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Inactivation of *Kalanchoë* latent virus strain 1 in *Kalanchoë* using heat treatment and meristem-tip culture

Inaktivering af Kalanchoë-*latentvirus linie 1 i* Kalanchoë *ved hjælp af varmebehandling og meristemkultur*

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Summary

Many *Kalanchoë blossfeldiana* cultivars are heavily infested and show viruslike symptoms, resulting in bad-looking plants and reduced quality and growth.

Several viruses have been found in infected plants, comprising a poty-like virus, a bacilliform virus, tobacco mosaic virus, and the *Kalanchoë* latent virus, which belongs to the carla group of viruses.

It was very difficult to diagnose the different viruses on account of low transmission rate to indicator plants and difficulties with the plant sap and plant tissue in the electron microscopical work.

The *Kalanchoë* latent virus, however, which is widespread in nearly all the *Kalanchoë* plants as a latent infection, is easy to diagnose by sap inoculation to *Chenopodium quinoa.*

Infection trials with the *Kalanchoë* latent virus strain 1 (KLV-1) showed that sap inoculation is preferable to dry and epidermal strip inoculation and can be used throughout the year.

Inactivation of the KLV-1 was achieved by meristem-tip culture and to an increasing degree combined with previous heat treatment.

Key words: *Kalanchoë, Kalanchoë* latent virus, heat treatment, meristem-tip culture, inactivation, infection trials.

Resumé

Mange *Kalanchoë blossfeldiana*-sorter er generet af viruslignende bladsymptomer, der forårsager et kedeligt udseende, samt en reduceret kvalitet og vækst.

Der er påvist adskillige virus i inficerede planter, blandt andet et poty-lignende virus, et bacilleformet virus, tobakmosaikvirus og *Kalanchoë*-latentvirus, hvor det sidstnævnte tilhører carla-gruppen af virus.

Det har vist sig at være meget vanskeligt at diagnosticere de enkelte virus på grund af en lav overførselsrate til indikatorplanter og yderligere vanskeligheder med plantesaften og plantevævet i forbindelse med det elektronmikroskopiske arbejde.

I modsætning hertil er *Kalanchoë*-latentvirus, som forekommer i næsten alle *Kalanchoë*-planter som en latent infektion, nem at diagnosticere ved saftinokulation til *Chenopodium quinoa.*

Infektionsforsøg med *Kalanchoë*-latentvirus linie 1 (KLV-1) har vist, at saftinokulation er bedre end tørinokulation og inokulation med strimler af overhudsvæv, og metoden kan yderligere anvendes året igennem.

KLV-1 er blevet inaktiveret ved meristemkultur og i stigende grad i kombination med en forudgående varmebehandling.

Nøgleord: *Kalanchoë, Kalanchoë*-latentvirus, varmebehandling, meristemkultur, inaktivering, infektionsforsøg.

Introduction

Kalanchoë commonly exhibit viruslike symptoms as yellow-green streaks or blotches, irregular rounded or circular areas or rings, chlorotic mosaic, necrotic spots and also deformed leaves.

Transmission of the causal pathogen to sensitive *Kalanchoë* species as *K. flammea* and *K. daigremontiana* has been achieved by grafting, but not by mechanical means (2, 13).

By ultrastructural studies and transmission tests using *Kalanchoë blossfeldiana,* viruslike particles have been found in leaf dip preparations and/or ultrathin sections. Thus a carla-like virus (mechanically transmitted) was found in symptomless plants, a poty-like virus in plants with mild mosaic, and a bacilliform virus in plants with leaf spotting (4).

The carla-like virus has been diagnosed as a member of the carla virus group called *Kalanchoë* virus 1, and later on, 2 strains of the virus called *Kalanchoë* latent virus 1 and 2 (5, 6).

By investigations, carried out at the Institute of Plant Pathology, Lyngby, the following viruses have been found in *Kalanchoë blossfeldiana:* A virus with bacilliform particles in 1980, a tobacco strain of tobacco mosaic virus in 1982, the *Kalanchoë* virus 1 and a poty-like virus in 1983.

Further Danish investigations showed virus symptoms in 17 out of 24 varieties. The most serious symptoms were seen in the yellow varieties. However, the most important red variety 'Annette' did not show any leaf symptoms.

Seasonal influence on the development of virus symptoms in 14 varieties showed a variation from 21 % with symptoms in August, 29 % in October to 43 % in February.

Meristem-tip culture and storage for one year of plantlets in tubes at 12°C with 16 hours' illumination was carried out with success.

The carla-like virus has been diagnosed by biological, physical and serological means (8, 9, 10, 11).

None of the sap-inoculated *Chenopodium quinoa* plants used for all these experiments have ever shown systemic symptoms, which indicates the presence of the KLV-1 strain only (6). Furthermore, the virus used for the inactivation experiment has been diagnosed serologically as strain 1 of the KLV by the ISEM method (7) using antisera against the 2 KLV strains received from *S. S. Hearon,* U.S.A. (unpublished).

