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An infectious virus isolated from soybeans.

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大豆萎縮病原 바이러스에 關한 硏究

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ABSTRACT

Soybean stunt virus (SSV) was newly isolated in Korea from naturally infected soybeans (*Glycine max*). The main symptoms caused by this virus on soybean cultivars are crinkling, mild mottling and reduction in plant size.

This virus induced local lesion on the inoculated leaves of *Chenopodium amaranticolor*, *C. quinoa* and *Vigna sinensis*, and mosaic symptoms on *Nicotiana tabacum* (Bright yellow, KY-57).

The virus was inactivated at 60C, and was infectious at dilution of 10^3. Extract juice became infective 3 days later at room temperature. The virus was transmitted by green peach apid (*Myzus persicae*). This virus closely is related serologically to cucumber mosaic virus.

The virus particles observed in the electron microscopy were spherical types of 30mm in diameter.

INTRODUCTION

Soybean plants showing crinkle and stunt symptoms were noted initially in a late planting in Suweon during 1977 season. Although soybean stunt virus has not been recognized previously in Korea, the virus has the potential of becoming epidemic. Symptoms of stunt disease can be easily confused with common bean virus such as soybean mild mosaic virus (SMMV) and peanut stunt virus (PSV) although SMMV or PSV were not detected from indicater plant and serological test.[1,2,3] Soybean stunt virus was named firstly by Koshimizu et al.[3] Previously this virus was described as cucumber masaic virus by Hagedorn (1954), Klesser (1960), and Komuro (1961).

This virus was recently indentified as a strain of cucumber mosaic virus due to its serological relationship with CMV.[4,5] This paper is dealing with serological relationships between cucumber mosaic virus and this virus as well as some of the characteristics of the virus.

MATERIALS AND METHODS

Soybean stunt virus was maintained in soybean (cultivar: Columbus) and tobacco (KY-57) by mechanical inoculation. All plants were grown at 25C greenhouse. Inoculum was prepared by grinding

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soybean leaf tissue in a mortar and pestle, expressing the sap with gauze, and diluting with 0.01M potassium phosphate buffer 7.0. Plants were inoculated by rubbing inoculum with a pestle on leaves previously dusted with 600 mesh carborandum. After inoculation the plants were washed and maintained at 25C greenhouse. Physical properties of this virus were determined with fresh extracted juice of young soybean leaves. SSV inoculum of soybean dilution 1 : 10 was used for the study of thermal inactivation point (TIP),dilution end point (DEP) and longevity in vitro (LIV) of the virus. Necrotic lesion production on *C. amalanticolor* was used as a measure of virus activity. Each physical property test was repeated two times.

TIP studies were conducted by grinding each sample just before heating because of the rapid inactivation of the virus. Each 1m*l* sample was heated for 10 minutes at 45C, 50, 55, 60 and 65C, immediately cooled in running tap water, and inoculated on the assay plants. Dilutions were made from 1 : 10 to 10^5 to determine DEP. LIV of the virus was determined by grinding the leaf tissue sample in buffer and holding the diluted sap in a test tube at room temperature.

Myzus persicae (Sulz.) and *Auracorthum solani* were allowed to feed the inoculated soybean plants for 24 hours. Fifteen individuals per plant were transferred on ten healthy seedlings for one day, and they were killed by spraying insecticide.

Serological reaction and electron microscopy

CMV antiserum used in this experiment was introduced from Institute for Plant Virus Research (Japan). Partially purified virus suspension was used as antigen. Purifying procedure is as follows: *Nicotiana tabacum* (KY-57) infected with SSV was harvested one week after inoculation and maintained for 20 hours at 4C in sealed polyethylene bags.

The leaves were homogenized with same volume of 0.5M citrate buffer. The extract was prepared through two sheets of cheesecloth and emulsified with 30% cold chloroform by stirring in a warming blender for 1 minute. The emulsion was then centrifuged at 9,000 rpm for 20 minutes in a Hitachi RPR 12~15M rotor. The clear supernatant liquid was collected and centrifuged for 150min utes at 28,000rpm in a Hitachi RP 30~535 rotor. Pellets were resuspended in 3m*l* of 0.005M EDTA. The resuspended virus was used as antigen. Leaf extract from healthy tobacco plant was used as control after the same procedure of partial purification in order to determine the optimum concentration of antigens and CMV antiserum reaction. Following combinations were tested; one third antigen against one fifth and one twentieth antiserum, original antiserum against one fifth and one twentieth antiserum. Partially purified virus was also for electron microscopy.It stained with uranyl acetate.

