# Transmission Characteristics and Cytopathology of a White fly-Transmitted Virus Isolated from Sweet Potato Leaf Curl Disease

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# Transmission Characteristics and Cytopathology of a Whitefly-Transmitted Virus Isolated from Sweet Potato Leaf Curl Disease*

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## Abstract

A leaf curl disease of sweet potato was found occasionally in field sweet potato crop in Osaka. Symptoms of this disease, including upward leaf curl and stunting, occurred in young plants in the spring which remained almost symptomless at other times of the growing season. The virus, which had an apparently narrow host range within the family Convolvulaceae, had several characteristics in common with sweet potato leaf curl virus (SPLCV) previously described by Shinkai (1983). The virus was transmitted by a whitefly (*Bemisia tabaci*), but not by aphids or mechanically. In virus-vector relationships, the minimum acqusition-access period and inoculation-access period were 1h and 30 minutes, respectively. A latent period of 21−24 h was demonstrated. The virus was retained by its whitefly vector for 10 days. Electron microscopic observations of thin sections from infected sweet potato, *Ipomoea setosa* and *Pharbitis nil* leaves indicated that geminivirus like particles, approximately 16 nm in diameter, were located in the nuclei of phloem parenchymal cells. The cytopathic effects typically observed in geminivirus infected dicotyledonous plants were noted in nuclei in phloem associated cells of SPLCV infected plants. These data suggest that SPLCV may belong to the subgroup B in geminivirus group.

## Introduction

A leaf curl disease of sweet potato was first reported in Japan by Shinkai[1] who showed that the causal virus, referred to as sweet potato leaf curl virus (SPLCV), was transmissible by the whitefly, *Bemisia tabaci*. Further informations concerning the transmission, host range or symptomatology of the virus has been briefly reported[2,3]. But the virus was not characterized in detail because no virus-like particles have so far been detected in negatively stained leaf-dip preparations from infected plants. We report here some additional data on insect transmission and host range of the virus designated here as SPLCV-J and show that the diseased plants may contain geminated virus particles.

## Materials and Methods

*Virus isolate* : An isolate, SPLCV-J, was obtained from naturally infected sweet potato (Kokei, No, 14) plant with typical leaf curl symptoms growing in the University farm, College of Agriculture, University of Osaka Prefecture for the study. Virus isolation was made by means of the vector *B. tabaci* to *Ipomoea setosa* seedlings. The virus

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was maintained in *I. setosa* by grafting and/or transmitting by the vector *B.tabaci*.

*Transmission tests* : Whiteflies used in transmission experiments were obtained from a virus free colony kept on soybean. To test the whitefly transmissibility, 30−50 insects were placed in muslin-covered cages and allowed feed for 1−2 days on diseased plants. Afterwards, they were placed on healthy plants for 2−5 days. Mechanical inoculation was done triturating leaves of *I.setosa* showing typical leaf curl symptoms in a motar and pestle with 0.05 M phosphate buffer (pH 7.6) containing 0.5% Celite and rubbing the sap over leaves. Aphid transmissibility was determined using *Myzus persicae*, *Aphis gossipium* and *Rhopalosiphum maides* by standard techniques for ability to transmit in the non-persistent, semipersistent and persistent modes. Mechanical inoculation and transmission tests through aphids were done on *I.setosa* and *P. nil* cv. Scarlet O'Hara and groupes of 10−15 plants were inoculated.

## Results

*Symptoms induced in sweet potato* : In the field, the affected plants (Kokei, No. 14 and Kogane-Sengan) showed severe stunting and leaf curling of new growth (Plate I-1). New shoots were stunted and had short internodes. The margins of the leaves curled up and conspicuous greening and thickening of the veins and veinlets were frequently formed on the underside of the leaves(Plate I-8). The leaf curl symptoms were most evident in the first flush of growth in the spring and tended to be masked with further growth and warmer weather. The effects of infection on yield have not been investigated but it appeared to be of minor economic importance because of low incidence.

Eight clonal lines from seedlings of sweet potato (DJI-7A) were tested for their reaction to the virus. Seedlings were graft inoculated with leaf curl infected *I. setosa* sprouts. Typical leaf curl symptoms were observed in all of these sweet potato seedlings. Leaf curl symptoms were markedly greater in clones with leaf shape of ovate (Plate I-5) than in those of palmate (Plate I-6) or triangular (Plate I-7).

