JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 47, No. 4 (2007)

SEROLOGICAL DETECTION AND VARIABILITY OF *TOMATO YELLOW LEAF CURL VIRUS* ISOLATES FROM TANZANIA

Boniface D. Kashina[1], Robert B. Mabagala[1], Anatolia A. Mpunami[2]*

[1] Department of Crop Science and Production, Sokoine University of Agriculture, Tanzania
[2] Agricultural Research Institute, Mikocheni, Dar es Salaam, Tanzania

Received: June 5, 2007
Accepted: October 5, 2007

Abstract: Tomato farms in Arusha, Morogoro, Dodoma, Iringa, Kilimanjaro and Coast regions of Tanzania were surveyed to assess the incidence of the yellow leaf curl disease, and to collect infected tomato leaf samples for sero-diagnosis. The triple antibody sandwich enzyme linked immunosorbent assay (TAS-ELISA) format was adopted for the detection of disease using commercial polyclonal antiserum and monoclonal antibodies SCRI 17, SCRI 20, SCRI 23 and SCRI 33. ELISA readings were rated on a scale of 0–4. The results of the tests indicated that all the *Tomato yellow leaf curl virus* (TYLCV) isolates recorded high reaction values (4) with the polyclonal antibody. However, the Dodoma and Arusha isolates were rated highest in optical density (OD) reading with MAb SCRI 20 and 23. The remaining isolates produced lower OD values. All the isolates rated low (2) when tested with SCRI 33. The differences in reaction to the monoclonal antibodies of TYLCV indicated that variability exists between the coat protein epitopes of TYLCV and *Tomato yellow leaf curl Tanzania virus* (TYLCTZV) on one hand, and among the TYLCTZV isolates on the other. Only the isolates from Arusha and Dodoma share a high sequence homology in coat protein with the European and related TYLCV isolates. Furthermore, the reaction with either SCRI 20 or SCRI 23 show that the isolates from Arusha and Dodoma share a high degree of homology, and could belong to one serotype. The other isolates from Morogoro, