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Strain of tomato bushy stunt virus, causing chlorotic mosaic and chlorosis in the leaves, demonstrated in *Kalanchoë blossfeldiana*

En linje af tomat-dværgbuskvirus påvist i Kalanchoë blossfeldiana *med klorotisk mosaik og klorose i bladene*

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Summary

Kalanchoë blossfeldiana plants often show conspicuous leaf symptoms resembling virus attacks.

Many different virus particles have been found in diseased plants, probably associated with the developed symptoms, but so far only one latent virus has been sufficiently diagnosed.

With the results described in this paper a strain of tomato bushy stunt virus (TBSV), causing chlorotic spots and vein clearing in two cultivars, has been diagnosed in *Kalanchoë blossfeldiana.*

The *Kalanchoë* virus found is serologically related closest to the type strain of TBSV and based on symptoms in indicator plants closest to the pepper strain of TBSV.

The *Kalanchoë* virus is apparently a new strain of TBSV (*Kalanchoë* strain), the *Kalanchoë* plant being a new host for the TBSV.

This is the first description of TBSV occurring in Denmark.

Key words: *Kalanchoë,* tomato bushy stunt virus, diagnosis, infection trials.

Resumé

Kalanchoë planter viser ofte tydelige bladsymptomer, som minder om virusangreb.

Mange forskellige viruspartikler er fundet i syge planter med de udviklede symptomer, men indtil nu er kun et latent virus blevet endelig diagnosticeret.

Med de her beskrevne resultater er en viruslinje af tomat-dværgbuskvirus (TDBV) blevet diagnosticeret i *Kalanchoë blossfeldiana.* Viruset forårsager klorotiske pletter og nervelysning i bladene af to inficerede sorter.

Kalanchoë-viruset er nærmest beslægtet med den originale TDBV-linje og forårsager lignende symptomer i indikatorplanter som TDBV-peberlinjen.

Kalanchoë-viruset formodes at være en ny linje af TDBV (*Kalanchoë*-linje), og *Kalanchoë* er en ny værtplante for TDBV.

Dette er den første beskrivelse af TDBV i Danmark.

Nøgleord: *Kalanchoë,* tomat-dværgbuskvirus, diagnosticering, infektionsforsøg.

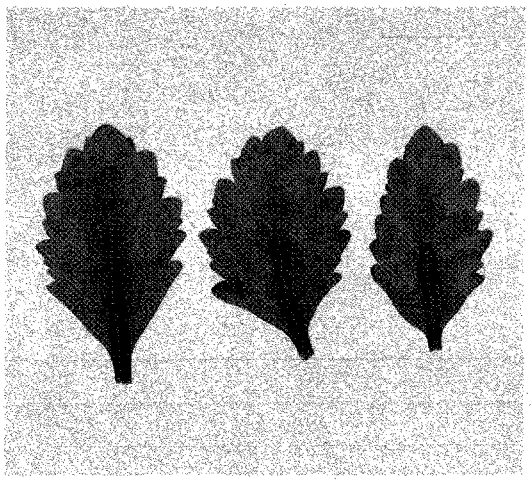


Fig. 1. *Kalanchoë blossfeldiana* 'Attraktion' showing chlorotic mosaic caused by natural infection of tomato bushy stunt virus (TBSV).
Kalanchoë blossfeldiana 'Attraktion' *med klorotisk mosaik forårsaget af angreb af tomatdværgbuskvirus (TDBV).*

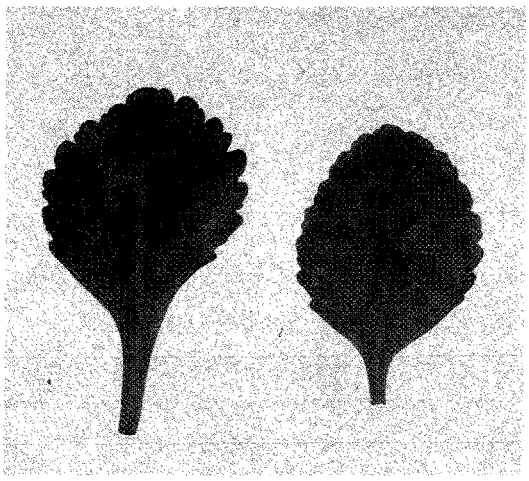


Fig. 2. *Kalanchoë blossfeldiana* 'Kristina' showing chlorotic spots and vein clearing caused by natural infection with TBSV.
Kalanchoë blossfeldiana 'Kristina' *med klorotiske pletter og nervelysning forårsaget af angreb af TDBV.*