RESULTS

1. Host range.

As shown in Table 1, plants of 12 species were infected with this virus. No infection occurred on *Datura metal*, *G. globosa*, *P. sativum*, *V. faba*, *P. hybrida*. Soybean cultivars such as Buseuk, Bongeui, Columbus, and Ou 13 showed vein clearing, crinkle and developed stunt symptom meanwhile Dongsan 65 showed necrotic lesion on inoculated leaves and mottling symptoms on upper leaves. Leaves of *Vigna sinensis* also developed necrotic ring spots. Systemically infected *N. glutinosa* and *N. tabacum* (KY-57) stripe-like mosaic patterns on leaves. General symptoms on naturally infected soybean cultivars by this virus were mild mottling, slight crinkling of leaves and reduction in plant size. The virus remained infective for 3 days in soybean leaf sop. The thermal inactivation point was 60C and infectivity was retained at dilutions of 10^3.

2. Serological reaction and electron microscopy

All four different combinations of antigen and antiserum formed precipitin band.One third dilution of antigen against one fifth dilution of CMV-antiserum showed most strong reaction among four different combinations. The precipitin bands by SSV and CMV antigen against CMV-antiserum did not formed any spur. Electron microscopy showed a lot of spherical particles from partially purified virus suspension. Average size of the particles wa

nm.

3. Vector transmission

Myzus persicae and *Auracorthum solani* were nployed to test insect transmission. *M. persicae* ansmitted this virus sixty percent of the test p-nts in this experiment, while *A. solani* marked ero.

DISCUSSION

A causal virus showing crinkle and stunt sympt-n on soybean cultivars was identified as soybean unt virus through serological test, physical prop-ties, host range and electron microscopy. Symp-ms are closely resembled those of the peanut s-nt virus or a strain of soybean mosaic virus. H-vever, the reactions on indicator plants (Table 1) ggested that this virus is soybean stunt virus r-her than soybean mosaic virus or peanut stunt rus in soybean[1,2,5]. This virus on soybean culti-rs showed different symptoms such as mosaic inkle, leaf crinkle and stunt[2,3,5]. The physical operties of the virus were closely related with ybean stunt virus (Koshimizu et al, 1963). The sults obtained from this experiment in soybean p are; 60C thermal inactivation point, 10^3 dilution d point and 3 day longevity in vitro at room t-mperature.

Koshimizu[2] also found that SSV was successfu- transmitted by 3 species of aphids. *Myzus per-ae*, one of the aphids used in this experiment, nsmitted the virus about 60 percent of test pla-s, although the transmission percentage obtained s not so high as expected. The data still indica-this virus can be transmitted by the green p-ch aphids.

t would be presumed that if the environmental ndition has been more favorable for the develo-ent of the disease, it would be have got higher e of transmission.

Takahashi (4) et al reported that soybean stunt us was serologically related to cucumber mosaic us.

Both the relative and absolute concentration of us and antiserum are the most important factor determining the speed of precipitation and the amount of precipitate produced by serological reaction between CMV and this virus has formed most strong precipitation band in one third dilution of antigen against one fifth dilution of CMV-antiserum. It is suggested that this particular combination is the optimum concentration of precipitation reaction between antiserum of CMV and antigen of the virus. There was no evidence of spur formationtion. This phenomenon presence due to these two virus has similiar results antigenic structure.

In electron microscopy, this virus showed spherical particle which was similar to that of CMV described byFrancki, R.I.B[1].

要 約

水原近郊의 大豆圃場에서 採集한 自然發病된 罹病株로부터 大豆萎縮病原바이러스가 되었다. 이 바이러스에 罹病된 大豆의 病徵은 Crinkling, 弱한 Mottling 및 品種에 따라서 甚한 萎縮증상을 나타내었다.

分離된 大豆萎縮病原바이러스를 指標植物에 汁液接種한 結果 명아주(*Chenopodium amaranticolor*), 명아주(*C. quinoa*), 동부(*Vigna sinensis*)에서는 局部病斑이 나타났고 담배(Bright yellow), 담배(KY-57)에서는 모자익病徵이 나타났다.

이 바이러스의 物理的 性質은 耐熱性이 60C, 耐稀釋性이 10^3으로 나타났으며 耐保存性은 3日이었다. 虫媒傳染試驗結果 복숭아혹진딧물은 이 바이러스를 비교적 쉽게 傳染시켰다.

또한 이 바이러스는 血淸學的으로 오이모자익바이러스와 密接한 關係를 가졌으며 電子顯微鏡에서 바이러스의 檢鏡結果 오이모자익바이러스와 같은 形態의 粒子가 觀察되었다.