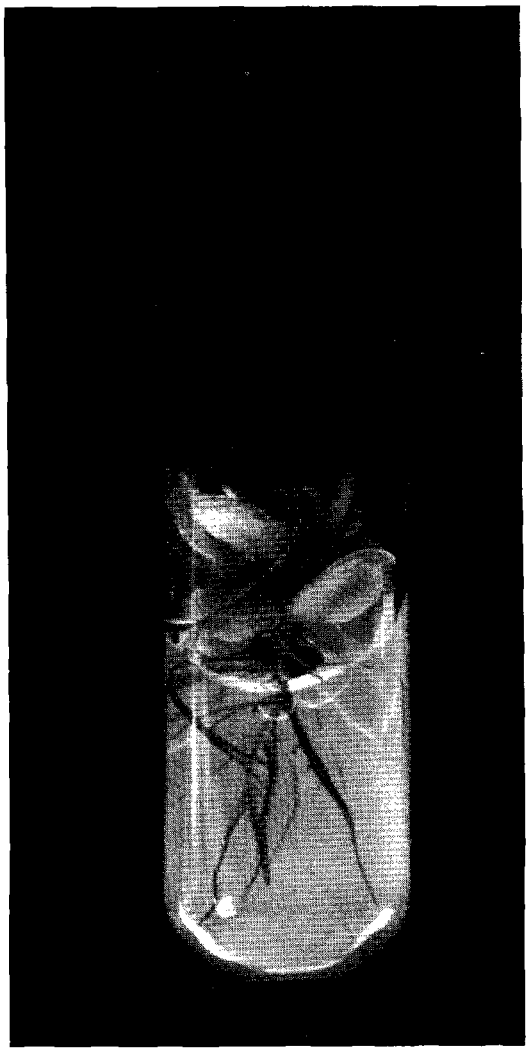

Fig. 1. Established meristem plant of *Kalanchoë* with leaf and root formations after 5 months of culture.
Etableret meristemplante af Kalanchoë *med blad- og rod-dannelse efter kultur i 5 måneder.*

This paper deals with infection trials and with the possibility of inactivating KLV-1 in *Kalanchoë blossfeldiana* by means of meristem-tip culture and heat treatment.

Method

The plant material consisted of selected clones of *Kalanchoë blossfeldiana,* 2 from the variety 'Annette' and 1 from 'Annette Frej', all received from the Institute of Glasshouse Crops at Årslev (1).

All the plants were infected with KLV-1 by grafting. Small shoots from naturally infected plants of *Kalanchoë daigremontiana* were used.

After controlled virus infection was achieved, the plants were grown partly under normal greenhouse conditions (20°C during the day and 18°C during the night), and partly in a thermostatically controlled growth chamber (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 for 8 hours at 20°C).

Meristems were excised from untreated or heat-treated plants after 3.5 months of treatment.

The size of the meristems was 0.25 mm including 2 primordia, and the medium used was a modified MS-62 as used by *Gibbert* and *Schmelzer* (3), without casein hydrolysate, glycine, meso inositol, nicotinic acid and pyrridoxin HCl, but with 1 mg thiamin HCl only. As growth substances, 0.5 mg/l furfuryl amino purin and 1 mg/l idolyl butyric acid were used. The explants were transferred every other month to the same medium. Plantlets ready for potting were obtained after about 5 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 an 8-hour night at a temperature of 18°C.

The established meristem-tip plants were tested twice by sap inoculation to *Chenopodium quinoa.* The results were assessed on the basis of the development of local lesions.

Another experiment was carried out during the year with inoculum from 10 different *Kalanchoë*

Fig. 2. *Kalanchoë blossfeldiana* 'Annette' established by meristem-tip culture.
Kalanchoë blossfeldiana *'Annette' etableret ved meristemkultur.*

Fig. 3. Local lesions in *Chenopodium quinoa* 2 weeks after sap inoculation caused by the *Kalanchoë* latent virus-1.
Lokale læsioner udviklet i Chenopodium quinoa *2 uger efter saftinokulation med* Kalanchoë *latent virus 1.*
Photos: Jens Begtrup.

plants, all showing virus symptoms. The trials included sap, dry and epidermal strip (12) inoculation, respectively, to *Chenopodium quinoa*. Carborundum powder (400 mesh) and a phosphate buffer pH 7.6 including 4 % polyethylene glycol (M 6000) were added to the inoculum.

Furthermore, virus particles were revealed with the ISEM technique described by *Milne* and *Lousoni* (7), trapped and decorated with antiserum. The sap was diluted 1:10, and antiserum 1:1000 (trapping) and 1:100 (decoration). For staining 2% uranyl acetate was used, and extraction was done with PO_4-buffer 0.1 M, pH 7.0 and with 2% polyethylene glycol.

