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Natural Infection of *Datura stramonium* L. By an Unusual Strain of *Pepper veinal mottle virus* Genus *Potyvirus* in Nigeria

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Abstract: A disease causing mosaic and leaf distortion was observed in 80% of *Datura stramonium* L. plants in a garden in Ibadan, Nigeria. The causal agent had flexuous rod-shaped particles of about 750-800 nm length. The virus was readily mechanically transmissible but was not seed borne in the natural host. The virus was not transmissible to any other host genera and was restricted to *D. stramonium* and *D. metel*. In serological tests using protein A-sandwich enzyme-linked immunosorbent assay (PAS-ELISA) and antisera to ten members of the family *Potyviridae*, the virus from *D. stramonium* reacted only with the antiserum to *Pepper veinal mottle* virus (PVMV) genus *Potyvirus*. Optical density readings of 3.564 and 0.466 at 405 nm were obtained in reactions between PVMV antiserum and virus infected and healthy *Datura* sp. samples respectively. No serological relationship was detected between the virus from *D. stramonium* and *Blackeye cowpea mosaic* virus (BICMV) genus *Potyvirus*, *Cowpea aphid-borne mosaic* virus (CABMV) genus *Potyvirus* and *Yam mosaic* virus (YMV) genus *Potyvirus* in double antibody sandwich ELISA (DAS-ELISA) and immunosorbent electron microscopy (ISEM) tests. Although some of the data on symptomatology, host range and serological relatedness could implicate *Datura shoestring* virus (DSV) genus *Potyvirus*, the virus may actually be an unusual strain of PVMV.

Key words: *Potyviridae*, *Datura* viruses, *Pepper veinal mottle* virus, serological diagnosis, *Pepper* viruses

INTRODUCTION

The genus *Datura* contains weeds and ornamentals commonly found in the tropics and subtropics (Ivens *et al*., 1978; Thonner, 1962). Some of its species also serve as sources of poisons, dyes, intoxicants and medicines and they assist in virus survival through adverse conditions. In West Africa, *D. stramonium* grows abundantly in wasteland, usually near habitations (Ivens *et al*., 1978). They serve as natural and alternative hosts to several viruses of crop plants.

Datura spp. are natural hosts of several members of the *Potyviridae*, especially in India (Brunt and Kenten, 1972) and are alternative hosts for several economically important viruses including Potato virus Y (PVY) genus *Potyvirus*, *Tobacco etch virus* (TEV) genus *Potyvirus*, *Cucumber mosaic* virus (CMV) genus *Cucumovirus*, *Pepper veinal mottle* virus (PVMV) genus *Potyvirus* and *Okra mosaic* virus (OMV) genus *Tymovirus* (Atiri, 1984; Brunt *et al*., 1990).

This study identifies the causal agent of the mosaic symptoms on *D. stramonium* and establishes its serological relationship with some of the economically important potyviruses of food crops in Southwest Nigeria. An abstract has been published (Taiwo *et al*., 2004).

MATERIALS AND METHODS

Virus isolation, host range studies and test for seed transmission: The virus was isolated from young leaves of *D. stramonium* collected from Ibadan and Lagos, Nigeria between Jan. 1996 and Dec. 2000. It was propagated and maintained by mechanically inoculating carborundum dusted young healthy *Datura* sp. with virus inocula prepared by triturating the infected leaves in 0.05 M potassium phosphate buffer pH 7.5.

For the host range studies, five plants each of fourteen plant species were mechanically inoculated with the *Datura* virus. The plants tested included *D. stramonium* L., *D. metel* L., *Chenopodium amaranticolor* Coste and Reyn., *Nicotiana tabacum* L., *N. benthamiana* Domin, *Celosia argentea* L., *Lycopersicum esculentum* Mill, *Ocimum gratissimum*, *Talinum triangulare* Willd, *Phaseolus vulgaris* L., *Capsicum annuum* L., *Petunia hybrida* Vilm., *Amaranthus hybridus* L. and *Vigna unguiculata* L. Walp.

The inoculated plants were maintained in the greenhouse located at the University of Lagos with temperature at 28-34°C and examined daily for four weeks for symptom development. Symptomless plants were back-inoculated to *D. stramonium*.

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Seedlings were grown and observed for four weeks from 200 seeds collected from 10 naturally infected *D. stramonium*, for seed transmission studies.

