Danish Research Service for Plant and Soil Science
Research Centre for Plant Protection
Institute of Plant Pathology
Virological Department
DK-2800 Lyngby

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Inactivation of tobacco mosaic virus in *Aeschynanthus hildebrandii* by means of heat treatment, chemotherapy and meristem-tip culture

Inaktivering af tobakmosaikvirus i Aeschynanthus hildebrandii *ved hjælp af varmebehandling, kemoterapi og meristemkultur*

Niels Paludan

Summary

Aeschynanthus hildebrandii is a rather new pot plant in Denmark.

Like many other genera of the gesneriads, *Aeschynanthus* is infected with tobacco mosaic virus (TMV), which is probably widespread in the culture.

In a few exceptional cases TMV-infected *Aeschynanthus* plants show viruslike symptoms such as narrow, deformed leaves.

Different TMV strains have been found in *Aeschynanthus* comprising the common tobacco strain (U_1), the para-tobacco strain (U_2), the tomato strain and the pepper 8 strain, which is identical with the pepper mild mottle virus, according to Dr. *C. Wetter*.

The TMV strain used in this experiment reacted serologically by the immunosorbent electron microscopy (ISEM) method with antisera against both the tobacco (U_1), tomato and pepper 8 strains.

In the present experiment, the dry inoculation method using *Chenopodium quinoa* as indicator plant has been most successful as bioassay.

Meristem plants have been successfully established in 6–8 months of culture using modified MS-62 media.

The TMV was inactivated by meristem-tip culture. The inactivation was further increased by a previous heat treatment during 1 month, contrary 8 months treatment without any effect. The addition of antiviral chemicals to the medium has shown only a limited inactivation effect, and only in combination with meristems excised from plants heat treated during 8 months.

The virusfree plants are established as nucleus stock plants and bear the name *Aeschynanthus hildebrandii* 'Gloria' Dafo.

Key Words: *Aeschynanthus*, tobacco mosaic virus, bioassay, inactivation, heat treatment, meristem-tip culture, chemotherapy.

Resumé

Aeschynanthus hildebrandii er en temmelig ny og ekspanderende potteplantekultur i Danmark.

Som i mange andre slægter af familien *Gesneriaceae* er *Aeschynanthus* inficeret med tobakmosaikvirus (TMV), der øjensynligt er udbredt i kulturen.

TMV-inficerede *Aeschynanthus*planter viser kun undtagelsesvis viruslignende symptomer i form af smalle, deforme blade.

Forskellige TMV-linjer er fundet i *Aeschynanthus* omfattende den almindelige tobaklinje U_1, paratobaklinjen U_2, tomatlinjen og peber-8-linjen, der er identisk med peber mild mosaikvirus ifølge dr. *C. Wetter*.

TMV-linjen, der blev brugt i dette forsøg, har reageret serologisk ved anvendelsen af immunosorbent-elektronmikroskopi-metoden (ISEM) med antisera mod både tobak- (U_1), tomat- og peber-8-linjerne.

De opnåede resultater har vist, at tørinokulationsmetoden til *Chenopodium quinoa* har været den biologisk mest følsomme metode.

Meristemplanter er blevet fremstillet efter 6–8 måneders kultur i modificerede medier af MS-62.

TMV er blevet inaktiveret ved meristemkultur og i stigende grad ved en forudgående kortvarig varmebehandling af planterne. Længere tids varmebehandling øgede derimod ikke inaktiveringen.

Anvendelsen af antivirale kemikalier i mediet har kun vist en begrænset effekt, og kun i forbindelse med meristemer skåret fra planter varmebehandlet gennem længere tid.

De virusfrie planter er etableret som kerneplanter og har fået navnet *Aeschynanthus hildebrandii* 'Gloria' Dafo.

Nøgleord: *Aeschynanthus,* tobakmosaikvirus, biologisk test, inaktivering, varmebehandling, meristemkultur, kemoterapi.

Introduction

Aeschynanthus hildebrandii, is a fairly new and popular pot plant culture in Denmark. The plant material was introduced in 1976 (3).

Until recently, little attention has been paid to viral diseases of gesneriads. In the case of tobacco mosaic virus (TMV), it was first described in the genus *Achimenes* in 1973 (6) and later in several genera including *Aeschynanthus*. Furthermore, TMV was found to be widespread in many cultivated gesneriads, but TMV infection was never found in Saintpaulia, which is probably immune (9, 13, 14).

Different TMV strains in the gesneriads were diagnosed. In general the U_2 strain was found to be more common than the U_1 strain. In *Aeschynanthus* the presence of the U_1 as well as the U_2 TMV strains were demonstrated (10, 14).

