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Occurrence of a Severe Mosaic Disease Infecting African Eggplant (*Solanum macrocarpon* L.) and its Pathogens in Cameroon

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Abstract: African eggplant (*Solanum macrocarpon* L.) is commonly distributed in Cameroon as a vegetable crop for its edible leaves. A severe mosaic disease symptomized as systemic mosaic on leaves and fruits, leaf mottling and crinkling, fruit necrosis, plant stiffing and stunt were commonly observed. Forty-one virus isolates were obtained from field samples and two viruses were detected. Isolate Au27 caused similar symptoms as the natural infection on *S. macrocarpon*. The principal virus (Au27) was identified as eggplant mottle crinkle virus that reported from S. melongena, an eggplant species well distributed from other countries. Isolate Au24 was identified as the complex of Au27 and tobacco mosaic virus (TMV) according to biological, molecular, morphological and serological tests. 14.6% isolates were found belonging to Au24 and 85.4% to Au27. No additional symptom was caused by TMV infection when co-inoculated with Au27 onto *S. macrocarpon*.

Key words: *Solanum macrocarpon*, severe mosaic disease, eggplant mottle crinkles virus, tobacco mosaic virus, Cameroon

Introduction

African eggplant (*Solanum macrocarpon* L.) is originated from West Africa where it seems to have been domesticated long ago (Stevels, 1990). In Cameroon, this plant is cultivated for its edible leaves. This vegetable crop is very popular in South Cameroon and the coastal lowlands, but less frequently found in West and North Cameroon. Some varieties are also cultivated for their fruits. In Cameroon, African eggplant is a herbaceous or suffrutescent plant, annual or biennial, depending on the area grown. .

Occurrence of virus from this particular species has never been documented. But a number of viruses infecting the commonly distributed eggplant, *S. melongena* L., have been reported from many parts of the world. The reported viruses are eggplant mottled dwarf virus in Tunisia, Turkey, Iran, Algeria, Jordan, Morocco, Italy, and Portugal (Musa, 1990); eggplant mosaic virus in West India, Bolivia, Colombia, Venezuela (Briand *et al.*, 1977); eggplant mild mosaic virus in Nigeria (Skotnicki, 1993); eggplant mottle crinkle virus in India (Cherif and Mateli, 1992); eggplant severe mottle virus in Nigeria (Danesh and Lockhart, 1989); eggplant yellow mosaic virus in Hungary and Japan (Gupta *et al.*, 1988); pepper venial mottle virus in Ghana, Nigeria, Ivory Coast, Kenya and South Africa (Lapido *et al.*, 1988) and tomato spotted wilt virus worldwide (Martelli and Cherif, 1987). Most of the above mentioned viruses were simply described according to field symptoms or in some, with host reactions but without a detailed study on the molecular, morphological and serological characteristics. Thus most of the above names are synonyms of each other (Brunt , 1990). During 1994 - 1997, a survey of the viruses infecting *Solanum macrocarpon* was done in Cameroon, a coastal country in West Africa, and this crop was found generally affected by a "severe mosaic" diseases which was widely distributed. The identification resulted for two groups of virus isolates firstly reported from this country.

Materials and Methods

Biological tests: Disease survey was done in vegetable fields at Yaounde (Central Cameroon), Obala (Central Cameroon), Mbamayou (Central East), Dschang (West) and Douala (Coastal South). Leaf tissue from typically infected plants was used as inoculum for isolation onto host plants. Mechanical inoculation was applied for isolation and host reaction tests. Single-lesion-separation was used for purifying the isolates during host reaction test (Chen, 1999).

Viral-specific protein analysis by gel-electrophoresis: SDS-polyacrylamide electrophoresis was used to detect viral specific proteins from infected plants (Alper *et al.*, 1984). Ten grams of infected leaf tissues of *S. macrocarpon* were used for each test.

Partial purification of virus particles: The following protocol was used for partial purification of virus. Fresh leaf tissue (100 g) inoculated with virus for 10 ~ 12 d were homogenized in 100ml of 0.5 mol/L potassium phosphate buffer (pH 7.4 containing 0.1% 2-mercaptoethanol, 2% Triton X-100, 0.01mol/L EDTA), 10% cold chloroform/pentanol (v/v = 1:1) was added and well mixed. The mixture was centrifuged at 8000g, for 20 min at 4°C. The aqueous phase was collected and 6% PEG-8000, 0.1mol/L NaCl were added. The solution was well mixed and kept at 4°C for 6 h. Pellets were collected after centrifuging under the same conditions as above, and it was repeatedly suspended in 5ml 0.2 mol/L sodium phosphate buffer (pH 7.2). The suspension was centrifuged at 12,000g for 20 min, at 4°C, during and after re-suspension. The suspension was used as partially purified virus.

