Some Properties of a Tobacco Mosaic Virus Strain Isolated from Pepper (*Capsicum annuum*) in Nigeria

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ABSTRACT

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A new strain of tobacco mosaic virus (TMV), serologically related to the type strain, was isolated from severely stunted and mottled pepper (*Capsicum annuum* 'California Wonder') plants from Ankpa, Benue State, Nigeria. Host range and physical properties were similar to those of previously described strains of TMV, except that this strain caused local lesions followed by systemic necrosis in *Nicotiana glutinosa*, *N. rustica*, and *N. tabacum* 'Burley 21,' 'Havana 425,' 'Samsun-NN,' and 'Xanthi' and *Datura stramonium*; defoliation and death of young plants of *D. metel*, *D. ferox*, and *Physalis floridana*; and wilting followed by defoliation of necrotic leaves of Tabasco pepper. This virus is considered to be a previously undescribed strain of TMV because of the unique and severe symptoms it causes on several hosts. The name "defoliation strain of TMV" (TMV-DS) is suggested for this virus.

Tobacco mosaic virus (TMV) is highly variable, especially in host reactions and symptomatology (1,8,14) and has a wide host range that includes important economic crops like peppers and tomatoes (2,6,9,10). In Nigeria, peppers (*Capsicum annuum* L. and *C. frutescens* L.) are widely cultivated because of their flavor and high vitamin C content.

In 1979, *C. annuum* 'California Wonder' plants showing severe stunting, leaf mottle, and reduced fruit set and size were obtained from a farm in Ankpa, Benue State, Nigeria. These plants contained TMV-like particles. Except for pepper veinal mottle virus, which causes a disease of peppers (5), viruses causing leaf mottle and mosaic in peppers in Nigeria remain unknown. Efforts were made to identify the virus causing this apparently new pepper disease at Ankpa. Evidence that the disease is caused by a severe strain of TMV is presented.

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MATERIALS AND METHODS

The virus isolate used in this study was maintained in tobacco (*Nicotiana tabacum* L. 'NC 95,' 'Xanthi') or in *Physalis floridana* Rydb. The inoculum was prepared by grinding systemically infected leaves in 0.05 M phosphate buffer, pH 8.0. Leaves to be inoculated were dusted with Carborundum and rubbed with a cheesecloth pad dipped in the inoculum. Plants were rinsed with tap water and observed for at least 6 wk. Back-inoculations were made on *N. glutinosa* L. to detect symptomless hosts of the virus.

Inoculum prepared from systemically infected leaves of *P. floridana* and containing 0.05 M phosphate buffer, pH 8.0 (1:2,w/v), was used to determine the dilution end point, thermal inactivation point, and longevity in vitro, as described by Ross (12).

Particle morphology of the virus was determined from leaf-dip preparations of naturally infected California Wonder pepper or artificially infected *Datura metel*. Samples were prepared on carbon-coated copper grids and were negatively stained with 2% neutralized potassium phosphotungstic acid before examination with an electron microscope.

Immunodiffusion tests were performed

Table 1. Reaction of plants inoculated with the defoliation strain of tobacco mosaic virus isolated from pepper in Nigeria

Plant	Symptoms[a]	
	Local	Systemic
Amaranthacea		
Amaranthus spinosus	–	–
Gomphrena globosa	NLL, VN	NLL, VN
Chenopodiaceae		
Chenopodium amaranticolor	CLL, DF	–
C. quinoa	CLL, DF	–
Compositae		
Helianthus annuus	–	–
Zinnia elegans	–	–
Cucurbitaceae		
Cucumis sativus		
Supermarket	–	–
Small Sugar Pumpkin	CLL	–
Euphorbiaceae		
Ricinus communis	–	–
Leguminosae		
Arachis hypogaea	–	–
Canavalia ensiformis	–	–
Phaseolus mungo	–	–
P. vulgaris		
Black Turtle-1	NLL	–
Black Turtle-2	NLL	–
Bountiful	–	–
Pinto	NLL	–
Pisum sativum		
Ranger	–	–
Vigna unguiculata		
Early Ramshorn	–	–
New Era	NLL	–
Solanaceae		
Capsicum annuum		
California Wonder	–	MOT
Nsukka Yellow	NLL, DF	NLL, DF
Yolo Wonder	NLL, DF	NLL, DF
Jalapeno M	NLL, DF	NLL, DF
C. frutescens		
Tabasco	LC, WLT, NLL, DF	NLL, DF
Usuede	NLL, DF	NLL, DF
Datura ferox	LC, NLL	LSN
D. metel	LC, NLL	LSN
D. stramonium	LC, NLL	LSN
Datura spp.	–	–
Lycoperaicon esculentum		
Beauty	–	MOT, GVB
Marglobe	–	MOT, FL
Money Maker	–	MOT, FL
Oxheart	–	MOT, FL
Roma	–	MOT, FL, GVB
Nicotiana clevelandii	WLT, DT	WLT, DT
N. glutinosa	LC, NLL	LSN
N. megalosiphon	–	MO
N. occidentalis	–	MO
N. rustica	LC, NLL	LSN
N. tabacum		
Burley 21	LC, NLL	LSN
Havana 425	–	MO
NC 95	–	MO, GVB
Samsun	LC, NLL	LSN
Xanthi	LC, NLL	LSN
White Burley	LC, NLL	LSN
Petunia hybrida		
Fire Chief	–	MOT
Physalis floridana	LC, VN, DF	MO, LD
Solanum melongena		
Black Beauty	LC, NLL, VN	LSN
Lokoja	LC, NLL, VN	LSN
S. nigrum	–	MOT