In other collections of *Aeschynanthus* unspecific reactions were achieved with TMV isolates reacting like both tomato and tobacco strains in *Nicotiana tabacum* 'White Burley' while other TMV strains reacted serologically like both pepper 8 strain and the tomato or tobacco strains (10).

An investigation showed that all collected plant material of *Aeschynanthus hildebrandii* was TMV-infected. The collected plant material descend from 2 Danish nurseries, the Royal Veterinary and Agricultural University, Copenhagen, the Royal Botanical Gardens, Kew, London, England, and the Royal Botanical Garden, Edinburgh, Scotland. The plant material did not show any noticeable virus symptoms. The only symptoms which have been seen in a few exceptional cases were narrow, deformed leaves with faint chlorosis.

Although the virus infection was more or less symptomless, it was decided to establish virusfree plants if possible, to avoid the risk of TMV contamination to other pot plants.

This paper deals with infection trials and the production of TMV-free plants by means of heat treatment, meristem-tip culture and chemotherapy. The experiments were carried out over the period 1983–84.

Method

In collaboration with the Institute of Glasshouse Crops, Årslev, the plant material of *Aeschynan-*

thus hildebrandii Hemsl. used in this investigation was received in 1983 from the Royal Botanical Garden, Edinburgh, Scotland. The plants released to the Danish growers in 1976 by the Royal Veterinary and Agricultural University, Copenhagen, also descend from the Royal Botanical Garden, Edinburgh (3).

An examination of the *Aeschynanthus hildebrandii* grown in Danish nurseries in 1982 led to the conclusion that no genetic selection before production of virusfree plants was necessary (*O. V. Christensen,* 1985, personal communication).

The TMV isolate reacted serologically with antisera against the TMV tomato strain (DK 87:55), the TMV tobacco strain (ATCC-135(U_1)) and the TMV pepper 8 strain (antiserum received from Dr. *D. Z. Matt,* Wageningen, the Netherlands). The results were estimated by the immunosorbent electron microscopy method (ISEM) (8,10).

The infected plants were grown partly under normal greenhouse conditions (20°C during the day and 18°C during the night) and partly in a thermostatically regulated growth chamber either at 30 or 34 ± 1°C for 16 hours with additional illumination (Philips 30 W/33 fluorescent lamp) with 3.6 Wm^{-2}, followed by a night temperature of 20°C for 8 hours.

Meristem-tip culture

Meristems were excised from untreated or heat-treated plants after 1 and 8 months of treatment.

The size of the meristems was 0.25 mm including 1 pair of leaf primordia.

Two different modifications of the MS-62 medium were used. One of the media has been described by *Gippert* and *Schmelzer* (2). It has 100% macro- and microelements and omission of most of the proteins except thiamin.HCl. The other medium has been described by *Horst et al.* (4) with 75% concentration of the MS-62 macro- and microelements. It was used in this experiment as a weaker medium and was further modified by omitting the coconut milk, using an agar content of 7 g/l, replacing the EDTA and the iron sulphate with 40 mg/l Na Fe EDTA and reducing the NH_4NO_3 to 638 mg/l.

As growth regulators, different concentrations between 0.5 and 1 mg/l of furfuryl amino purin (FAP) and 0.2-1 mg/l of indolyl butyric acid (IBA) were used. The meristems were transferred every month or every other month to a new tube containing the same medium. After establishment of the growth, the meristem plants were transferred to a medium with 0.4 mg FAP and 0.2 mg IBA/l.

Antiviral chemicals 'Amantadine' (1-adamantanamine) and ribavirin (1-B-ribofuranosyl-1,2,4-triazole-3 carboxamide) (Virazole) were added to the medium in quantities of 10, 50 or 100 mg/l during the whole period of the meristem culture in order to stop virus replication (1, 5, 7, 11).

Plantlets ready for potting were achieved after about 6–8 months of culture in a growth room at 20°C and 16 hours' illumination (Philips TLF 40 W/33 cool white fluorescent lamp) with 10 Wm^{-2}, followed by 8 hours of night temperature at 18°C.

Infection trials: Provisional infection trials were carried out by means of sap inoculation to detached leaves of *Nicotiana tabacum* 'Xanthi' and by sap and dry inoculation to *Chenopodium quinoa* plants, respectively, using carborundum powder (400 mesh) and a phosphate buffer pH 7.6, including 4% polyethylene glycol (M 6000).

Virus test: The established meristem-tip plants were all tested twice on *Chenopodium quinoa* using sap and dry inoculation, respectively. The results obtained were estimated according to the development of local lesions which indicated the presence of virus infection.

The ISEM method was used in a few cases.

Results

Infection trials

Established meristem plants were tested by sap inoculation both to detached leaves of *Nicotiana tabacum* 'Xanthi' and to *Chenopodium quinoa* plants. Of 24 samples tested, no reactions were registered in *N. tabacum* 'Xanthi', while 2 samples caused local lesions in *Chenopodium quinoa.* The negative samples were tested on *C. quinoa*

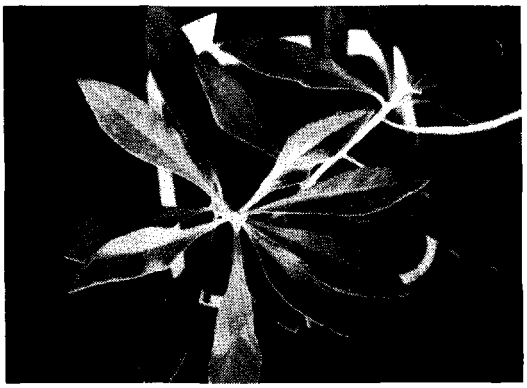

Fig. 1. *Aeschynanthus hildebrandii* plant infected with the tobacco strain of TMV.
Aeschynanthus hildebrandii *plante inficeret med tobaklinjen af TMV.*

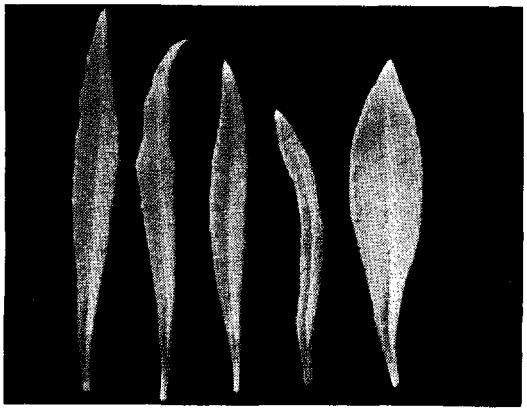

Fig. 2. *Aeschynanthus* leaves from plant infected with both the tomato- and the pepper 8 strain of TMV showing narrow, deformed leaves. Healthy leaf to the right.
Aeschynanthus-*blade fra plante inficeret med både tomat- og peber-8-linjen af TMV med smalle, deforme blade. Sundt blad til højre.*

by dry inoculation. No reaction occurred in 17 samples.

TMV-infected stock plants of *Aeschynanthus* were furthermore tested by different methods. In 1 of 5 plants tested, positive reactions were achieved by the ISEM method. With *Chenopodium quinoa* reactions were achieved in 4 out of 4 by dry inoculation, in 4 out of 5 by sap inoculation, in 3 out of 5 using sap from young leaves as inoculum, and in 4 out of 5 using sap

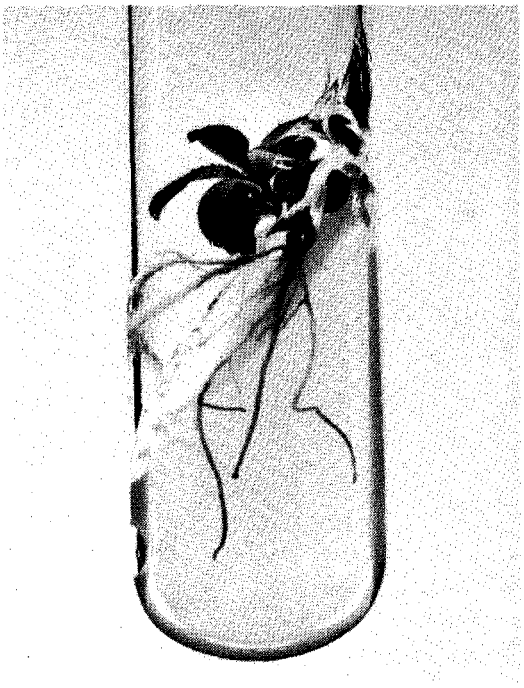

Fig. 3. Established meristem plant suitable for potting after 6 months of culture.
Etableret meristemplante klar til potning efter 6 måneders kultur.

from more mature leaves. Out of the 5 stock plants tested on *Chenopodium quinoa,* 3 reacted in all 13 tests, while 1 reacted in 4 out of 6 tests, and 1 in 5 out of 9 tests.

Meristem-tip culture

Independent of the media used, the different combinations of growth regulators and the addition of antiviral chemicals, normal meristem plants were established in 6–8 months of culture corresponding to about half the number of excised meristems.

