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A Newly Discovered Mosaic Disease of Bush Basil (*Ocimum basilicum*) in Taiwan

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ABSTRACT

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An unusual disease occurred in bush basil (*Ocimum basilicum*) fields mainly in Pingtung County in southern Taiwan. Foliar symptoms of affected plants included mosaic and leaf distortion. A virus isolate was obtained from the symptomatic basil and subsequently tested for its infectivity on 21 plant species from 5 families by mechanical inoculation. Three species in Chenopodiaceae and one of the plants from Solanaceae were infected. Inoculation of the isolated virus on basil seedlings induced symptoms similar to those observed from the fields. Two aphid species, *Aphis gossypii* Glover and *Myzus persicae* Sulzer, were found to transmit the virus in a non-persistent manner to healthy basil seedlings. The virus was purified. By electron microscopy, isometric virus particles, approximately 30 nm in diameter, were visible. The thermal inactivation point of the virus isolate was 60℃-70℃, dilution end-point was 10^{-3} to 10^{-4} and the longevity *in vitro* of infected sap was 8 days at 24℃ and more than 10 weeks at -70℃. Two protein species with molecular weights of about 42 and 26 kDa were detected after the purified virus was denatured with sodium dodecyl sulfate (SDS) and analyzed by polyacrylamide gel electrophoresis. The purified virus and crude sap from infected *Chenopodium quinoa* leaves reacted specifically with *Broad bean wilt virus* (BBWV) antiserum in SDS-agar immunodiffusion test but not with *Cucumber mosaic virus* (CMV) antiserum. In indirect enzyme linked immunosorbent assay (ELISA), CMV polyclonal antibody did not show correlation with purified preparations. The virus was identified as *Broad bean wilt virus*, a member of the genus, *Fabavirus* belonging to the family *Comoviridae* on the basis of its particle morphology and size, manner of aphid transmission, serology and the molecular weights of capsid proteins. This was the first report of the natural occurrence of *Broad bean wilt virus*, a member of the genus *Fabavirus* to cause a disease on bush basil in Taiwan and also in the world.

Key words : Bush basil, mosaic, *Fabavirus*, *Broad bean wilt virus*

INTRODUCTION

Bush basil (*Ocimum basilicum* L.) is a herbaceous annual plant of the family Lamiaceae (Labiatae). It is used fresh, dried and processed as spice, perfume, cosmetic, fly repellent, medicine, snuff and as an ornamental [(19)].

Basil is an economically important herb crop in several Mediterranean countries. Approximately 80 ha of the herb were grown annually in Italy, 30 ha in France, and 20 ha in Israel [(10)]. Basil was grown in the United States throughout the year both in greenhouses with natural light and ambient CO_2 and in hydroponic facilities with artificial lights and enriched atmosphere with CO_2 [(21)].

A report showed that there were more than 20 ha of basil planted, producing more than 1,800 t in 1986 in the northern part of the country, Taipei, Taiwan [(7)]. In southern Taiwan, Pingtung County was considered another major area of basil production. There, more than 40 ha of bush basil were grown on specialized farms with large acreage and non-specialized farm on small acreage. All the farms were typically family-managed. Basil in Taiwan is a year-round crop. Typically it is grown from seeds but occasionally from cuttings. Fresh-

market basil was produced by both specialized and non-specialized farms, harvested either mechanically or by hand (Biing-Shan Fuh, *personal communication*). The types of bush basil grown in Taiwan were local selections of the green-stem and purple-stem cultivars introduced many years ago from Mainland China [7].

During the growing season of 1997, an unusual disease occurred in basil fields in Pingtung County and to a lesser extent in Kaohsiung County in southern Taiwan. This appeared to be the new record of this disease in Taiwan. Foliar symptoms of affected plants included mosaic and leaf distortion.

Cucumber mosaic virus (CMV) [15], *Lucerne mosaic virus* (LMV) (previously known as *Alfalfa mosaic virus*) [9] and *Tomato spotted wilt virus* (TSWV) [11] are the only three viruses reported so far to infect basil naturally.

Since basil is an herb crop and the marketable product includes fresh leaves, damage caused by the virus directly affects the grower's income. Currently, in Taiwan the extent of losses caused by this disease with virus-like symptoms is unknown. Cotton aphid (*Aphis gossypii* Glover) was found associated with basil plants. This situation prompted research aimed at isolation, identification and characterization of the casual agent of the unreported virus disease of *O. basilicum* in Taiwan, including mode of disease transmission, host range, electron microscopy, virus purification, serology and capsid protein analysis. The results may lead to the development of strategy for effective disease suppression, which is the objective of this study.

MATERIALS AND METHODS

Virus source and isolation

Basil plants exhibiting virus-like symptom were collected in Pingtung County. The virus was isolated by mechanical inoculation from infected basil to healthy green stem basil following a series of three successive single lesion transfers on *Chenopodium quinoa* Willd. Plants were dusted with carborundum (400 mesh) and an inoculation buffer (0.1 M phosphate-buffered saline (PBS) containing 0.1% 2-mercaptoethanol, pH 7.0) was used. Symptoms on inoculated basil were similar to those from infected basil collected from the field. The virus was subsequently maintained in basil and *C. quinoa* plants in the laboratory. This basil virus isolate was tentatively designated as Basil mosaic virus (BsMV) for further study.