Electron microscopy: Virus particles were prepared for electron microscopy from leaf dips stained with 2% potassium phosphotungstate (KPT) pH 6.8. The grids were examined under Philips type E.M. 208 transmission electron microscope at the International Institute of Tropical Agriculture (IITA) and virus particle size was estimated by comparison with those of *Tobacco mosaic* virus (TMV) genus *Tobamovirus* taken at the same magnification.

Serological tests: The serological relationships between the *Datura* virus and other potyviruses were determined by PAS-ELISA, DAS-ELISA and ISEM at IITA.

PAS-ELISA was performed using polyclonal antisera raised against the following members of the family *Potyviridae*: *Bean yellow mosaic* (BYMV), *Dasheen mosaic* (DMV), *Pepper veinal mottle* (PVMV), *Tobacco etch* (TEV), *Bean common mosaic* (BCMV), *Sweet potato mild mottle* (SpMMV), *Potato* virus *Y* (PVY), *Sweet potato chlorotic stunt* (SpCSV), *Sweet potato virus* (SpV-IITA) and *Blackeye cowpea mosaic* virus (BICMV) according to Hobbs *et al.* (1987). All the antisera were obtained from IITA. The ELISA plates were coated with 100 μL of protein A (1 μg mL^{-1}) before the addition of 100 μL of the polyclonal antisera (10^{-3} dilution). Test samples (100 μL) were added, before 100 μL of the polyclonal antisera (1:1000 dilution) was again added. The addition of protein A alkaline phosphatase conjugate (100 μL) was followed by 200 μL of ρ-nitrophenyl phosphate (1 mg mL^{-1}) for reaction detection.

DAS-ELISA was performed using antisera to CABMV and BICMV prepared by Huguenot *et al.* (1993). The plates were coated with 100 μL of polyclonal antisera (1 μg mL^{-1}) and blocked with 100 μL of 1% milk before the addition of the test samples. Detection of reaction was by the addition of monoclonal antibodies (10^{-3}-10^{-5} dilution) followed by the addition of goat antimouse alkaline phosphatase conjugate (10^{-4} dilution) (Sigma Chemical Co. St. Louis, USA). The substrate ρ-nitrophenyl phosphate (200 μL) at a concentration of 1 mg/1 mL was then added. Each sample was tested in at least four wells and each plate included positive and negative controls from IITA's stock. Absorbance at 405 nm (A405) was measured after 60-90 min. with Dynex microplate reader (Dynex Technologies, USA).

Immunosorbent electron microscopy was performed with antisera to YMV and CABMV according to Richter *et al.* (1994).

RESULTS

Virus properties: The *Datura* virus (Ibadan and Lagos isolates) was readily transmissible by mechanical inoculation. Both *D. stramonium* and *D. metel* developed symptoms which consisted of vein clearing, mosaic, leaf distortion and suppression of leaf lamina on either side of the midrib, leading to the formation of filiform or shoestring type of leaves (Fig. 1). Both virus isolates had a highly restricted host range, limited to *Datura* spp. in which they induced similar symptoms. The two isolates were therefore regarded to be biologically similar (Table 1).

None of the seedlings grown from seeds obtained from infected *D. stramonium* plants developed symptoms of infection. Electron microscopy of negatively stained sap from infected plants revealed flexuous rod -shaped particles of about 750-800 nm length (Fig. 2).

Serological test: In serological tests, the *Datura* virus (Ibadan isolate) reacted only with the antiserum to PVMV in PAS-ELISA. Optical density readings of 3.564 and 0.466 were obtained for virus infected and healthy *Datura* samples, respectively. Virus concentration was higher in recently infected plants than in plants infected for a longer time (Table 2). The relatively high A405 values for healthy *Datura* sap tested with antisera to BYMV and SpMMV suggest a high level of homologous host plant proteins in virus preparations used in antisera production.

The DAS-ELISA and ISEM did not reveal any serological relatedness between the *Datura* virus and CABMV, BICMV and YMV.

Table 1: Reactions of test plants to mechanical inoculation with the *Datura* virus

Test plant	Datura virus	Back inoculation[a]
Datura stramonium	M, LD, SL	+
D. metel	M, LD, SL	+
Chenopodium amaranticolor	NS	-
Nicotiana tabacum	"	-
N. benthamiana	"	-
Celosia argentea	"	-
Lycopersicum esculentum	"	-
Ocimum gratissimum	"	-
Talinum triangulare	"	-
Phaseolus vulgaris	"	-
Capsicum annuum	"	-
Petunia hybrida	"	-
Amaranthus hybridus	"	-
Vigna unguiculata	"	-