*Transmission tests* : Typical leaf curl symptoms were observed in 17 out of 20 *I. setosa* seedlings on which *B. tabaci* was allowed acquisition- and inoculation-access feeding for 2 and 5 days, respectively. Symptoms did not develop in any of the mechanically inoculated test plants. Similarly, transmission through aphids were also negative.

*Host range* : The host range was determined by infesting at least 10 seedlings of each species tested with 30−50 viruliferous whiteflies for 2 days. The virus had an apparrently narrow host range within the family Convolvulaceae; *Calystegia japonica*, *Calonyction aculeatum*, *Convolvulus tricolor*, *Ipomoea batatas*, *I. setosa* (Plate I-2), *Pharbitis nil* (Plate I-3), *P. purpurea* and *Quamoclit pennata* (Plate I-4) were infected. The virus induced severe stunting and leaf curl on all species of Convolvulaceae tested. *B.tabaci* transmission of selected test species outside the Convolvulaceae resulted in no infection as determined by symptomatology and the virus could not be recovered from inoculated plants by whitefly transfer to *I. setosa* seedlings. These apparently immune species included *Ageratum conyzoides*, *Cucumis sativus* cv. Suyo, *Cucurbita maxima*, *Datura stramonium*, *Glycine max*, *Lycopersicon esculentum* cv. Ponderosa, *Nicotiana glutinosa*, *N. rusutica*, *N. sylvestris*, *N. tabacum* cv. Xanthi, *Phaseolus vulgaris* cv. Yamashiro-kurosando, *Vigna sesquipedalis* cv. Kurodanesanjaku and *Zinnia elegans*.

*Virus-vector relationships* : After different acquisition-access periods on infected plants, groups of 20−25 female whiteflies were kept for 48h on healthy plants. A single

case of transmission (1/15) was observed after an acquisition-access feeding period of 1 h on the test plants. Transmission rate established by 4-, 6-, 24-h acquisition-access feedings were 13% (2/15), 27% (4/15), and 73% (11/15), respectively. Following a 48 h aquisition feeding, 5% (1/20), 10%(1/10), 10% (1/10), 30% (3/10), and 60%(6/10) transmission rate were reached following 0.5-, 4-, 6-, and 24-h inoculation feedings, respectively.

Caged females after acquisition feeding periods of 3- or 21-h on infected plants were transferred in groups of 20−25 to healthy plants. They were then transferred to new test plants at 3 h intervals during the daytime. Whiteflies given an acquisition feed of 3 h were able to transmit the virus between 21−24 h after the start of the acquisition feeding period and after the acquisition feeding of 21 h, whiteflies transmitted the virus immediately (Table 1).

For the tests to virus persistence in the vector, whiteflies were given an acquisition feeding of 2 days. A group of 10−30 whiteflies was transferred on a single plant. The

Table 1. Rate of transmission of SPLCV-J during serial transfers of whiteflies after different periods of aqusition feeding.

| Time from start of acquisition feeding (hours) | Transmission after the indicated aquisition feeding period | |
|---|---|---|
| | 3 hours | 21 hours |
| 3-6 | 0/15* | |
| 6-9 | 0/15 | |
| 9-12 | 0/15 | |
| 21-24 | 2/15 | 3/15 |
| 24-27 | 1/15 | 3/15 |
| 27-30 | 2/15 | 5/15 |

*No. of *I. setosa* plants infected/no. of plants tested

Table 2. Serial transmission of SPLCV-J to *I. Setosa* plants following 2 days aquisition access period by groups of *B. tabaci* transferred at intervals of one day.

| Group no. of *B. tabaci* | Successive transfers to test plants (days after the start of acquisition) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13-22 |
| 1 | +(10) | +( 7) | −( 5) | +( 3) | +( 2) | −( 2) | −( 2) | * +( 9) | +( 7) | −( 6) | −( 5) |
| 2 | +(40) | +(32) | +(24) | −(12) | +(11) | −( 8) | −( 7) | | | | |
| 3 | +(60) | +(49) | −(43) | +(35) | −(23) | −(16) | +(12) | −( 7) | +( 4) | −( 3) | −( 2)[d] |
| 4 | −(30) | −(27) | +(22) | −(19) | −( 8) | −( 5) | −( 2) | −( 1)[d] | | | |
| 5 | −(10) | −( 6) | +( 6) | −( 3) | −( 9) | +( 5) | +( 4) | −( 3) | −( 3) | −( 2) | −( 1)[d] |
| 6 | +(10) | +( 8) | +( 8) | −( 6) | | | | | | | |
| 7 | +(10) | −( 6) | −( 4) | −( 4) | +( 2) | −( 7) | −( 6) | +( 6) | −( 5) | −(13) | −(11) |
| 8 | −(10) | +( 9) | +( 9) | −( 8) | −( 5) | | | | | | |
| 9 | −(40) | −(32) | +(29) | −(24) | +(19) | −(15) | −(11) | −(10) | −( 8) | | |
| 10 | −(20) | −(14) | −(10) | −( 8) | −( 6) | −( 3) | −( 6) | −( 5) | −( 2) | −( 2) | −( 2)[d] |
| 11 | −(10) | −( 7) | −( 7) | +( 5) | −( 3) | −( 3) | | | | | |