Plants and growing conditions

Basil (Local green stem variety) and other plant seeds were sowed on a bi-weekly basis. Nine days after sowing, seedlings were transplanted into individual containers with a mixture of peat moss, vermiculite, perlite and organic fertilizer and grown under greenhouse condition. Care included watering and application of foliar fertilizer on a regular basis. Insect pest problem, when occurred, was controlled chemically on a timely basis.

Aphid colonies, source and test plants

Cotton aphids (*Aphis gossypii* Glover) collected from basil field in Wan Dan, Pingtung County, were reared on healthy basil plants in a screen cage. Green peach aphids, *Myzus persicae* Sulzer, were obtained from a colony started a couple of months earlier from apterous aphids reared in screen cage on tobacco (*Nicotiana tabacum*) plants. Source plants for transmission test were those basil plants inoculated mechanically with BsMV and the test plants were one-month old basil seedlings.

Transmission characteristics of BsMV

After the inoculation access period, aphids were removed from test plants with a camel hairbrush. These plants were placed under observation for a period of two weeks to allow symptoms to develop at room temperature with supplemental fluorescent light providing a photoperiod of 12L:12D. Diseased plants were identified visually within two weeks of inoculation. The experiment was conducted in a randomized complete block design with five replicates over time for a total of 20 plants for each treatment.

Preacquisition fasting time : The effect of starvation times of 0, 0.5, 1, 1.5, 2, 3, 4, and 6 h on transmission efficiency by cotton aphid was tested. Two apterous cotton aphids were starved on moisten tissue paper in 4-fl.oz. dixie cup for each starvation time treatment. After the starvation time, the aphids were placed on BsMV infected plants for an acquisition access time of 5 min, then removed to test plants for a 2 min inoculation access period.

Acquisition access time : The ability of aphids to transmit BsMV after acquisition access times of 0, 10, 30 sec; 1, 2, 5, 10, 30 min; and 1, 2, and 6 h was evaluated. Two apterous cotton aphids were starved on moisten tissue paper in 4-fl.oz. dixie cup for a 2 h preacquisition fasting period. After each acquisition access treatment on BsMV infected plant, aphids were transferred to test plants for a 2 min inoculation access period.

Inoculation access time : Two apterous cotton aphids were subjected to a 2 h starvation period on moisten tissue paper in 4-fl.oz. dixie cup followed by a 5 min acquisition access period on BsMV infected plant. Aphids were then placed individually on test plants for the inoculation access times of 0, 10, 30 sec; 1, 2, 5, 10, 30 min; and 1, 2, and 6 h.

Retention time : Two apterous cotton aphids were starved for 2 h on moisten tissue paper in 4-fl.oz. dixie cup and then allowed for an acquisition access period of 5 min on BsMV infected plant. Differences in transmission efficiency were measured over an interval of 3 h by transferring aphids to each test plant after a 30 min inoculation access period.

Number of aphids : The efficiency of different number of

aphids to transmit BsMV was evaluated using 1, 2, 3, 4 and 5 aphids. In each case apterous cotton aphids were starved on moisten tissue paper in 4-fl.oz. dixie cup for a 2 h starvation period. After the 5 min acquisition access time on BsMV source plant, aphids were transferred individually to the test plant for a 2 min inoculation access period.

Green peach aphid transmission efficiency of BsMV

Apterous green peach aphids were removed from the stock colony, confined in a 4-fl.oz. dixie cup, and starved for 2 h. Two aphids were then placed on the abaxial surface of an BsMV-infected basil leaf for a 5 min acquisition access time. Aphids were then transferred to test plants for a 2 min inoculation period.

Test of host range

To characterize the biological properties of the virus, several differential test plants (Table 6) of *Broad bean wilt virus* (BBWV) [2], *Lamium mild mosaic virus* (LMMV) [6] and several species of the Lamiaceae family were used for inoculation assays. Leaf tissue from infected basil was ground in inoculation buffer (0.1 M phosphate-buffered saline (PBS) containing 0.1% 2-mercaptoethanol, at pH 7.0), and extracts were rubbed on test plants previously dusted with carborundum (400 mesh). At least five plants of each species were inoculated with BsMV. Each plant species was tested three times. The plants were kept at room temperature and observed for two weeks.

Physical properties of BsMV

Thermal inactivation point (TIP), dilution end-point (DEP), and the longevity of the virus *in vitro* (LIV) at two different temperatures were tested using infected basil leaves and *C. quinoa* as assay plants [8].

Dilution end-point : The inoculum prepared from the infected basil leaves was diluted at various serial tenfold dilutions (10^{-1} to 10^{-7}) [8].

Thermal inactivation point : The sap was subjected to the following temperature: 30, 40, 50, 60, 70, 80, 90 and 99℃ in a water-bath for 10 min [8].

Longevity *in vitro* : The sap was divided into two for each temperature treatment at 24 and -70℃. A few drops of a 1:1 mixture of penicillin G (30 mg/ml) and streptomycin sulphate (30 mg/ml) were added and mixed thoroughly in the sap for treatment at 24℃. At the following days 0, 1, 2, 4, 8, 15, 22 and 30, individual *C. quinoa* plants were inoculated. For treatment at -70℃ sap was inoculated onto *C. quinoa* on the following weeks 1, 2, 4, 6, 8 and 10 [8].