[a]CLL = chlorotic local lesions, DF = defoliation, DT = death, FL = fernleaf, GVB = green veinbanding, LC = leaf curl, LD = leaf deformation, LSN = lethal systemic necrosis, MO = mosaic, MOT = mottle, NLL = necrotic local lesions, VN = vein necrosis, WLT = wilting, – = no symptoms.

with crude antigen preparations (expressed sap from *N. tabacum* 'Xanthi'), using agar plates containing 0.8% Bacto Noble agar, 0.5% sodium dodecyl sulfate (SDS), 1.0% sodium azide, and 0.85% sodium chloride dissolved in distilled water. TMV and potato virus X (PVX) antisera were gifts from D. E. Lesemann, West Germany, and tobacco etch virus (TEV) and potato virus Y (PVY) antisera were donated by D. E. Purcifull, University of Florida, Gainesville.

RESULTS

The reactions of test plants mechanically inoculated with the virus are shown in Table 1. The virus caused severe and possibly diagnostic symptoms in *N. glutinosa, N. rustica, N. tabacum* 'Burley 21,' 'Havana 425,' 'Samsun-NN,' and 'Xanthi' and *D. stramonium* L. On these plants, the earliest symptoms were downward curling of inoculated leaves 2–3 days after inoculation followed 1–2 days later by light brown local lesions. The lesions rapidly turned dark brown or necrotic and coalesced. They covered the entire leaf lamina, giving it a dried appearance. Inoculated leaves soon wilted and died but remained attached to the stem (Fig. 1A) and sometimes turned white. Seven to 10 days later, the stem portion to which the dead leaves were attached developed necrotic areas that quickly spread, causing systemic stem necrosis (Fig. 1A). Fourteen to 20 days after inoculation, plants usually died. Occasionally, *N. glutinosa* or Samsun-NN tobacco plants with systemic necrosis developed many side shoots near the soil level that also died. Development of systemic necrosis in *C. annuum*, *D. metel*, and *D. ferox* was preceded by complete defoliation of inoculated leaves (Fig. 1B, C). Infected defoliated plants usually collapsed near the top or at the soil line because of severe necrosis in this region (Fig. 1C,D). Infected older plants of *D. metel, D. ferox*, or *N. glutinosa* sometimes developed longitudinal stem cracking. Leaf mottle followed by wilting and death was characteristic of infected *N. clevelandii* L. *Physalis floridana* plants inoculated before the six- to eight-leaf stage usually developed leaf necrosis followed by defoliation and systemic necrosis. Inoculated older plants developed leaf mosaic, puckering, and deformation. In *Lycopersicon esculentum* Mill. 'Roma,' the earliest symptom was malformation of leaflets in the basal portion of the leaf. Leaflets later developed fernleaf, shoestringing, and chlorotic mottle. Within 24 hr after inoculation, leaves of *C. frutescens* 'Tabasco' wilted but soon recovered. Five to 6 days after inoculation, the plants showed stem necrosis and inoculated leaves developed necrotic areas, necrotic veins, curls, and finally abscised. The plants eventually died. *Phaseolus vulgaris* L. 'Black Turtle-1' and 'Black

Turtle-2' developed pinpoint necrotic lesions on inoculated leaves 2–3 days after inoculation. The lesions enlarged and coalesced, forming extensive necrotic areas. Black Turtle-2 produced fewer lesions than Black Turtle-1, and the tendency for these lesions to enlarge and coalesce was also less in Black Turtle-2.