+ : infection − : no infection d : death of whiteflies

( ) : number of surviving whiteflies at each transfer

* : surviving whiteflies were mixed, and transferred

whiteflies were removed daily to a new test plant. Number of live insects in each transfer was noted. The data (Table 2) indicated that the virus persisted for at least 10 days, and that transmission occured in an intermittent manner. The life span of female whiteflies fed for 2 days on infected plants was 25 days.

*Electron microscopic observations* : Tissue samples consisted of small fragments of mid-vein with adjacent mesophyll tissues excised from SPLCV-infected sweet potato, *I. setosa* and *P. nil* plants, and from healthy control plants were prefixed in 5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol and embeded in Spur's resin. Thin sections were double stained with uranyl acetate and lead citrate before examination with a Hitachi H-300 electron microscope.

In infected tissues, cytopathological changes were observed in cells of the vascular bundles of tissues from naturally and artificially infected plants. The major changes occurred in the nuclei of phloem parenchyma and companion cells. Some cells exhibited apparent segregation of nucleolar material into fibrillar and granular regions (Plate II-1) or ring structures with an amorphous or finely fibrillar matrix (Plate II-2).

Virus-like particles were observed in the nucleoplasm of some of modified nuclei. In the nucleoplasm, the particles were scattered at random (Plate II-6) or occurred in clumps (Plate II-3, 4, 5A,7). In certain areas, where a better resolution was achieved, some of the virus particles appeared to be paired (PlateII-5B, 6, 8). They were not detected in the cytoplasm of infected cells nor in seemingly normal nuclei. The above cytological modifications were consistently present in all types of infected samples observed, but their incidence was very low.

Virus-like particles could not be observed under the electron microscope in crude sap preparations from field-infected sweet potato, or greenhouse-inoculated *P. nil*, *I. setosa*, or sweet potato plants fixed in 2% glutaraldehyde, after 2% PTA (pH 7.0) or 2% UA (pH 5.0) staining. Some attempts were made to purify a virus by conventional procedures from *I. setosa* plants, but we have not so far been able to success any virus-like particles in any of the scores of variously prepared high-speed preparations.

## Discussion

Based on similarities in host range, symptomatology and transmission characteristics, SPLCV-J was shown to be the same as SPLCV described by Shinkai[1] in Japan. Seven other whitefly-transmitted viruses, including Georgia mosaic (USA)[4], vein clearing (Israel)[5], yellow dwarf (USA)[6], yellow dwarf (Taiwan)[7], Sheffield's virus B (East Africa)[8,9], mild mottle (East Africa)[10,11] and leaf curl (Taiwan)[12] are known to occur naturally in sweet potato.

In each of these viruses, sweet potato mild mottle virus (SPMMV) is the most throughly investigated. Hollings *et al.*[10,11] reported that SPMMV is sap-transmissible, infect hosts outside the Convolvulaceae and has flexuous particles of 950 nm length. It is suggested that SPMMV is probably the same as Scheffield's virus B[10,11,13]. The virion morphology of sweet potato yellow dwarf virus (Taiwan)[7] is similar to that of SPMMV but sufficient differences have been reported as separate virus. SPLCV-J is thus morphologically different from SPMMV and SPYDV (Taiwan). Campbell *et al.*[14] suggested that mosaic, vein clearing and yellow dwarf (USA) viruses may be the same or similar entities. These viruses have not been investigated in detail, but it is possible that they are distinct from SPLCV-J on the basis of their symptoms and host range.