Fig. 3. *Kalanchoë blossfeldiana* 'Annette' showing chlorotic and necrotic local lesions as a reaction from sap inoculation with TBSV.
Kalanchoë blossfeldiana 'Annette' *med klorotiske og nekrotiske pletter som en reaktion fra saftinokulation med TDBV.*

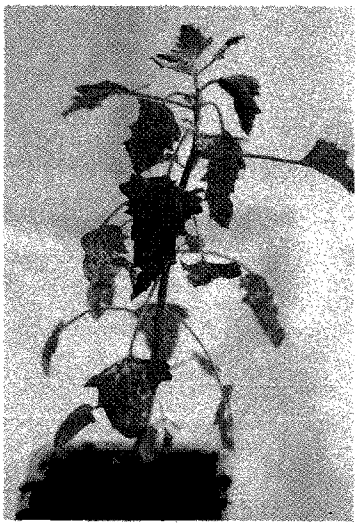


Fig. 4. Systemic infection and local lesions in *Chenopodium quinoa* caused by TBSV.
Systemisk infektion og lokallæsioner i Chenopodium quinoa *forårsaget af TDBV.*

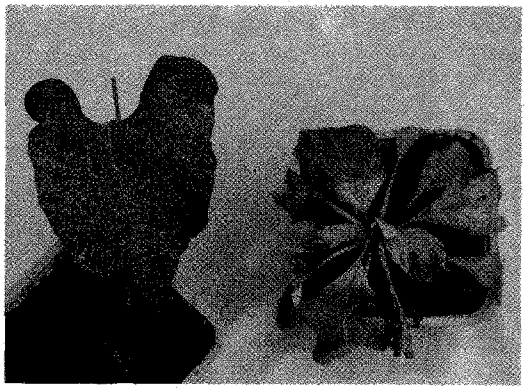
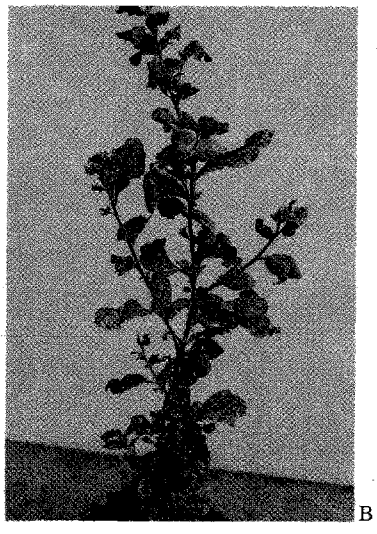


Fig. 5. Local lesions in *Phaseolus vulgaris* and dying *Nicotiana clevelandii* caused by TBSV.
Lokale læsioner i bønne og visnende Nicotiana clevelandii *forårsaget af TDBV.*

A



B

Fig. 6. *Nicotiana benthamiana* with strong vein clearing and rugosity caused by TBSV. A: Two weeks after inoculation. B: A half year old infection
Nicotiana benthamiana *med tydelig nervelysning og rynkning forårsaget af TDBV. A: To uger efter inokulation. B: Seks måneder gammel infektion.*

Introduction

The *Kalanchoë* plant production is one of the most important in the Danish pot plant industry.

During the cultivation period, some of the cultivars show leaf symptoms which resemble virus symptoms. These symptoms can vary from very weak chlorotic mosaic spots, rings and vein clearing to severe yellow-green streaks or blotches, necrotic spots and deformed leaves (1,3,5,10,11, 12).

So far only the *Kalanchoë* latent virus (KLV) has been identified, but many different virus particles have been demonstrated in *Kalanchoë* plants showing different leaf symptoms (3,4,5,11).

This paper deals with the diagnosis of and infection trials with the tomato bushy stunt virus (TBSV) (7) found for the first time in two *Kalanchoë* cultivars. The leaf symptoms were diffuse chlorotic mosaic, chlorotic spots, and vein clearing. The work was carried out in 1986–87 at the Department of Virology, Lyngby.

Method

Plant material and growth conditions

The plant material consisted of two cultivars of *Kalanchoë blossfeldiana,* namely 'Kristina' showing clear chlorotic mosaic, chlorotic vein clearing, chlorotic spots and slightly deformed leaves and 'Attraktion' showing diffuse chlorotic mosaic as pale green areas with green blotches and chlorotic 2–4 mm spots.

The plant material was received from the Danish Government Plant Protection Service and the Horticultural Advisory Service on account of the leaf symptoms.