Electron microscopy

Negative staining : Virus particles from *C. quinoa* and basil leaf extracts and purified preparations were floated onto electron microscopy Formvar-fronted, carbon-coated, 200-mesh copper grids and incubated for five minutes. The grids were washed with distilled water, negatively stained with 2% aqueous uranyl acetate (pH 5.0) and air dried before examination with a Hitachi 7500 electron microscope, and photographed. Particle size was determined by measuring the diameter of 50 virions on an electron micrograph and calculating the mean diameter.

Virus purification

The BsMV isolate was purified according to the method described by Mossop *et al*. [16] with modification as follows: inoculated *C. quinoa* leaves with chlorotic local lesions were ground with a mortar and pestle in extraction buffer (0.1 M Na_2HPO_4, 0.1% (v/v) thioglycollic acid; 0.1% (w/v) Na-DIECA adjusted to pH 8.0) with liquid nitrogen (1g/3ml). After filtering through cheesecloth, the extract was centrifuged at 8,000 g for 10 min. The supernatant was mixed with Triton X-100 to a final concentration of 2% (v/v) for 15 min at 4℃. After clarification the mixture was centrifuged at 78,000 g for 2 h. The pellet was resuspended in extraction buffer approximately 1/10 of the original volume of extraction buffer. After clarification at 5,500 g for 5 min the supernatant was layered onto half its volume of 10% (w/v) sucrose in resuspension buffer (0.1 M Na_2HPO_4, pH 8.0) and centrifuged at 144,000 g for 45 min. The pellet was resuspended in resuspension buffer. After clarification at 5,500 g for 5 min, the preparation was purified by density-gradient centrifugation in 5%, 15%, 25% and 35% sucrose dissolved in 0.1 M Na_2HPO_4 centrifuged in swinging-bucket rotor at 96,000 g for 2.5 h. The upper and lower partitions were collected and concentrated separately by centrifugation at 78,000 g for 2.5 h and the virus particles were resuspended in storage buffer (10 mM sodium borate buffer adjusted to pH 7.5). Purified virus preparations obtained from sucrose density gradients were inoculated onto healthy basil and *C. quinoa* plants for symptom development. Also, the purified virus components were negatively stained with 2% uranyl acetate on Formvar carbon-coated copper grids, examined with a Hitachi 7500 electron microscope, and photographed.

Determination of the molecular weight of viral coat protein by polyacrylamide gel electrophoresis

Purified virus preparations and bovine serum albumin were disrupted in protein sample buffer (0.5 M Tris-base, pH 6.8, 10% sodium dodecyl sulfate (SDS), 0.1% 2-mercaptoethanol, 10% glycerol, and 0.05% bromophenol blue) for 4 min in boiling water. The extracts were analyzed by a SDS-13% polyacrylamide gel electrophoresis (SDS-PAGE) using a discontinuous buffer system. Protein bands were stained with 0.2% Coomassie Brilliant Blue R-250 in 7% acetate and 45% methanol for 2 h. Gels were de-stained with 7% acetate and 5% methanol solution. The molecular weight of the viral coat protein was determined by comparison with the following molecular weight standards: myosin, 200 kDa; β-galactosidase, 116 kDa; phosphorylase B, 97.4 kDa; albumin, 66 kDa; ovalbumin, 45 kDa; carbonic

anhydrase, 31 kDa; soybean trypsin inhibitor, 21.5 kDa; lysozyme 14.5 kDa; and bovine pancreas aprotinin 6.5 kDa (Bio-Rad Laboratories, Hercules, CA.) [13].

Serological assays

Indirect ELISA : The indirect enzyme-linked immunosorbent assay (ELISA) conditions were as described by Koenig [12]. The crude antigen was diluted 1:10 in coating buffer, the *Cucumber mosaic virus* (CMV) polyclonal antibody (ADGEN, Agrifood Diagnostics) was diluted 1:1,000 and the enzyme-labeled goat anti-rabbit antibody was diluted 1:4,000. The initial coating was with 100 µl of each sample, positive and negative control in duplicates and incubated at 4℃ for 16 h. After washing with phosphate buffered saline, pH 7.2 and 0.05% Tween 20 (PBST) three times, 200 µl of blocking buffer was added to each well and incubated at 37℃ for 1 h. After washing as described above, 100 µl of diluted polyclonal CMV antibody was added to each well and incubated at 37℃ for 2 h. The goat anti-rabbit IgG, coupled with alkaline phosphatase was then added in conjugate buffer for 1 h at 37℃ after washing. Substrate (p-nitrophenyl phosphate) was dissolved in substrate buffer (5 mg/5 ml) and 100 µl of this was added to each well, incubated in the dark for 1 h. The absorbance at 405 nm was read at 1 and 2 h using a Dynatech MR500 Microplate reader.

Ouchterlony test : Sodium dodecyl sulfate-agar containing 0.8% Noble agar; 1.0% NaN_3; 0.5% SDS was prepared and allowed to solidify before the pattern was cut in the agar and the agar disks removed by aspiration. The *Cucumber mosaic virus* and *Broad bean wilt virus* antisera were used undiluted. Purified BsMV, BBWV and CMV infected crude sap, healthy *C. quinoa* crude sap, pre- immune serum and PBS buffer were used. The plates were incubated in a sealed plastic moist container at room temperature for 24 h and observed for the development of precipitation bands. The results were recorded and photographed [17].

RESULTS

Virus isolation

Basil plants exhibiting virus-like symptom collected in Pingtung County included mosaic and leaf distortion (Fig. 1A). Healthy basil plants mechanically inoculated with fresh sap from *C. quinoa* plants infected mechanically with the third single-lesion isolate showed systemic symptoms 7 days after inoculation. Symptoms in mechanically inoculated basil plants with the third single-lesion isolate included mosaic and leaf distortion (Fig. 1B).

Cotton aphid transmission characteristics of BsMV

The source plants were those basil seedlings mechanically infected with BsMV and showed systemic mosaic and leaf distortion symptoms (Fig. 1B). Those basil seedlings that were infected with BsMV by cotton aphid transmission also developed mosaic and leaf distortion symptoms (Fig. 1C).

Preacquisition starvation time : A highest transmission efficiency of BsMV by *Aphis gossypii* Glover was obtained after a 2 hr preacguisition starvation time as 12 of 20 test plants were infected (Table 1)

Acquisition access time : The 5 min acquisition access time resulted in the highest transmission efficiency of 8 infected plants from a total of 20 plants tested when compared with the other treatments. The aphids were able to acquire the virus within 10 sec. No transmission was obtained in the 1, 2 and 6 h time intervals (Table 2).

Inoculation access time : *A. gossypii* was able to transmit the virus within 10 seconds after the inoculation access time. At 1 min there was no transmission but the aphids were able to transmit the virus efficiently for all other treatments, except for the control. The highest transmission efficiency of 13 infected plants from a total of 20 plants tested was obtained after 2 h inoculation access time (Table 3).

Retention Time : Cotton aphid transmitted the virus at 1 h retention time; however, at or after 1.5 h of the retention time no aphids transmitted the virus (Table 4).

Number of aphids : Two aphids obtained the highest transmission efficiency with 10 plants infected from a total of 20 plants tested (Table 5).

Green peach aphid transmission characteristics of BsMV

Transmission of BsMV by the green peach aphid resulted in 11 infected plants from a total of 20 plants tested. It transmitted BsMV in a non-persistent manner: a mode of transmission characterized by a short acquisition time of 5 min and a very short inoculation time of 2 min.

Host range

Only the three Chenopodiaceae species and *N. rustica* produced local and systemic infection, respectively (Table 6).

Physical properties

Inocula prepared from basil lost infectivity after being diluted to 10^{-4} but not when diluted to 10^{-3}. *C. quinoa* produced local lesions at the highest dilution of 10^{-3}. The infectivity of the extract after treatment for 10 min at 70℃ was completely lost but not at 60℃. Hence the temperature range in which virus activity ceased was 60-70℃. *C. quinoa* plants inoculated with infected crude sap kept at 24 ℃ remained infective for 8 days. When the inoculum was stored at -70℃ virus infectivity was retained during the 10 weeks of storage.

Electron microscopy

Crude saps of basil and *C. quinoa* infected with third single-lesion isolated BsMV after the and purified

Fig. 1. Basil plants showing mosaic and leaf distortion. (A) field symptom; (B) symptom caused by inoculation with the third single-lesion Basil mosaic virus isolate; (C) symptom caused by Basil mosaic virus through cotton aphid transmission.

preparations of BsMV contained isometric virions of similar morphology with mean diameters of approximately 30 nm (Fig. 2).

Virus purification

BsMV was purified following Mossop *et al.* [16] virus purification procedure with little modification. Electron microscopic examination of negatively stained purified preparation showed isometric virus particles approximately 30 nm in diameter (Fig. 2).

Molecular weight of virus coat protein

Two major protein bands with molecular weights of approximately 42 and 26 kDa were observed in SDS-denatured purified BsMV preparations that were analyzed by SDS-13% PAGE (Fig. 3, lane 3).

Table 1. Efficiency of transmission of Basil mosaic virus by *Aphis gossypii* Glover after various preacquisition fasting time intervals

Preacquisition fasting time	Transmission efficiency [1]
0	8/20
30 min	3/20
1 h	5/20
1.5 h	7/20
2 h	12/20
3 h	3/20
4 h	8/20
6 h	12/20

[1.] Number of plants infected /total number of plants tested.

Table 2. Efficiency of transmission of Basil mosaic virus by *Aphis gossypii* Glover after various acquisition access time intervals

Acquisition access time	Transmission efficiency [1]
0	0/20
10 sec	2/20
30 sec	5/20
1 min	3/20
2 min	7/20
5 min	8/20
10 min	7/20
30 min	3/20
1 h	0/20
2 h	0/20
6 h	0/20

[1.] Number of plants infected /total number of plants tested.

Table 3. Efficiency of transmission of Basil mosaic virus by *Aphis gossypii* Glover after various inoculation access time intervals

Inoculation access time	Transmission efficiency [1]
0	0/20
10 sec	5/20
30 sec	3/20
1 min	0/20
2 min	2/20
5 min	5/20
10 min	5/20
30 min	7/20
1 h	10/20
2 h	13/20
6 h	12/20

[1.] Number of plants infected /total number of plants tested.s

Table 4. Efficiency of transmission of Basil mosaic virus by *Aphis gossypii* Glover after various retention time intervals

Retention time	Transmission efficiency [1]
30 min	10/20
1 h	7/20
1.5 h	0/20
2 h	0/20
2.5 h	0/20
3 h	0/20

[1.] Number of plants infected /total number of plants tested.

Table 5. Efficiency of transmission of Basil mosaic virus by different numbers of *Aphis gossypii* Glover

Number of aphid	Transmission efficiency [1]
0	0/20
1	2/20
2	10/20
3	8/20
4	8/20
5	8/20

[1.] Number of plants infected /total number of plants tested.

