First Report of *Ageratum conyzoides* L. and *Sida acuta* Burm F. as New Weed Hosts of *Tomato Yellow Leaf Curl Tanzania Virus*

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Abstract

Kashina B.D., Mabagala R.B., Mpunami A.A. (2003): **First report of** *Ageratum conyzoides* L. **and** *Sida acuta* Burm F. **as new weed hosts of** ***Tomato yellow leaf curl Tanzania virus***. Plant Protect. Sci., **39**: 18–22.

Tomato farms in the Dodoma region of Tanzania where a high incidence of *Tomato yellow leaf curl Tanzania virus* has been reported were visited to survey for weed hosts. Weeds exhibiting symptoms of infection by the virus were collected and identified. Lysates of leaf samples of the weed species were prepared and clarified by centrifugation. The clarified sap was dotted on nylon membranes and hybridised with a DIG-labelled probe. The putative viral DNA was extracted from the samples by phenol-chloroform procedures, and amplified by polymerase chain reaction methods using a primer pair designed to amplify a 1.2 kb fragment of the virus. Strong hybridisation signals were observed when sap from *Ageratum conyzoides* and *Sida acuta* were hybridised to the labelled probe. Similarly, the expected fragment size was obtained after amplification of DNA from both samples. It is concluded that these weeds are new hosts of *Tomato yellow leaf curl Tanzania virus*. An extensive search for yet undiscovered weed hosts is advocated, while the practice of farm sanitation is encouraged to eliminate reservoirs of the virus and vector.

Keywords: *Tomato yellow leaf curl Tanzania virus*; weed hosts; amplification; hybridisation

Tomato, *Lycopersicon esculentum* Mill., is the major fruit vegetable crop cultivated by commercial and small scale farmers in Tanzania. However, fruit yields continue to dwindle in spite of a steady increase in acreage. This has been attributed to many factors, including pests and diseases. Of the latter, viral infections contribute significant economic losses to tomato fruit yield. So far, four virus diseases have been reported on tomatoes in Tanzania. Of these, *Tomato yellow leaf curl Tanzania virus* (TYLCTZV) (Fauquet *et al*. 2000) is the most economically damaging virus disease of tomatoes in Tanzania with incidences as high as 100% in some regions (Nono-Womdim *et al*. 1996).

In nature, the virus is exclusively transmitted and spread by the aleurodid *Bemisia tabac*i Gennadius and in a persistent manner (Cohen & Harpaz 1964; Cohen & Nitzany 1966). It has become increasingly difficult to control the disease. In Tanzania, records of high incidences of the disease continue unabated with grave consequences of huge losses in quality and quantity of tomatoes. Nono-Womdim *et al*. (1996) revealed the economic importance and spread of TYLCTZV in Tanzania.

Tomato yellow leaf curl virus (TYLCV) is reported to have a narrow host range. It is endemic on tomato, but it is not seed transmitted. Several weed plants have been reported as hosts of the virus in Israel, Jordan, Cyprus, Italy and southern India (Cohen & Nitzany 1966; Ioannou 1987; Saikia & Muniyappa 1989; Rapisarda 1990; Mansour & Al-Musa 1992; Jorda 1993).

Three weed hosts of TYLCTZV, *Achyranthes aspera* L., *Euphorbia heterophylla* L. and *Nicandra physaloides* (L.) Gaertn., have been reported in Tanzania. However, there is a great diversity of

weed species in and around tomato farms, with the possibility that many of them could serve as alternative hosts of the virus. Attempts to manage the disease by controlling the insect vectors through the application of insecticides have not been very successful. An understanding of the epidemiology of the virus is a necessary pre-requisite for effective control of the disease. This paper reports the findings of an investigation that aimed to identify alternative hosts of TYLCTZV.

MATERIALS AND METHODS

Weed samples. Tomato farms in the Dodoma region of Tanzania were visited during the 2000/2001 cropping season. This region has a history of high TYLCTZV incidence (Nono-Womdim *et al*. 1996). Two weeds common within and outside the tomato farms and showing TYLCTZV-related symptoms (Cohen & Harpaz 1964; Mansour & Al-Musa 1992) were collected. These weeds were identified as *Ageratum conyzoides* L. and *Sida acuta* Burm. F. (Ivens 1967; Akobundu & Agyakwa 1987).

Labelling of TYLCV-DNA probes. The viral DNA was eluted from a clone of *Tomato yellow leaf curl Tanzania virus* (pTYA58) and labelled with DIG-11-dUTP using a DIG-High Prime labelling kit (Boehringer, Mannheim) according to manufacturer's instructions. Twenty micro litres of the viral DNA (7 ng/µl) was added to a sterilised 1.5 ml Eppendorf tube. The DNA was denatured at 95°C for 10 min and quickly chilled in an ice/ethanol bath. Four micro litres of DIG-High Prime mix (1 U/µl Klenow enzyme, 1mM dATP, 1mM dCTP, 1mM dGTP, 0.65mM dTTP, 0.35mM DIG-11-dUTP) was added to the denatured DNA and incubated at 37°C overnight. The reaction was stopped by heating for 10 min at 65°C; then 2.5 µl of 4M LiCl, 0.5 µl herring sperm and 75 µl of pre-chilled (–20°C) ethanol were added. After thorough mixing and incubation for 2 h at –20°C, the mixture was centrifuged at 12 000 xg for 15 min (4°C). The pellet was washed in 500 µl of 70% ethanol at room temperature for 5 min, centrifuged as above, vacuum-dried and dissolved in 50 µl TE buffer.

Dot blot hybridisation assay. Leaf samples of the weed species were macerated and incubated in 0.5N NaOH for 30 min at 50°C (Czosnek *et al*. 1988). After neutralisation in 1N HCl and centrifugation at 14 000 rpm for 5 min, 1 or 3 µl of the supernatant was dot-blotted on marked squares on nylon membrane (Hybond N^+). After air-drying the membranes to fix the viral DNA, they were washed for 30 min in transfer solution (0.6M NaCl; 0.4M NaOH) to denature the DNA and then neutralised for 15 min in neutralisation solution (1.5M NaCl; 0.5M Tris, pH 7.5). The membranes were dried briefly between two pieces of Whatman paper and fixed in UV-cross-linker (700 heat units for 1 min) (Amersham Pharmacia). Pre-hybridisation was done for 3 h at 65°C in 20 ml of pre-hybridisation solution (0.2 g glycine, 3.2 ml 20XSSPE, 0.2 ml sonicated herring sperm (10 mg/ml), 1 ml 100X denhardt, 10 ml de-ionised formamide, 0.6 ml 10% SDS, 3.2 ml sterile water) in a hybridisation oven (Biometra AT line, Germany). For hybridisation, the pre-hybridisation solution was replaced with a solution containing 0.1 g/ml of dextran sulphate and 0.1 µl/ml of the DIG-labelled probe, but without glycine. Hybridisation was allowed overnight at 65°C. Post-hybridisation washes included one brief washing in 2X SSPE, four 15 min washes in 2X SSPE and 0.3% SDS at 65°C, 5 min washing in wash buffer [0.3% Tween 20 prepared in buffer 1 (0.1M maleic acid, 0.15M NaCl, pH 7.5)] at room temperature and 30 min wash in buffer 2 (blocking reagent diluted in buffer 1/1:10 v/v). Further washing was done as follows: 30 min in antibody-conjugate solution (anti-Dig alkaline phosphatase in buffer 2 at ratio 1:10 000), two washes (15 min each) in washing buffer and one 5-min wash in buffer 3 (100mM NaCl; 50mM $MgCl_2$, pH 9.5). The membrane was soaked in the substrate solution (CDP Star diluted 1:100 in buffer 3, w/v) for 15 min and dried afterwards between two pieces of Whatman paper (not completely). The semi-dry membrane was placed between pieces of transparent plastic sheets and sealed (Electric Petra Vacuplus, Germany). The sealed membranes were incubated for 15 min at 37°C and exposed for 25 min to X-ray films (Fujifilm, Tokyo). The films were developed for observation of hybridisation signals.