Morphological observation of the virus particles: Partially purified virus particles were fixed with 2% formalin for 4 h and dialyzed against double distilled water for over 24h before stained with 2% phosphotungstate (PTA). The morphological characteristics were examined and measured with transmission electron microscope under 20,000 to 35,000-fold (Chen, 1999).

Agarose diffusion test for serological relationship of the viruses: Serological tests were carried out using OUCHETERLONY agarose diffusion (Chen, 1999).

Antigens were prepared by direct extraction from infected leaf tissue with 0.5 mol/L potassium phosphate buffer (pH 7.4) or

partially purified virus fixed with 2% formalin. A known TMV isolate Be-1, kindly provided by Zhang C. L. from Institute of Plant Quarantine, Agricultural Ministry of China, was used in serological tests.

Results

Field symptom of naturally infected African eggplants, virus distribution and infection tests: Severe mosaic on leaves and fruits, leaf mottle and crinkle, plant stiffing and stunt were commonly observed from field African eggplant in our survey. Vein clearing was observed on young seedlings as evidence for early infection, stem and fruit necrotic lesions were occasionally observed as late effects on whole plants.

Two groups of virus isolates resembling isolate Au24 and Au27 respectively, were obtained by sap inoculation onto *Nicotiana tobaccum* and virus-free seedlings on *S. macrocarpon*.

Two virus isolates, AuX and AuT, derived from single lesion of Au24 were compared with their original isolate and also with Au27. The host reaction on 20 test plant species by four isolates is presented in Table 1.

Six among 41 isolates, 14.6% percent were assigned into group I resembling isolate Au24, and the other 35 isolates, up to 85% of the total, resembling Au27 were assigned into group II. Au24 and Au27 caused similar symptom on *S. macrocarpon* just like the natural infected plants. The addition of Xt into Au27 resulted no additional symptoms with back inoculation but the combination caused necrosis on *N. tobaccum* Than Xanthi NC and *N. clevilendii*. Symptoms of virus infection were presented in Fig. 1 ~ Fig. 3.

Table 1: Host reaction of virus isolates obtained from African eggplant

Test Plants	Au24	Au27	AuX	AuT
Chenopodium quinoa	LN	LN	LN	LN
C. amaranticolor	LN	LN	LN	LN
Datura stramonium	ChS, SN	SM	SM	LN, SM
Nicotiana glutinosa	LN, SM	SM, D	SM, D	LN
N. tobaccum	SM, SV, D	SM, SV, D	SM, SV, D	SM, SV
N. tobaccum Thanxi NC	SN, D, TN	SM	SM	TN
N. rostical	TN	SM	SM	TN
N. clevelendii	SN	SM	SM	LN
N. debneyi	SM, LL	SM, LL	SM, LL	SM
Nicandra physalodes	SM, D,LL	SM, D	SM, D	SM, D
Physalis floridana	LN, SM,YL, IF	SM,YL	SM,YL	LN, SM
Celosia cristata	LN	LN	LN	LN
Antirrhinum majus	LN	LN	LN	LN
Cucumis sativus	SN; Smt	SN; Smt	SN; Smt	SN
Trifolium hybridum	LN	LN	LN	LN
Aracjis hypogea	-	-	-	-
Lycopersicon esculentum	SM, LL	SM, LL	SM, LL	SM, LL
Brassica campestris ssp.pekinensis	-	-	-	-
B. chinensis	-	-	-	-
Solanum macrocarpon	SM, Cr, SN, St	SM, Cr, SN, St	SM, Cr, SN, St	SM, Cr, SN, St

LN: Local necrotic lesion; ChS: Chlorotic spots; SN: Systemic necrosis; SM: Systemic mosaic; SV: Systemic vein clearing; D: Distortion; TN: Top necrosis; LL: Linear leaf; YL: Yellow leaf; LF Leaf falling; SMt: Systemic mottle; Cr: Crinkle; St: Stunt; -:

Fig.1: Natural-infected African eggplant, with symptoms of crinkle, stiffing, systemic mosaic and fruit necrosis.

Fig.2: Seedling of African eggplant inoculated by the isolate Au27, with symptoms of crinkle, stiffing, systemic mosaic and vein clearing.

Fig.3: *Nicotiana rostica* inoculated by Au24, with symptom of completely top necrosis and plant died off.

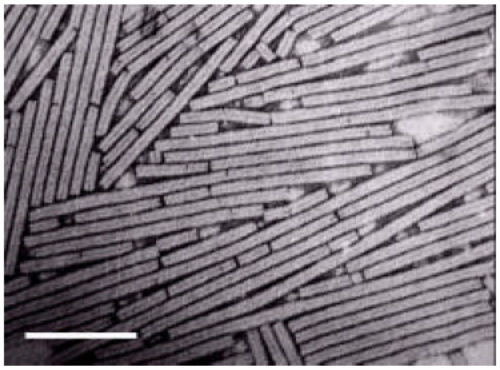

Fig.4: Virus particles of isolate AuT (bar equals to 200nm)

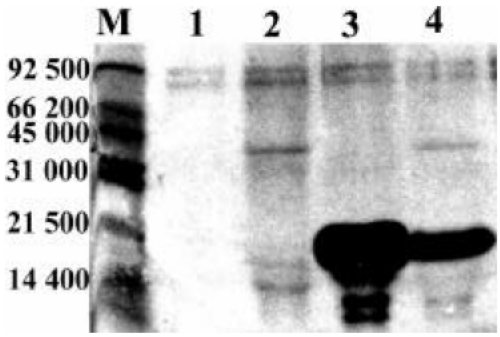

Fig.5: SDS polyacrylamide gel electrophoresis of the viral specific proteins. M: marker proteins, 1: healthy plant control, 2: African eggplant inoculated with isolate Au27, 3: African eggplant inoculated with isolate AuT, 4: African eggplant inoculated with isolate Au24

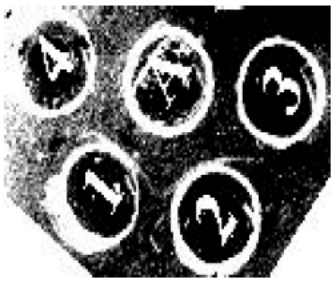

Fig.6: Ouchterlony serological double diffusion test of virus isolates. A: Antiserum of TMV Be-1; 1: Leaf tissue of Be-1 infected African eggplant; 2: Leaf tissue of Xu infected African eggplant; 3: Leaf tissue of Au27 infected African eggplant; 4: Healthy plant of African eggplant

Virus particles observed from the partial purification of the virus: Icosahedral particles, about 30nm in size, were observed from isolate Au27, and AuX. Rod-shaped virus particles with Tobamovirus characterization were observed from isolate Au24 and AuT. Virions of this isolate were observed as elongated rigid cylinders, up to 300 x 18nm in size, as shown in Fig. 4.

Viral specific protein detected from the partial purification of the virus: Three protein patterns were observed in SDS-PAGE for the tested isolates obtained from *S. macrocarpon* as shown in Fig. 5. Protein pattern A: containing one protein band of about 41kD, as that of isolate Au27. Pattern B: containing one dominating protein band of about 17-18 kD, as that of AuT. Pattern C: protein molecules of about 17-18kD and 41kD were both included, as that of isolate Au24. The health control eggplant contained no obverse protein band when prepared with the same schedule.

All the above-listed protein patterns included two to three small polypeptides less than 15kD in the sample inoculated with eggplant isolates but never with that of healthy plants.

The 41kD viral specific protein equals to the coat protein of eggplant mottle crinkle virus and the 17.4 kD protein equal to the coat protein of Tobamovirus.

The protein pattern is constant with the biological grouping of the isolates by host reaction, which indicated that all the isolates with a Tobamovirus addition demonstrated the protein pattern of Au24 while those without Tobamovirus revealed the protein pattern of Au27.

Serological relationship of isolate AuT with known TMV isolates: To confirm the existence of Tobamovirus as one of the pathogenic viruses infecting *S. macrocarpon* plants, OUCHTERLONY agarose diffusion test was applied in comparison with TMV Be-1 isolated from China. The results showed the smoothly jointed precipitation bands between AuT and Be-1. The same result was obtained for the isolates Au24 as well as for other similar isolates among this group. Isolate AuT was identified as a strain of TMV.

Discussion

The symptom presented on *S. macrocarpon* as a result of back inoculation with isolates Au24 and Au27 indicated that the group Au27 virus is the principal virus causing severe mosaic disease on African eggplant in Cameroon. Among the isolates obtained from the field, 85% were assigned into Au27 group, which could be considered as eggplant mottle crinkle virus (EMCV) with the above characteristics. The remainders all gathered into the other group as complexly infection of EMCV and another virus, which must be considered as tobacco mosaic virus (TMV). This is the first time for TMV to be detected as one of the natural pathogenic virus infecting eggplants. The proteins less than 15 kD detected from viral specific protein tests, do not agree with coat proteins of any virus reported from eggplant species, such as eggplant severe mosaic potvirus (Lapido *et al.*, 1988), eggplant mosaic virus (Osorio-Keese *et al.*, 1989) and eggplant mottle dwarf virus (Cherif and Mateli, 1992). They are regarded as the commonly existed "Pathogen-Related Protein" by virus infection (Szyperski *et al.*, 1998, Pennazio and Roggero, 2000). The relationship of the above two viruses with viruses obtained from other continents need to be investigated for their identity and variation, and the influence of these virus on crop production need to be evaluated with more tests.

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