To determine if development of systemic necrosis rather than normal local lesions in *N. glutinosa* inoculated with the virus was caused by a temperature effect (10), groups of eight *N. glutinosa* plants were inoculated and placed in growth chambers at 22 and 32 C; uninoculated tobacco plants were included as checks. All plants inoculated with the virus developed systemic necrosis at 22 and 32 C in 16–21 and 8–10 days, respectively. All check plants remained symptomless.

The following plants were not infected after mechanical inoculation with the virus: *Amaranthus spinosus, Helianthus annuus, Zinnia elegans, Cucumis sativus* 'Local,' *Ricinus communis, Canavalia ensiformis, Phaseolus mungo, Phaseolus vulgaris* 'Bountiful,' *Pisum sativum* 'Ranger,' *Vigna unguiculata* 'Early Ramshorn,' and *Datura* spp.

The virus had a dilution end point between 10^{-5} and 10^{-6}, a thermal inactivation point between 85 and 90 C, and longevity in vitro in excess of 5 wk at 20–22 C. Infected leaf pieces of Samsun-NN tobacco stored at 4 C over $CaCl_2$ in sealed tubes retained infectivity for more than 2 yr.

Rigid rod-shaped particles 15 × 300 nm were found in leaf-dip preparations of infected *D. metel* and *C. annuum* 'California Wonder' showing typical symptoms.

No reaction occurred between the virus and antisera to PVY, PVX, or TEV when crude extracts of infected tissue were used in SDS-immunodiffusion tests. The virus formed a strong precipitin line when reacted with TMV antiserum. Crude extracts of healthy tissue failed to react with TMV antiserum. PVY, PVX, and TEV antisera reacted with homologous antigens but not with extracts of healthy tissues.

DISCUSSION

On the basis of host range, symptomatology, physical properties, and particle morphology, the pepper virus was