Results

Infection trials

The results of the infection trials are shown in table 1:

Table 1: *Kalanchoë* infection trials on *Chenopodium quinoa*.
Kalanchoë infektionsforsøg med *Chenopodium quinoa*.

Inoculation method *Inokulationsmetode*	Number of samples producing local lesions of all plants tested in the months of: *Antal prøver, hvor lokale læsioner er udviklet af i alt testede i månederne:*					
	March	May	July	Sept.	Oct.	Dec.
Sap *Saft*	7/10	5/9	6/9	5/9	3/10	7/10
Dry *Tør*	–	3/9	4/8	–	–	–
Epidermal strips *Epidermis strimler*	3/10	2/9	–	–	0/10	6/10

–: Experiments were not carried out *forsøg ikke udført*.

The sap inoculation was more sensitive than the other methods used.

The 10 different *Kalanchoë* plants caused variations in the indicator plant reaction. With sap inoculation only, 3 plants did not cause any development of local lesions in the indicator plants, 2 plants caused reactions in 4 out of 6 inoculations, 1 plant in 3 out of 4, 1 plant in 5 out of 6, and 3 plants caused reactions in all 6 inoculations.

If the 3 plants which did not cause any reactions are left out of account, the seasonal variation in percentage of infection may be calculated as follows: March: 100; May: 83; July: 100; September: 83; October: 43; and December: 100.

Inactivation experiment

The results from the inactivation experiment are shown in table 2:

Table 2: Inactivation experiment with *Kalanchoë* latent virus strain 1 using meristem-tip culture and heat treatment at 34°C.
Forsøg med inaktivering af Kalanchoë *latentvirus linie 1 ved hjælp af meristemkultur og varmebehandling ved 34°C.*

Clones *Kloner*	Heat treatment in months *Varmebehandling i måneder*	No. of meristem tips *Antal meristemer* excised *skåret*	No. of meristem tips *Antal meristemer* established *etableret*	Virus-free plants % *Virusfrie planter %*
'Annette' No. 3	0	50	20	15
	3.5	50	33	97
'Annette' No. 6	0	50	28	21
	3.5	50	27	96
'Annette Frej' No. 28	0	50	32	69
	3.5	50	33	100

The inactivation of the KLV-1 increased with the previous heat treatment of the mother plants. The results obtained with the 2 clones No. 3 and 6 were very similar, whereas the 'Annette Frej' showed a higher degree of virus-free plants among the meristem tips.

The reliability of the test results was demonstrated by the number of samples causing local lesions at the second testing of all the negative ones from the first test. Of all 113 negative samples only 1 caused lesions in *Chenopodium quinoa* at the second test.

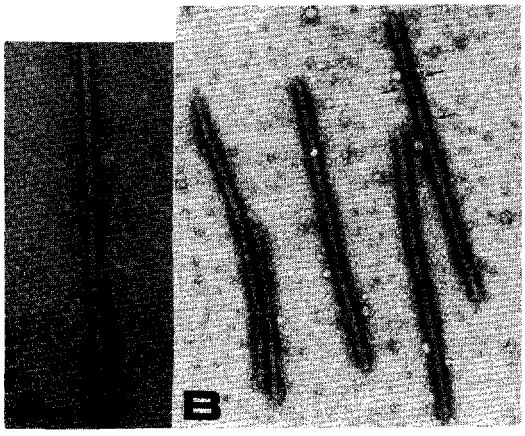

Fig. 4. *Kalanchoë* latent virus 1 diagnosed with ISEM technique:
A. trapped particle on formwar film.
B. trapped and decorated particles for final identification. Note the halo of antibodies (arrows) on both sides of the decorated particles. X 130.000.
Kalanchoë *latent virus 1 diagnosticeret ved ISEM-teknik:*
A. Fangne partikler på formwar film.
B. Fangne og dekorerede partikler for identifikation. Bemærk belægningen af antistoffer (pile) på begge sider af partiklerne. X 130.000.

Photo: Jens Begtrup.

Discussion and conclusion

It was possible to diagnose the KLV-1 throughout the year by sap inoculation to *Chenopodium quinoa,* and that method was preferable to other methods of bioassay.

The virus was found in most of the varieties tested, which corresponds with investigations by *Hearon* (5) showing that the virus is widespread in the *Kalanchoë* culture.

The KLV-1 was latent in all the investigated, virus infected plants.

The influence of the *Kalanchoë* latent virus infection on *Kalanchoë* plants has still not been properly investigated. However, it is quite possible that the development of leaf symptoms is stimulated in combination with other viruses.

Kalanchoë daigremontiana has turned out to be naturally infested with the KLV-1.

The KLV-1 has been successfully inactivated by meristem-tip culture, preferably in combination with a previous heat treatment.

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