodium quinoa		0/3	–	–	1/7[1])
Philodendron bipinnatifidum		0/3	2/3[2])	3/3[2])	0/27
Dry inoculation					
Chenopodium quinoa		0/3	–	–	0/14
Chenopodium quinoa[3])		0/3	–	–	0/10
Philodendron bipinnatifidum		0/3	1/4	2/2	0/8
Philodendron selloum		0/3	1/6	3/3	0/12
Aphids (*Myzus persicae*)					
Acquisition feeding period:					
20 min:	*Philodendron bipinnatifidum*	0/3	0/3	–	0/6
	Philodendron scandens	0/3	1/1	–	0/1
24 hours:	*Philodendron bipinnatifidum*	0/3	0/3	–	0/6

[1]) Local lesion. Tobacco necrosis virus infection shown
[2]) Systemic vein bands and vein clearing. Dasheen mosaic virus infection shown
[3]) Plants placed 24 hours in darkness before inoculation
–: No test performed

Fig. 3. *Philodendron selloum* showing extremely yellow vein bands and leaf deformation, caused by sap inoculated dasheen mosaic virus. *Dieffenbachia* infector to the right.

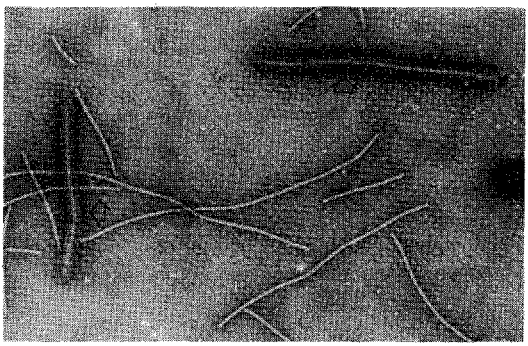

Fig. 4. Two decorated virus particles from *Dieffenbachia* (Dm 7) covered with antibodies from antiserum against dasheen mosaic virus. Not decorated PVY-particles added as control. × 63.7000.

Virus particles in several samples of Dm 8 were not observed using the EM-leaf dip preparation method.

Pinwheels and dense bands were detected in the cytoplasm of parenchymatic tissue of *Dieffenbachia* isolate Dm 7 (Fig. 5). The amount of pinwheels and dense bands was lower than most found in the patato virus Y-group, but even if complete pinwheels were not seen, the pattern of pinwheels were convincing. This result together with the ISEM-test leave no doubt about the presence of dasheen mosaic virus.

Dm 11, 12, 13 and 14 isolates

None of the inoculated *Philodendron* varieties have shown any virus symptoms and neither virus particles nor pinwheel formations have been observed in *Dieffenbachia* isolates using EM-dipp preparations and thin sections respectively.

In *Chenopodium quinoa* a single local lesion occurred once by sap inoculation from the Dm 13 isolate. This infection showed by several transfers to other indicator plants and by double immunodiffusion test to be a strain A of tobacco necrosis virus (TNV).

The TNV was back inoculated to *Philodendron* varieties using sap from *Nicotiana t.* 'Xanthi' with local lesions. However no virus symptoms could be assessed and no virus infection could be shown by return inoculation to *N. t.* 'Xanthi'.

Despite several similar inoculations the development of local lesions in *C. quinoa* could never be repeated.

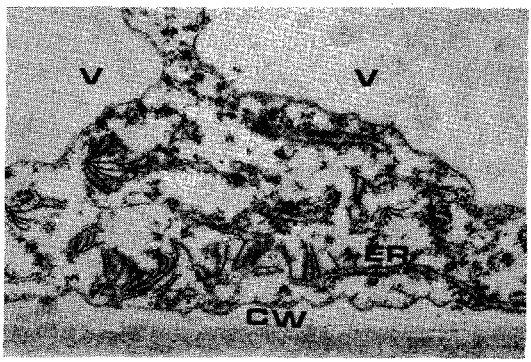

Fig. 5. Partly pinwheel inclusions, dense bands in close contact with endoplasmatic reticulum (ER) and pinwheels scattered in the cytoplasm of *Dieffenbachia* infected with dasheen mosaic virus. Cell wall=CW. Vacuole=V. × 40.000.

Foto:Jens Begtrup.

Conclusion and discussion

Danish cultures of *Dieffenbachia* are almost virus-free, where attack of dasheen mosaic virus has only been found in 1 out of 11 visiting nurseries. The virus is not at the present time of any problem for the culture, where virus infected plants apparently are disgarded during the cultivation on account of the retarded growth and leaf symptoms, which are visible through the year. This does not correspond with *Hakkaart* and *Waterreus* (1976), who found that the symptom manifestation may change through the year.

A careful selection of healthy looking plants shows to be an exceptable method for establishment of virus-free mother plants. This means, that there is no demand for virus elimination work by meristem-tip culture.

However the selection work must be followed up by a testing programme based on sap or dry inoculation of young plants of *Philodendron* varieties, this test method being the most effective one.

The serological method can be used only for a verification of the virus and only after a sufficient virus transmission to young plants showing clear leaf symptoms.

The appearance of a periodic vein clearing, specially in combination with the propagation, might be caused by either physiological conditions or a partly latent virus infection. The presence of tobacco necrosis virus in such plant material, did not give an explanation for the vein clearing.

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