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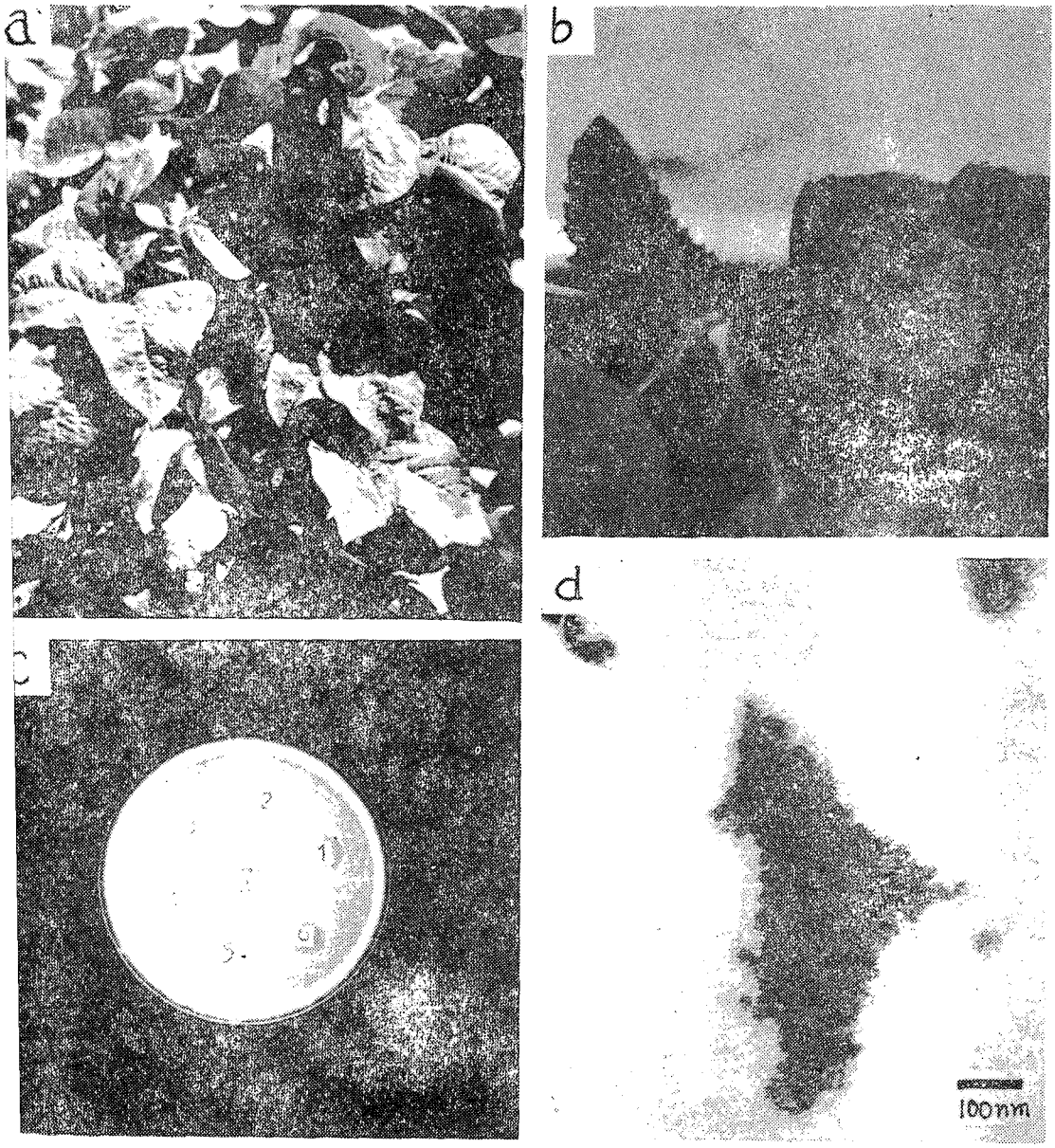

General symptoms on soybean cultivar caused by this virus at field.
Symptom of SSV on *Chenopodium amaranticolor* by sap inoculation.
Agar gel diffussion plate. Well 1, 2, 3, 4 contain this virus in the form of partial purification and CMV isolated from different crops. (1). CMV(red pepper), (2) this virus(soybean), (3) CMV(cucumber), (4) CMV(tomato), Well 5 and 6 contain healthy tobacco and soybean preparation as control. Well 7 contains CMV antiserum.
The SSV particles by means of partial purification in electron microscopy.