Table 6. Host reaction to Basil mosaic virus

Test plants	Reactions [1]
Chenopodiaceae	
Chenopodium amaranticolor	cll
C. quinoa.	cll
Spinacia oleracea	sl, w
Cucurbitaceae	
Cucumis sativus	-
Cucurbita moschata	-
Summer squash cv. Tender Finger	-
Winter squash cv. Eastern Rise	-
Lagenaria siceraria	-
Lamiaceae	
Melissa officinalis L.	-
Mentha arvensis	-
Nepeta sp.	-
Salvia farinacea Benth.	-
Salvia splendens Ker-Gawl	-
Leguminosae	
Phaseolus vulgaris	-
Vigna unguiculata	-
Solanaceae	
Capsicum annuum cv. - hot pepper	-
C. annuum cv. Blue Star - sweet pepper	-
Datura stramonium	-
Lycopersicon esculentum cv. Red round tomato	-
Nicotiana tabacum cv. Samsun	-
N. tabacum L. cv. White Burley	-
N. rustica	rs
Petunia x hybrida Vilm.	-

[1.] cll: chlorotic local lesion; rs: ringspot; sl: shoe-lace; w: wilting; - : no visual symptom.

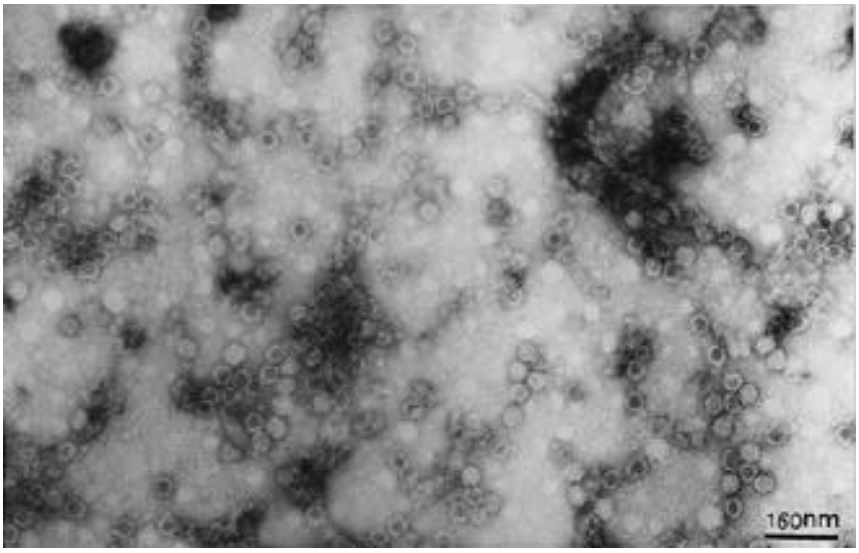

Fig. 2. Electron micrograph of negatively stained Basil mosaic virus particles from purified preparation.

Serology

Indirect ELISA : Antiserum has not yet been prepared for BsMV; hence, healthy and infected basil and *C. quinoa*, purified BsMV preparations and tobacco sample infected with CMV were tested by indirect ELISA with CMV polyclonal antibody. Strong positive reaction was obtained with tobacco tissues infected with CMV. No evident reactions occurred with the healthy and BsMV infected basil and *C. quinoa*, and the purified BsMV preparations.

Ouchterlony test : In agar gel diffusion tests purified BsMV preparation formed one major precipitin band near both wells with undiluted BBWV antiserum. But did not form any precipitin band with CMV antiserum, pre-immune rabbit serum and phosphate-buffered saline buffer (Fig. 4 left). BBWV antiserum reacted with BBWV antigen that was prepared from crude sap of BBWV infected *C. quinoa* leaf and purified BsMV preparations forming major precipitin bands that fused upon meeting each other (band of identity) near the antiserum well. No band was observed for tobacco leaf infected with CMV, healthy *C. quinoa* crude sap, pre-immune rabbit antiserum and phosphate-buffered saline (Fig. 4 right).

DISCUSSION

We concluded that the virus disease infecting basil, in Taiwan is caused by *Broad bean wilt virus*, a member of the *Fabavirus* genus. Our conclusion is based on six primary lines of evidence as follows: mode of transmission, host range and symptoms; *in vitro* properties in crude sap; electron microscopy; serology; and coat protein analysis.

BsMV was readily sap-transmitted by mechanical inoculation from basil to basil. Back- inoculation of purified