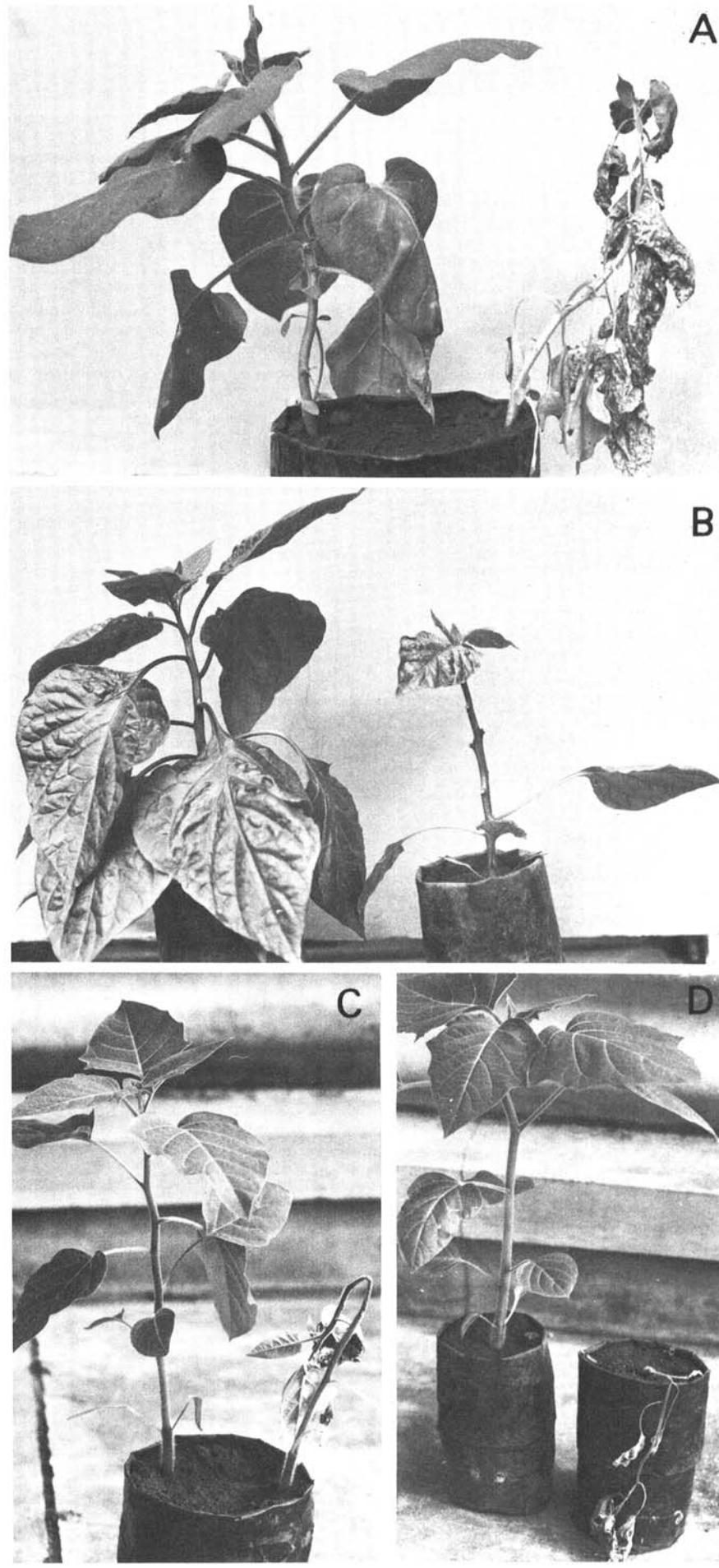

Fig. 1. Symptoms induced by the defoliation strain of tobacco mosaic virus isolated from pepper in Nigeria. **(A)** *Nicotiana glutinosa* plant showing inoculated dead leaves drooping from necrotic portion of the stem; healthy plant on the left. **(B)** *Capsicum annuum* 'Nsukka Yellow' plant showing abscission of inoculated necrotic leaves; healthy plant on the left. **(C)** *Datura metal* plant showing abscission of inoculated leaves and collapse of upper half of the stem; healthy plant on the left. **(D)** *D. metel* plant killed as a result of systemic necrosis; healthy plant on the left.

identified as a defoliation strain of TMV (TMV-DS). Serological tests confirmed the identity of the virus.

TMV is extremely variable, and several strains that are pathogenic to *Nicotiana* species but not to Pinto bean are known (6,9–11). Because TMV-DS infected Pinto bean, it is not identical to these TMV strains. Of the TMV strains that infect Pinto bean (4,10,11), TMV-DS most closely resembles the ash strain described by Lana and Agrios (4), especially in host range. TMV-DS, however, caused defoliation and necrosis in *D. stramonium*, which is a nonhost of the ash strain. TMV-DS also caused necrosis in *N. glutinosa* and *N. tabacum* 'Havana 425,' whereas the ash strain caused local necrotic lesions only. One final difference is that TMV-DS incited wilt and systemic necrosis in Tabasco pepper but the ash strain caused only local necrotic spots. The most useful diagnostic symptoms of TMV-DS were necrosis in *N. glutinosa, N. tabacum* 'Burley 21,' 'Havana 425,' 'Samsun-NN,' and 'Xanthi,' *N. rustica, D. stramonium, D. metel*, and *D. ferox*; defoliation followed by necrosis in young plants of *D. metel, D. ferox*, and *P. floridana*; and leaf abscission in Tabasco pepper, *Chenopodium amaranticolor*, and *C. quinoa*.

Development of necrosis in inoculated *N. glutinosa* plants kept at low (22 ± 2 C) or high (32 ± 2 C) temperatures indicated that symptoms caused by TMV-DS were more necrotic than those of previously described TMV isolates. Furthermore, the development of systemic symptoms in Burley 21 and NC 95 tobaccos inoculated with TMV-DS indicated that it was not identical to either the tomato strain or the common strain of TMV (3). These unique properties of TMV-DS suggest that it is a previously undescribed strain.

The necrosis induced by TMV-DS in Samsun-NN tobacco is similar to that produced by a tobacco veinal necrotic strain of PVY (7) on this host. In addition, the wilting caused by TMV-DS on Tabasco pepper is diagnostic for TEV on this plant (2), but the shape and size of TMV-DS particles and failure to react with antisera to PVY, PVX, or TEV ruled out the involvement of these viruses in the pepper disease at Ankpa.

The origin of TMV-DS is unknown. Because TMV is seedborne in both tomato and pepper (3,13), it probably was introduced by infected seeds.

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