The *Kalanchoë* plants and the indicator plants were grown under normal greenhouse conditions, i.e. 18°C during the day and 16°C during the night in winter, and up to 30–32°C during the warmest summer period. The plants were illuminated during the winter season with four hours additional assimilation light to stimulate growth. The nutrient solution was distributed by the drip-water method and controlled after evaporation. The concentration of the nutrient, using the Hornum mixture of macro- and microelements, was one per thousand during the winter and 1.5–2 per thousand during the summer.

Bio-assay

Based on earlier experiences (11), sap inoculation to *Chenopodium quinoa* was used. Plant sap from *Kalanchoë* leaves with symptoms was macerated in a mortar together with 0.03 M phosphate buffer pH 7.6 including 4 per cent polyethylene glycol (M 6000).

Developed symptoms either as local lesions or systemic infection were further transmitted to other indicator plants, the developed symptoms being recorded. Latent infections were estimated by retesting to the local lesion host *Chenopodium quinoa.*

Physical properties

Determination of the physical proporties was carried out according to the described methods (6) using plant sap from systemic infected *Nicotiana benthamiana* originating from *Kalanchoë blossfeldiana* 'Kristina'. The plant sap was diluted 1:1 with 0.03 M phosphate buffer pH 7.6 added 4 per cent PEG (M 6000). The diluded plant sap was centrifugated at 7000 RPM for ten minutes before use in order to separate existing plant tissues from the sap. *Chenopodium quinoa* was used as a test plant reacting with local lesions as well as systemic symptoms.

Immuno-assay

Virus particles were revealed by ordinary leaf dip preparation using the immunosorbent electron microscopy (ISEM) technique described by *Milne* and *Lousoni* (9), trapped and decorated with antiserum. The sap was deluted 1:10, and antiserum 1:1000 (trapping) and 1:100 (decoration). For staining 2 per cent uranyl acetate was used, and extraction was done with 0.1 M phosphate buffer, pH 7.0 and with 2 per cent polyethylene glycol.

The concentration of virus particles was counted in a Philips 201 electron microscope at a magnification of 15000 by studying 100 fields (eyesight) each of 500μm^2.

The first symptoms developed in the indicator plants indicated a soil borne virus, and therefore ISEM tests were performed, using antisera from several soil borne viruses.

Subsequent physical examinations of the *Kalanchoë* virus indicated, however, a very stable virus, and the ISEM tests were therefore concentrated on members of the tombus virus group (7) and possible members of this group.

A total of 25 different antisera against isometric viruses were involved.

The tombus antisera used were kindly supplied by *Renate Koenig,* Braunschweig (*Pelargonium* leaf curl virus (PLCV)), *A. Brunt,* Littlehampton (*Cymbidium* ringspot virus, type strain (CyRSV) *Pelargonium* flower break virus (PFBV), tomato bushy stunt virus, type strain (TBSV)) and by *E. Luisoni,* Torino (Carnation mottle virus (CarMV), *Petunia* asteroid mosaic virus (PAMV)).

Infection trials

Healthy plantlets of the *Kalanchoë blossfeldiana* 'Annette' from the nuclear stock plant collection at the Institute of Glasshouse Crops, Årslev, were sap inoculated in April. Sap from systemic virus infected plants of *Nicotiana benthamiana* was used. The virus sources originated from the *Kalanchoë blossfeldiana* cultivars 'Kristina' and 'Attraktion'. The developed symptoms were recorded for three months.

Results

Bio-assay

The indicator plants used and the symptoms developed after sap inoculation from the virus infected *Kalanchoë blossfeldiana* cultivars 'Kristina' are shown in Table 1. From the cultivar 'Attraktion' similar symptoms were achieved in *Chenopodium quinoa, Cucumis sativus, Nicotiana clevelandii, Phaseolus vulgaris* and *Spinacea oleracea.*

Physical proporties

The virus infected sap from *Nicotiana benthamiana* could endure 10 minutes heat treatment at 85°C, but not at 90°C, a dilution end point over 10^{-6} and a longevity in vitro up to 64 days.

Immuno-assay

When looking for rod-shaped virus particles of the *Kalanchoë* latent virus in local lesions from *Chenopodium quinoa* isometric virus particles

Table 1. Developed symptoms in different indicator plants caused by sap inoculation from virus infected *Kalanchoë blossfeldiana* 'Kristina'.
Symptomudvikling i forskellige indikatorplanter efter saftinokulation fra virusinficeret Kalanchoë blossfeldiana 'Kristina'.