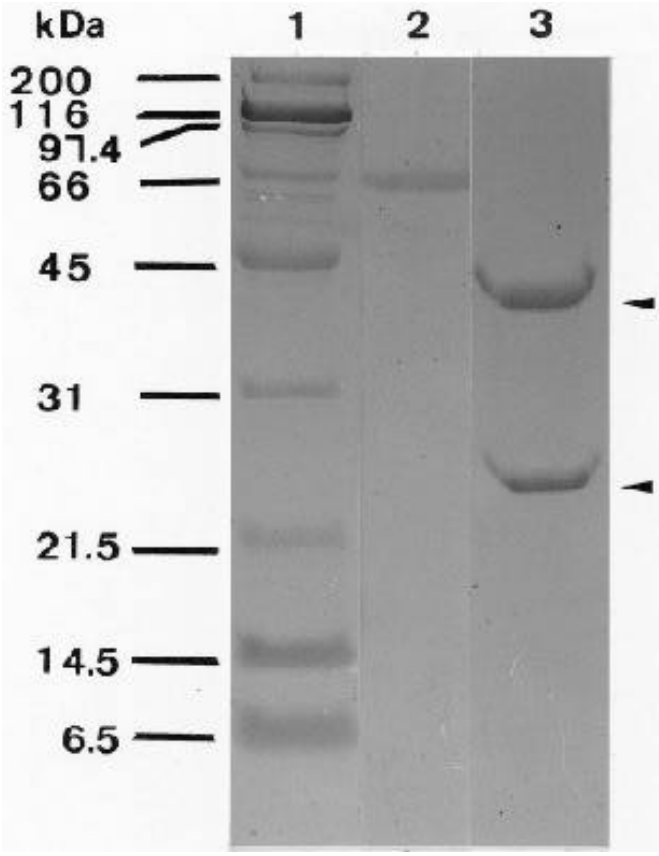

Fig. 3. Analysis of sodium dodecyl sulfate (SDS) denatured Basil mosaic virus capsid protein by electrophoresis in a 13% polyacrylamide gel permeated with SDS and stained with Coomassie Brilliant Blue R-250. Lane 1: SDS-PAGE protein weight standards (kDa) (Bio-Rad, Hercules, CA.). Lane 2: bovine serum albumin (66 kDa). Lane 3: purified Basil mosaic virus coat protein (two protein species with relative molecular weights (Mr) of 42 and 26 kDa).

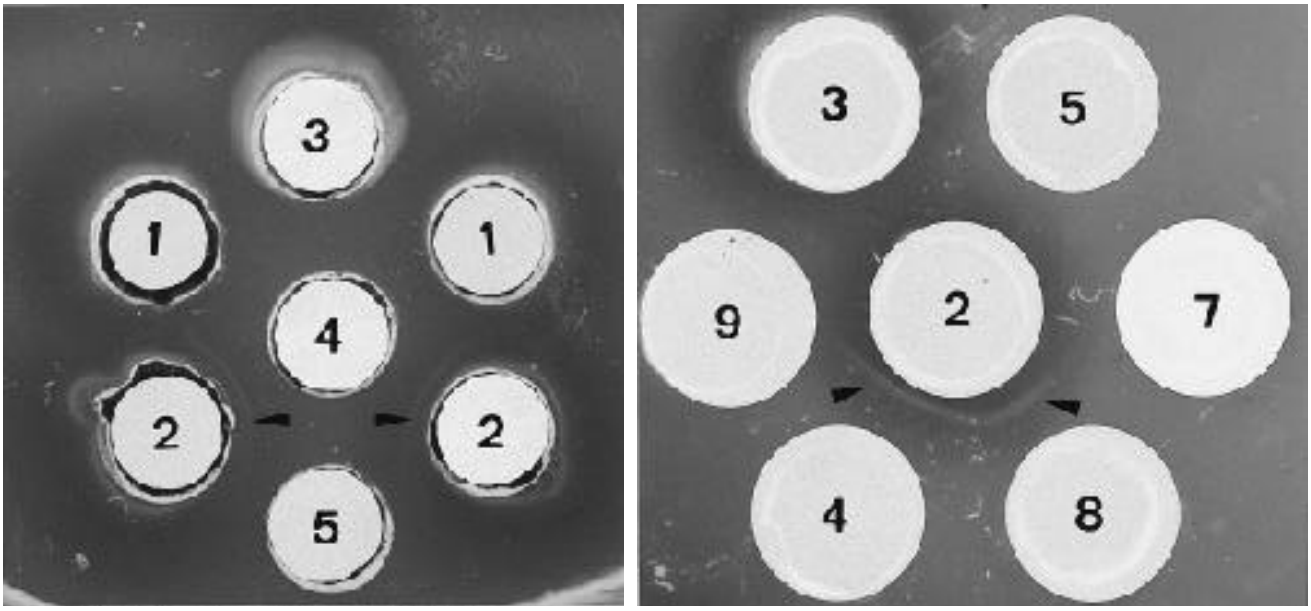

Fig. 4. Reaction in sodium dodecyl sulfate (SDS)-immunodiffusion tests of *Cucumber mosaic virus* (CMV) and *Broad bean wilt virus* (BBWV) antisera to Basil mosaic virus (BsMV) purified preparation. 1: CMV antiserum; 2: BBWV antiserum; 3: pre-immune rabbit serum; 4: purified BsMV; 5: phosphate-buffered saline buffer; 7: healthy *Chenopodium quinoa* leaf extract; 8: BBWV antigen; 9: leaf extract of tobacco infected with CMV. (left): purified BsMV and (right): undiluted BBWV antiserum in center wells. Arrow heads indicate the precipitin bands.

virus to the original host plant has been achieved. The basil virus was acquired and transmitted by cotton aphids in 10 sec, and was retained for less than 1.5 h since at that time the aphids were unable to transmit the virus. As an alternative method of natural transmission BsMV was transmitted in a non-persistent manner by a biological vector. Specifically BsMV was transmitted by *A. gossypii* and *M. persicae*. *M. persicae* has a wide host range but is not reported to colonize on basil. *Broad bean wilt virus* (BBWV) has been reported as transmissible by *M. persicae* but not by *A. gossypii* [(2)]. This paper shows cotton aphid as another important vector for BBWV in basil. Both aphid species therefore, may be important in BsMV epidemiology.