The symptoms incited on sweet potato by SPLCV-J are almost identical to those of sweet potato leaf curl virus described in Taiwan (SPLCV-T)[12]. SPLCV-J resembles SPLCV-T in other respects including lack of sap transmissibility, transmission characteristics by *B. tabaci*, symptoms in certain indicator hosts and host range. However, SPLCV-J is morphologically different from SPLCV-T or SPLCV isolated in Kyusyu, Japan (SPLCV-K). Yamashita *et al.*[15] indicated that rod-shaped particles (80−200×18 nm) were observed in thin sections of sweet potato leaf phloem cells infected with SPLCV-K. Chung *et al.*[12] also observed similar rod-shaped particles, approximately 18 nm wide, in thin sectioned materials of SPLCV-T infected sweet potato. However, we found no evidence to support the contention that SPLCV may be a rod-shaped virus. Our results indicate that SPLC disease is apparently caused by a geminivirus. Particle morphology, crystalline arrangement of virus-like particles in nuclei, phloem-limited cytopathic effects and a consistent association of fibrillar rings in the nuclei are ultrastructural characteristics similar to those occurring in other known whitefly transmitted geminivirus infections[16,17]. Moreover, we demonstrated that the probes for DNA 1 component of bean golden mosaic virus and mung bean yellow mosaic virus reacted in spot hybridization tests with extracts of tissue infected with SPLCV-J[3]. Similar relationships between the DNA 1 components of distinct whitefly-transmitted geminiviruses have been described by Roberts *et al*[18].

Several lines of evidence reported here support the view that SPLCV is incited by a geminivirus. However, the relationships between isolates of SPLC viruses will remain inconclusive until additional comparative information is available, and further work on the purification and characterising the nucleic acid and some other properties are needed before such relationships to this and other virus groups.

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## Explanation of Plates

**Plate I** Symptoms caused by sweet potato leaf curl virus (SPLCV-J).

1. Field sweet potato plant cv. Kokei No. 14 showing characteristic leaf curl symptoms.
2. *Ipomoea setosa* plant showing leaf curl symptoms 23 days after inoculation.
3. *Pharbitis nil* plant cv. Scarlet O'Hara showing leaf curl symptoms 19 days after inoculation.
4. *Quamoclit pennata* plant showing leaf curl symptoms 30 days after inoculation compared with healthy plant (left). Symptoms include reduced leaf size and stunting.

5−7. Reaction of selected clonal lines from seedlings of sweet potato (DJI-7A) to inoculation with SPLCV-J.

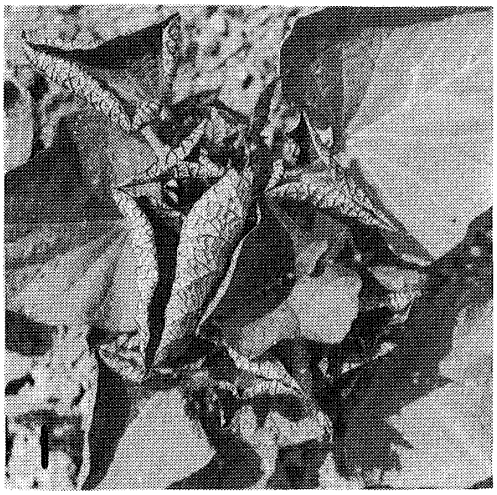
5. Severe leaf curl symptoms in clone with leaf shape of ovate.
6. Mild leaf curl symptoms in clone with leaf shape of palmate (5 lobed).
7. Leaf curl symptoms in clone with leaf shape of triangular.
8. Sweet potato leaf (Kokei, No. 14) showing vein thickenings (arrows) on the under surface.

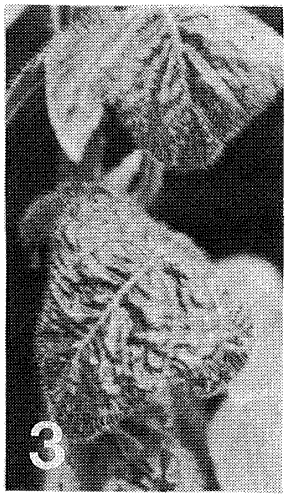
**Plate II** Electron micrographs of a nucleus in phloem associated cells of sweet potato plant infected with SPLCV-J.

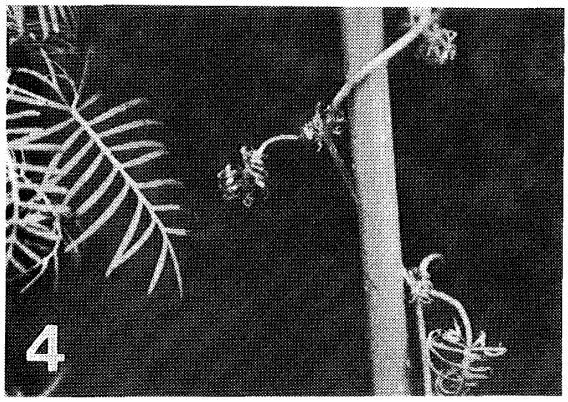
1. A segregated nucleolus exhibiting granular (G) and fibrillar (F) regions. U: unknown inclusion also observed in healthy cells. ×30,000.
2. Small fibrillar ring (FR) in the nucleoplasm. ×12,000.