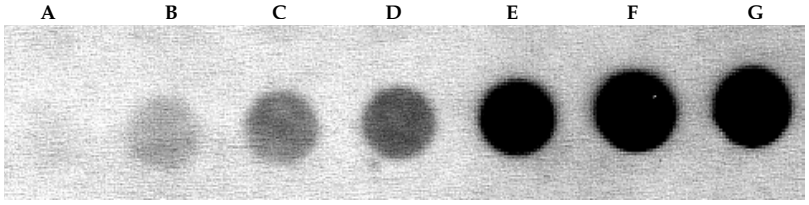
Extraction of viral DNA. The putative viral DNA was extracted from leaves of weed samples showing symptoms of infection by *Tomato yellow leaf curl Tanzania virus* using the phenol-chloroform method described by Sambrook *et al*. (1989). Leaf samples placed in 2 ml-Eppendorf tubes were dipped in liquid nitrogen and ground into powder using a sterile glass rod. One ml of extraction buffer (1M Tris, 1M NaCl, 100mM Na-EDTA, 1M DTT, 10% SDS) and phenol-chloroform (9:1 v/v) mixed in equal volumes was added. After shaking for 15 min and centrifuging for 5 min at 12 000 rpm, the aqueous

phase was transferred to new Eppendorf tubes, and the DNA extracted with an equal volume of phenol:chloroform:iso-amyl alcohol (25:24:1). After centrifugation at 12 000 rpm for 5 min, the aqueous phase was transferred into a new sterile tube and the DNA precipitated overnight at –20°C upon adding a tenth volume of 3M sodium acetate, pH 4.8 and 750 µl of absolute ethanol. The DNA was washed with 500 µl 70% ethanol for 5 min after centrifugation for 30 min at 14 000 rpm. The pellet was vacuum-dried for 10 min and re-suspended in 50 µl of double distilled water.

Polymerase chain reaction (PCR). One micro litre of sample DNA was amplified in a reaction medium containing 0.4 µl of dNTP mix, 4 µl of Q-Reagent, 0.4 µl of each primer, 2.0 µl of 10 × PCR buffer, 0.25 µl of Taq polymerase and sterile water to a total of 20 µl. Two negative control included replacing the primers with double distilled water or sample DNA with DNA from healthy tomato lieaf tissue. For positive control, sample DNA was replaced with DNA from infected tomato issues. The primers used were OTYA 2 (5-GTGAATCCCCAGTTCCTTCCT-3') and OTYA 6 (5'-CTACATGAGAATGGGGAACC-3') designed to amplify 1.2 kb fragment of TYLCV containing the viral coat protein, pre-coat protein and the replication-associated gene. DNA was amplified by 30 cycles in a Thermobaid TouchDown Cycler with melting, annealing and DNA extension conditions of 95°C at 1 min, 55°C at 1 min and 72°C at 2 min, respectively. This was followed by a final cycle of 95°C for 1 min, 55°C for 1 min and 72°C at 10 min. The amplified DNA was electrophoresed in 0.7% agarose gel in Tris-borate-EDTA buffer, stained in 0.5 µg/ml of ethidium bromide and photographed under UV light.

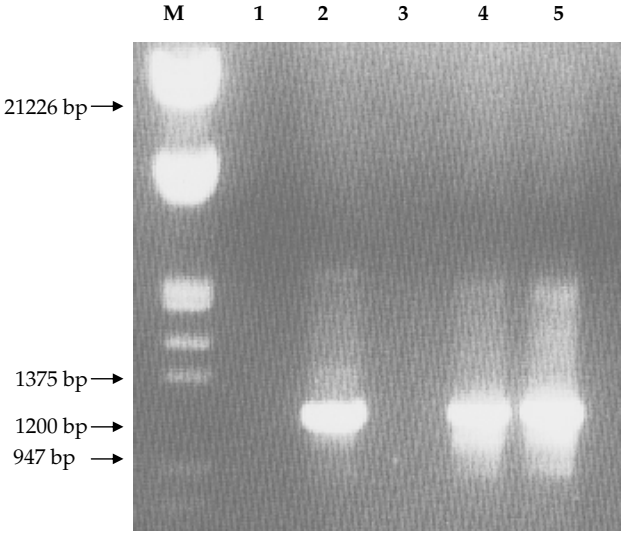
RESULTS

Strong hybridisation signals were obtained when 3 µl of test samples of *Ageratum conyzoides* and *Sida acuta* were hybridised (Figure 1). The expected 1.2kb fragment of the viral DNA was amplified



A = healthy *Lycopersicon esculentum*; B, C, D (1 µl) and E, F, G (3 µl), respectively, of *Ageratum conyzoides*, *Sida acuta*, and *Lycopersicon esculentum* var. Moneymaker infected with *Tomato yellow leaf curl Tanzania virus*

Figure 1. Dot blot hybridisation assay of weed samples using a DIG-labelled probe synthesised from full-length *Tomato yellow leaf curl Sardinia virus*



M = marker (λ DNA digested with Hind III), 1 = PCR medium without primers, 2 = *Lycopersicon esculentum* var. Moneymaker infected with *Tomato yellow leaf curl Tanzania virus*, 3 = healthy L. esculentum, 4 = *Ageratum conyzoides*, 5 = *Sida acuta*

Figure 2. Viral DNA amplified from weed samples using primer pairs OTYA2 and OTYA6

from *A. conyzoides*, *S. acuta* and infected *Lycopersicon esculentum* samples, while no fragment was produced from either PCR control medium or the healthy control (Figure 2).