Aphis gossypii is the only aphid species reported on basil [(1)]. *M. persicae* based on the results of the test is the second aphid vector of BsMV. Hence, a single aphid of either species moving between crops can infect several plants after one acquisition event. Both aphid species may acquire and transmit BsMV during short probes that occur during plant sampling as part of host-selection behavior. The use of insecticides therefore, will be ineffective because transmission generally occurs before the aphids obtain a lethal dose of insecticide.

The host range and symptoms produced by BsMV are important clues to its identity. BsMV infected three and one species belonging to Chenopodiaceae and Solanaceae, respectively. All four species and *O. basilicum* are the reported experimental host species susceptible to *Fabaviruses* [(5)]. The host range test indicated that BsMV has a narrower host range (three families, four species) than BBWV. BBWV has shown experimentally to infect more than 9 families [(3)]. Based on these results we speculate that BsMV may be a new strain with a narrow host range.

Within the *Fabavirus* genus there are four definitive species: *Broad bean wilt virus* (BBWV) 1 and 2 and *Lamium mild mosaic virus* (LMMV) and *Patchouli mild mosaic virus* (PatMMV) [(20)]. BsMV did not infect *Vigna unguiculata*, *Nicotiana tabacum* cvs. Samsun and White Burley, *Datura stramonium* and *Petunia x hybirda* [(14)]. BsMV like LMMV and BBWV did not infect *Lycopersicon esculentum* or *Capsicum annuum* [(2,6)]. The symptoms produced by BsMV in green stem basil variety were consisted of mosaic and leaf distortion. This description is similar to that made by Marini in 1955 when he found *Cucumber mosaic virus* (CMV) affecting basil naturally [(15)]. But, serology and protein analysis by SDS-PAGE of BsMV did not match those of *Cucumovirus*, specifically with CMV. CMV has only one virion protein with molecular weight of 24.247 kDa [(3)].

The temperature at which the virus activity ceased for BsMV was at 60℃. It is within the range (55-60℃) of that reported for *Broad bean wilt virus* [(3)]. A longevity *in vitro* of 8 days was observed for BsMV. This is longer than that reported for *Broad bean wilt virus*, 3-4 days [(3)]. Brunt *et al*. [(3)] did not specify whether the sap used in their study was treated with any antibiotics. However, in our test BsMV infected plant sap was treated with two antibiotics before storage at 24 ℃. We speculate that this treatment prolonged the infective life of BsMV in our test. At 10^{-3} BsMV was still infectious,

this is a little lower than that known for *Broad bean wilt virus* which is 10^{-4} to 10^{-5}.

Isometric virus particles of approximately 30 nm in diameter were identified as the causal agent of the BsMV observed in bush basil field in Taiwan. The disease was reproduced by inoculating healthy plants with crude sap and purified virus preparations, confirming the pathogenicity of the virus according to Koch's postulates [18]. The icosahedral symmetry, the size of approximately 30 nm for both virus particles are characteristics similar to that reported for *Broad bean wilt virus* [3,20].

Serological affinity of BsMV with CMV polyclonal antibody was not related as demonstrated with indirect ELISA. Compared to CMV, the absorbance reading for the positive control, that was tobacco infected with CMV was twice as much as the other samples. BBWV antiserum reacted with purified BsMV and BBWV antigen in SDS-immunodiffusion tests to produce distinct immuno- precipitin bands of identity. Our finding showed reactions of identity with a member of the Fabavirus genus but no reaction of identity was observed with CMV antiserum. These data support our finding further that BsMV is not a member of the genus *Cucumovirus*.

When disrupted BsMV was analyzed by SDS-PAGE, two proteins of approximately 42 and 26 kDa were consistently found in purified preparations. This is consistent with the molecular weights of the two proteins of *Fabavirus* genus 42-43.5-45 kDa and 26-27.25-28.5 kDa [5]. Unlike *Cucumovirus* genus members, they have only one major protein at 24.24-24.37-24.5 kDa [3,4].

This is the first report of the natural occurrence of *Broad bean wilt virus*, a member of the genus *Fabavirus* to cause a virus disease in bush basil, a Lamiaceae host plant in Taiwan and also in the world.