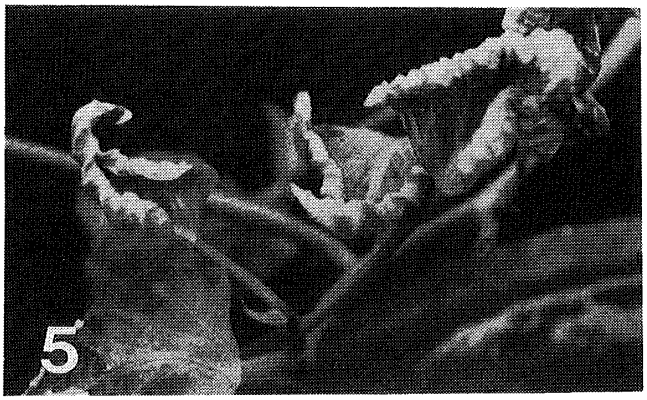
3. A large mass of virus-like particles (V) in the nucleoplasm. ×10,000.
4. Randomly arranged aggregates of virus-like particles (V) in the nucleoplasm. ×16,000.
5A. Crystalline arrays of virus-like particles (V) in the nucleoplasm. ×25,000.
5B. A higher magnification of the portion of virus aggregates in Plate II−5A. ×70,000.
6. Randomly scattered virus-like particles in the nucleoplasm in which the particles were often paired (arrows). Some particles associated with ring-shaped fibrillar body (F). ×120,000.
7. Virus-like particles in regular arrays (boxed). ×14,000.
8. A higher magnification of the boxed area in Plate II−7. Virus-like particles are linearly arranged. ×80,000.

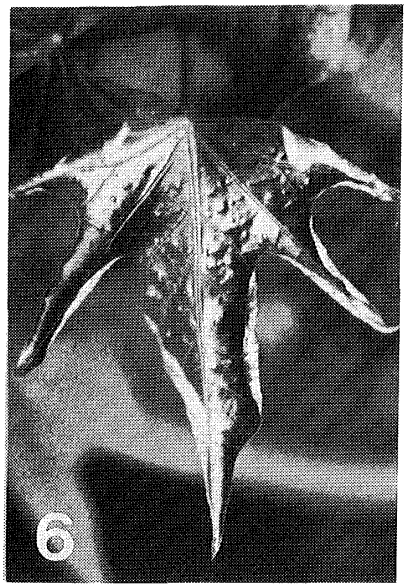
**Plate I**

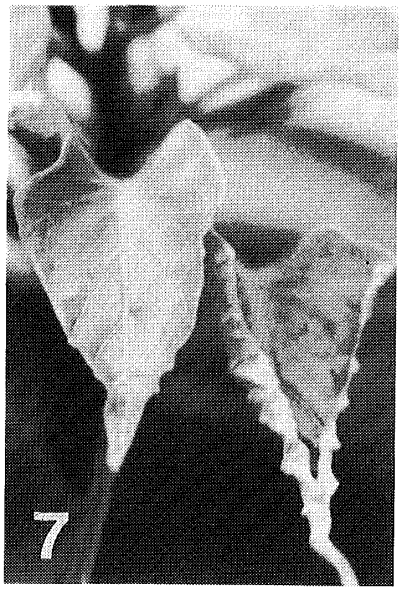


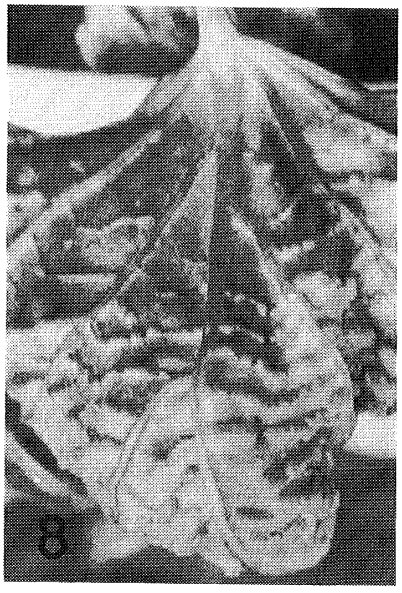



**Plate II**