Indicator plants *Indikatorplanter*	Symptoms *Symptomer*	
	local lesions *lokale læsioner*	systemic *systemiske*
Capsicum annuum	yellow stars 10 mm	yellow stars 10 mm
Chenopodium amaranticolor	white 1 mm	deform.
Chenopodium quinoa	white 2 mm	mottle, deform.
Cucumis sativus	white, necrot. 2 mm	0
Datura stramonium	necrotic 2 mm	0[1)]
Gomphrena globosa	white, 2–4 mm	0
Lycopersicon lycopersicum	chlorot. rings 2 mm	0[2)]
Nicotiana benthamiana	0	mosaic, deform.
– *clevelandii*	water soaked	vein clear., dying
– *rustica*	chlorot. 2 mm	0[2)]
– *tabacum* 'Samsun'		
– *tabacum* 'Xanthi NN'	white 2 mm	0
tabacum	white 2 mm	0
Ocimum basilicum	brown 4 mm	0[1)]
Petunia hybrida	white (rings) 2 mm	0[1)]
Phaseolus vulgaris	necrotic 2 mm	0[2)]
Spinacea oleracea	white 2 mm	vein clear., deform.

1) no latent infection
2) latent infection

were recorded by the ISEM test for the first time in *Kalanchoë*.

The following trapping and decoration of virus particles using antisera against different tombus viruses are shown in Table 2.

Infection trials

The development of symptoms in the *Kalanchoë blossfeldiana* cultivar 'Annette' after sap inoculation from the cultivars 'Kristina' and 'Attraktion' were recorded over a period of three months.

After one month (May), both virus strains caused chlorotic 2–4 mm spots in young leaves.

After two months (June) chlorotic vein spots occurred. The 'Attraktion' strain also caused white rings.

After three months (July) both strains showed only weak chlorotic vein spots and vein clearing.

Kalanchoë plants, which had not been inoculated, did not show any symptoms in this period.

Discussion

Spherical virus particles have been demonstrated for the first time in *Kalanchoë blossfeldiana* and differ from the earlier viruses described as the carla virus (KLV), the poty virus, the tobamo virus (TMV) and the bacille formed virus (3,4,5, 11).

The spherical virus particles were diagnosed as a strain of TBSV, which is a new virus occurring in *Kalanchoë* and in Denmark.

The virus was readily transmitted to many indicator plants causing mostly local lesions with systemic infection in only a few plants and furthermore a very stable virus. All these characteristics are typical for the tombus virus group (8).

The diagnosis was also based on ISEM tests, where the number of trapped and decorated virus particles indicated a very close relation to the TBSV type strain. The virus was futhermore distantly related to PAMV and PLCV, whereas the CyRSV, PFBV and CarMV did not cause sufficient decoration.

The developed symptoms in the indicator plants showed, however, some disagreement between the *Kalanchoë* virus and the tombus viruses including the TBSV, type strain, the PLCV and PAMV. This indicates the existence of another virus strain. The *Kalanchoë* virus only caused local lesions in *Datura stramonium,* latent systemic infection in *Lycopersicon lycopersicum* and *Phaseolus vulgaris* and no systemic reaction in *Petunia* hybrida (7,8).

A comparison with other described TBSV-strains found in many different hosts (2,8) shows that the *Kalanchoë* virus is most closely related to the pepper strain (8) with respect to symptoms developed in indicator plants, except, however, for a weaker reaction in pepper and spinach.

There is every indication that the *Kalanchoë* virus is another *Kalanchoë* strain of TBSV, for which there has been no previous documentation.

Table 2. ISEM-trapping and decoration of *Kalanchoë* virus particles, using antisera against tombus viruses.
Binding og dekorering af Kalanchoë *viruspartikler med antiserum imod tombus virus.*

Viruses *Virus*	No. of recorded particles[1)] *Antal registrerede partikler*[1)]	Decoration *Dekoration*
Tomato bushy stunt, type strain	39.4	full *(total)*
Petunia astroid mosaic	5.0	partly *(delvis)*
Pelargonium leaf curl	4.7	partly *(delvis)*
Cymbidium ringspot, type strain	1.5	traces *(spor)*
Possible members *Evt. tilhørende virus*		
Carnation mottle	2.4	none *(ingen)*
Pelargonium flower break	3.4	traces *(spor)*

1) Average of view of 100 fields of 500 μm^2.
Gennemsyn af 100 felter (gns.) af 500 μm^2.

Conclusion

A strain of TBSV has been diagnosed in *Kalanchoë blossfeldiana* as causing chlorotic mosaic, spots and vein clearing in two cultivars.

The diagnosis is based on indicator plants, physical properties and ISEM.

The *Kalanchoë* virus is closest related to the type strain of TBSV and causes very similar symptoms in indicator plants as the TBSV-pepper strain.

The *Kalanchoë* virus is apparently a new strain of TBSV (*Kalanchoë* strain), and the *Kalanchoë* plant is a new host for the TBSV in Denmark.

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