The best result (18 plants out of 25 excised meristems) was achieved by using *Gippert* and *Schmelzer*'s medium (2) containing 0.5 mg FAP and 1 mg IBA/l as the first medium, and by transferring the meristem plants to the weaker medium of *Horst et al.* supplemented with 0.4 mg FAP and 0.2 mg IBA/l after establishment.

The addition of the chemical ribavirin and

'Amantadine' did not have any appreciable influence on the growth of the meristems.

Inactivation experiment

The results from the inactivation experiments are shown in Table 1.

Table 1. Inactivation experiments with *Aeschynanthus* infected with TMV.

Heat treatment		Media			
			+ Ribavirin	+ 'Amantadine'	
months	°C	Basis medium	10 mg/l	50 mg/l	100 mg/l
0	20	16/119*)	3/16	4/9	–
1	30	0/15	0/11	–	0/12
8	34	3/14	1/19	–	0/21

*) Numerator: TMV-infected plants
Denominator: Numbers of plants tested

Conclusion and discussion

The TMV infection in *Aeschynanthus* plants was best shown by the use of dry inoculation to *Chenopodium quinoa*. In some cases it was not possible to demonstrate TMV infection in plants known to be infected even when different inoculation methods were used. This may indicate either a variation in the virus concentration, an unusual TMV strain or an inhibiting effect of the plant sap.

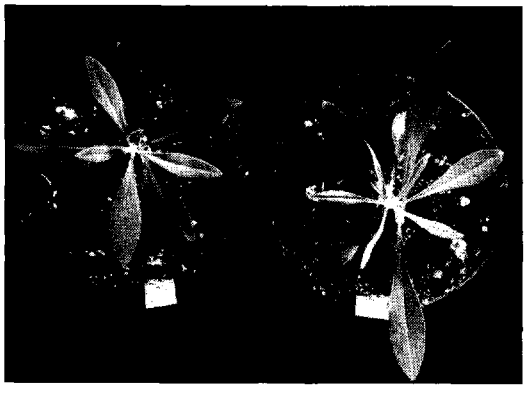

Fig. 4. Established meristem plants of *Aeschynanthus*. Virusfree plant to the left and TMV-infected to the right showing narrow top leaves.
Etablerede meristemplanter af Aeschynanthus. *Virusfri plante til venstre og TMV-inficeret til højre med smalle topblade.*

Fig. 5. Local lesions in *Chenopodium quinoa* caused by TMV from *Aeschynanthus* 1 week after sap inoculation.
Udviklede lokale læsioner i Chenopodium quinoa *fra TMV-inficeret* Aeschynanthus *1 uge efter saftinokulation.*

Fig. 6. Strains of TMV from *Aeschynanthus* used in the inactivation experiment diagnosed with ISEM-technique. Decorated virus particles obtained by the use of antisera against the following TMV-strains: Tobacco, Tomato, Pepper 8. × app. 140.000.
TMV-linjer fra Aeschynanthus, *der blev anvendt i forsøget med inaktivering, diagnosticeret ved ISEM-teknikken. Dekorerede viruspartikler opnået ved anvendelse af antisera imod følgende TMV-linjer: Tobak, Tomat, Peber 8. × ca. 140.000.*
(Photos *J. Begtrup*).

The effect of the TMV infection on *Aeschynanthus* plants has still not been investigated, but in this experiment only few plants showed viruslike symptoms such as narrow, deformed leaves.

The TMV strain involved in this experiment is either a mixture of TMV strains (tomato, tobacco and pepper 8) or quite another TMV strain. The U_2 strain (para-tobacco mosaic virus) earlier

found in *Aeschynanthus* (14) causes only local lesions in *Nicotiana tabacum* 'White Burley' NN (12), while the *Aeschynanthus* TMV isolate causes systemic green mosaic (10).

The causal TMV strain was inactivated by meristem-tip culture, and to an even greater extent when combined with previous short heat treatment contrary long time treatment. This might be due to differences in the growth of the heat treated plants, where plants grown at 30°C during 1 month developed new shoots in good condition, contrary plants grown at 34°C during 8 months with a very limited growth.

The addition of chemicals to the medium had only a limited inactivating effect and only in combination with meristems excised from plants heat treated during 8 months.

The virusfree plants have been established as nucleus stock plants at the Institute of Glasshouse Crops, Årslev, and will be tested for growth and flowering.

The plant material originating from this work bears the name *Aeschynanthus hildebrandii* 'Rubin' Dafo.

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