(a) Back inoculation of *Datura* virus to *D. stramonium*, + or - indicate symptom expression or absence of symptoms respectively in the plants. M = Mosaic, LD = Leaf Distortion, SL = Shoestring of Leaf, NS = No Symptom

Fig. 1: Mosaic and leaf malformation induced by the *Datura* virus in *Datura stramonium* (right) a healthy plant is on the left

Fig. 2: Electron micrograph of flexuous rod-shaped virus particles from infected *Datura stramonium*

Table 2: Reaction of the *Datura* virus to antisera of other potyviruses in protein A sandwich enzyme-linked immunosorbent assay

	Antigens		
Antisera	H	I_R[a]	I_o[b]
Bean yellow mosaic virus	1.862[c]	0.057	0.362
Pepper veinal mottle virus	0.466	3.564	2.154
Dasheen mosaic virus	0.177	0.220	0.173
Tobacco etch virus	0.714	0.341	0.285
Bean common mosaic virus	0.672	0.316	0.320
Sweet potato mild mottle virus	1.037	0.431	0.350
Potato virus Y	0.573	0.252	0.310
Sweet potato chlorotic stunt virus	0.601	0.312	0.342
Sweet potato virus (IITA)	0.755	0.315	0.348
Black eye cowpea mosaic virus	0.372	0.344	0.271

I_R - tests using sap from recently infected *D. stramonium*, I_o - tests using sap from *D. stramonium* that had been infected for some time, (c) Absorbance at 405 nm (averages), H tests using sap from healthy *D. stramonium*

DISCUSSION

The mechanical transmissibility, presence of filamentous particles of about 750-800 nm length and positive serological reaction with antiserum to PVMV indicate a potyvirus in the etiology of the mosaic and leaf distortion disease of *D. stramonium* in Nigeria. At least 10 members of the family *Potyviridae* have been reported to infect *Datura* sp. these include *Datura* (*Colombian*) virus (DCV) (Kahn and Bartels, 1968), *Datura distortion mosaic* virus (DDMV), *Datura mosaic* virus (DMoV), *Datura necrosis* virus (DNV) (Brunt *et al*., 1990), *Datura shoestring* virus (DSV) (Giri and Agrawal, 1971) and *Datura* virus-437 (DV-437) (Fauquet and Mayo, 1999), but most occur only in India.

The *Datura* virus isolate from Nigeria can be distinguished from DCV, DDMV, DMoV and DNV either on the basis of the type of symptoms induced in *Datura* sp. or on the basis of host range (Prasanna *et al*., 1996). While DCV occasionally induces chlorotic spots on inoculated leaves of *D. stramonium* and lesions and systemic wilting/death of *D. metel*, DmoV induces necrotic lesions, systemic mosaic and reduction of leaves in *Datura* sp. The *Datura* virus from Nigeria is fairly similar to DSV in the symptoms it induced in *D. metel*, in its host range (which excluded *C. annuum*) and its serological relatedness to PVMV.

PVMV spreads in West and South Africa causing mottle and foliar rugosity (Loebenstein and Thottappilly, 2003). It is made up of isolates that differ in host

range and symptomatology (Brunt and Kenten, 1972, Brunt *et al*., 1990). In Nigeria, PVMV causes natural infection of pepper (Lana *et al*., 1975), eggplant (Igwegbe and Waterworth, 1982), tomato and tobacco (Ladipo and Roberts, 1977, 1979). Although the isolates vary in host range, some like the *Datura* virus under investigation, failed to infect *C. fructescens* and *C. annuum* (Ladipo, 1987). The PVMV isolate from tomato (Ladipo, 1987) differs from this *Datura* virus by its wider host range which did not include *D. stramonium*. Although *Datura* spp. have been reported as natural hosts for PVMV (Brunt *et al*., 1990), PVMV differs from this *Datura* virus in its wider host range, some of its reported hosts were not susceptible to the *Datura* virus in this investigation. PVMV is serologically related to BYMV, PVY, TEV, *Clover yellow vein* virus (CYV), DCV, DSV and *Water melon mosaic* virus 2 (WMV-2) (Brunt and Kenten, 1972), but the *Datura* virus in this investigation is not serologically related to BYMV, PVY and TEV, suggesting that this *Datura* virus is different from the PVMV, TEV and PVY that have been reported to infect *Datura* spp. in Nigeria. The *Datura* virus is most likely to be an unusual strain of PVMV.

PVMV is widespread and destructive. A comprehensive comparative study of the different PVMV isolates is needed in order to unravel the genomic basis for the diversity in host range and symptomatology. This should assist in the development of an effective and durable control strategy for PVMV.

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