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LITERATURE CITED

1. Blackman, R. L., and Eastop, U. F. 2000. Aphids on the world's crops: an identification and information guide, 2nd ed. John Wiley & Sons Ltd., New York, 466 pp.
2. Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L., and Zurcher, E. J. 1996. Broad bean wilt fabavirus. Plant viruses online: Descriptions and lists from the VIDE database. Version 16th January 1997. URL http://biology.anu.edu.au/Groups/MES/vide.
3. Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L., and Zurcher, E. J. 1996. Cucumber mosaic cucumovirus. Plant viruses online: Descriptions and lists from the VIDE database. Version 16th January 1997. URL http://biology.anu.edu.au/Groups/MES/vide.
4. Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L., and Zurcher, E. J. 1996. Cucumoviruses: bromoviridae. Plant viruses online: Descriptions and lists from the VIDE database. Version 16th January 1997. URL http://biology.anu.edu.au/Groups/MES/vide.
5. Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L., and Zurcher, E. J. 1996. Fabavirus: comoviridae. Plant viruses online: Descriptions and lists from the VIDE database. Version 16th January 1997. URL http://biology.anu.edu.au/Groups/MES/vide.
6. Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L., and Zurcher, E. J. 1996. Lamium mild mottle fabavirus. Plant viruses online: Descriptions and lists from the VIDE database. Version 20th August 1996. URL http://biology.anu.edu.au/Groups/MES/vide.
7. Chen, Y. J. 1987. Plants used as vegetables as well as medicine: *Ocimum basilicum* L. Pages 70-74 *in*: Herb. Liang, E. ed. Harvest Publication , Taipei , 192 pp.
8. Dijkstra, J., and de Jager, C. P. 1998. Practical plant virology: protocols and exercises. Springer, New York, 459 pp.
9. Feldman, J. M., and Garcia, O. 1970. Two new natural hosts of alfalfa mosaic virus. Plant Dis. Rep. 54:722-723.
10. Garibaldi, A., Gullino, M. L., and Minuto, G. 1997. Diseases of basil and their management. Plant Dis. 81:124-132.
11. Holcomb, G. E., Valverde, R. A., Sim, J., and Nuss, J. 1999. First report on natural occurrence of tomato spotted wilt tospovirus in basil (*Ocimum basilicum*). Plant Dis. 83:966.
12. Koenig, R. 1981. Indirect ELISA methods for the broad specificity detection of plant viruses. J. Gen. Virol. 55:53-62.
13. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.
14. Lisa, V., Luisoni, E., Boccardo, G., Milne, R. G., and Lovisolo, O. 1982. Lamium mild mosaic virus: a virus distantly related to broad bean wilt. Ann. Appl. Biol. 100:467-476.
15. Marini, E. 1955. Una virosi apparsa sul basilico (*Ocimum basilicum*). Riv. Ortoflorofruttic. Ital. 39:360-362.
16. Mossop, D. W., Francki, R. I. B., and Grivell, C. J. 1976. Comparative studies on tomato aspermy and cucumber

mosaic viruses. V. Purification and properties of a cucumber mosaic virus inducing severe chlorosis. Virology 74:544-546.
17. Purcifull, D. E., and Batchelor, D. E. 1977. Immuno-diffusion tests with sodium dodecyl sulfate (SDS)-treated plant viruses and plant viral inclusions. Bull. No. 783, 39 pp. Agric. Exp. Stn. Uni. Fl., Gainesville.
18. Rivers, T. M. 1937. Viruses and Koch's postulates. J. Bacteriol. 33:1-12.
19. Ryding, O. 1994. Notes on the sweet basil and its wild relatives (*Lamiaceae*). Econ. Bot. 48:65-67.
20. van Regenmortel, M. H. V., Fauquet, C. M., Bishop, D. H. L., Carstens, E. B., Estes, M. K., Lemon, S. H., Maniloff, J., Mayo, M. A., McGeoch, D. J., Pringle, C. R., and Wickner, R. B. 2000. Virus taxonomy classification and nomenclature of viruses: 7th report of the international committee on taxonomy of viruses. Academic Press, San Diego, CA.
21. Wallick, K., and Zinnen, T. M. 1990. Basil chlorosis: a physiological disorder in CO_2-enriched atmospheres. Plant Dis. 74:171-173.

摘 要

Nerie T. Sanz [1]、陳滄海[2,4]、賴博永[3]. 2001. 台灣新發現之羅勒嵌紋病. 植病會刊 10:155-164. ([1.] Ministry of Agriculture, Fisheries and Cooperatives, Belize, Central America ; [2.] 屏東縣 國立屏東科技大學植物保護系 ; [3.] 屏東縣 國立屏東科技大學熱帶農業研究所 ; [4.] 聯絡作者, 電子郵件 : thchen@mail.npust.edu.tw ; 傳真 : 08-7740293

羅勒 (*Ocimum basilicum* L.) 俗稱九層塔, 為台灣地區常見之香辛蔬菜, 本文記載在南台灣高屏地區田間, 發現一種病毒性的羅勒新病害, 受感染的植株葉片呈現嵌紋及皺縮之病徵。將單斑分離所得之病毒機械接種於 5 科 21 種植物, 結果計有藜科植物 3 種及茄科植物 1 種受感染, 而且接種之羅勒幼苗呈現與田間相類似的病徵。棉蚜 (*Aphis gossypii*) 及桃蚜 (*Myzus persicae*) 等兩種蚜蟲可以非持續型方式媒介傳播此病毒。以電顯觀察純化病毒可見到直徑約 30nm 之球型粒子。病毒之物理性質包括熱不活化溫度介於 60-70℃、耐稀釋度為 10^{-3}-10^{-4}、室溫下耐保存性為 8 天、而 -70℃下其活性則可維持達 10 週以上。純化病毒經十二烷基硫酸鈉聚丙烯胺膠體電泳 (sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-PAGE) 可測得分子量分別為 42 和 26 kDa 之兩種蛋白質。凝膠雙向免疫擴散反應或酵素免疫分析法 (ELISA) 試驗結果顯示本病毒可與 *Broad bean wilt virus* 抗體發生特異性反應但卻不與 *Cucumber mosaic virus* 抗體發生反應。依據病毒粒子形態及大小、蚜蟲傳播模式、血清學及鞘蛋白特性等分類特徵, 初步判定此病毒應屬 *Comoviridae* 科 *Fabavirus* 屬之 *Broad bean wilt virus* ; 此為台灣地區田間羅勒病毒病害之首度報導。

關鍵詞 : 羅勒、九層塔、嵌紋病、*Fabavirus* 屬、*Broad bean wilt virus*