DISCUSSION

Ageratum conyzoides and *Sida acuta* are common weed species found in and around tomato farms in Tanzania. From the results of the investigation, the symptoms observed on *A. conyzoides* and *S. acuta* were confirmed to be due to TYLCTZV infection. Leaf samples of tomato infected with the TYLCTZV isolate described by Chiang *et al*. (1997) were tested as controls to confirm the results obtained. Nono-Womdim *et al*. (1996) had used hybridisation procedures to confirm that *Achyranthes aspera*, *Euphorbia heterophylla* and *Nicandra physaloides* are weed hosts of TYLCTZV. This new information has further increased the number of weed species that serve as alternative hosts of the virus. *Tomato yellow leaf curl virus* is able to survive on overlapping tomato crops and weed hosts. The knowledge of these hosts is very crucial to the understanding of the epidemiology of the virus and its control. *A. conyzoides* and *S. acuta* are potentially important TYLCTZV reservoirs because they occur in abundant numbers within the tomato farms, they are associated with whitefly vectors, and were found naturally infected with TYLCTZV. Elsewhere, *A. conyzoides* has been identified as host of *Ageratum yellow vein virus*, while *Sida* species are reported to harbour S*ida golden mosaic virus* from Costa Rica, Florida, Honduras and Jamaica (Wong *et al*. 1993; Hofer *et al*. 1997; Frischmuth *et al*. 1997; Fauquet *et al*. 2000). The results further indicate that TYLCTZV survives in weeds within and outside tomato farms, which are potent sources of virus inoculum for primary and secondary spread of the disease. Considering the great diversity of vegetation associated with tomato farms, there is a high possibility of the existence of more weed hosts of TYLCTZV. In some areas, tomatoes are grown all-year round supported by irrigation, thereby complicating the effective management and control of the disease.

Extensive research work is recommended to identify potential weed hosts of the disease, and to breed resistant tomato cultivars that can withstand infection and produce high yields. The practice of farm sanitation by keeping tomato farms free of weeds and surrounding vegetation is important to reduce TYLCTZ infection and spread. This can be effectively implemented if other farmers within the area co-operate.

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Souhrn

Kashina B.D., Mabagala R.B., Mpunami A.A. (2003): **Plevele** *Ageratum conyzoides* L. **a** *Sida acuta* Burm F. – **noví hostitelé *Tomato Yellow Leaf Curl Tanzania Virus***. Plant Protect. Sci., **39**: 18–22.

Byl proveden průzkum plevelných hostitelů *Tomato yellow leaf curl Tanzania virus* na farmách produkujících rajčata v oblasti Dodoma v Tanzanii, kde byla hlášena vysoká četnost viru. Byly odebrány a určeny plevele s příznaky virové infekce. Byly připraveny lyzáty vzorků listů jednotlivých druhů plevelů a vyčištěny odstředěním. Čistá šťáva byla nanesena na nylonovou membránu a hybridizována se sondou značenou digoxygeninem. Virová DNA byla extrahována ze vzorků metodou chloroform-butanol a amplifikována polymerázovou řetězovou reakcí s párem primerů vymezujících 1.2 kb fragment virové DNA. Při hybridizaci šťávy z *Ageratum conyzoides* a *Sida acuta* se značenou sondou byl pozorován silný hybridizační signál. Podobně byl z obou vzorků získán fragment DNA o předpokládané velikosti. Bylo zjištěno, že uvedené druhy plevelů jsou novými hostiteli viru. Doporučuje se rozsáhlý průzkum dosud nezjištěných plevelných hostitelů viru a sanitace farem eliminací hostitelů viru a vektorů.

Klíčová slova: *Tomato yellow leaf curl Tanzania virus*; plevelní hostitelé; amplifikace; hybridizace

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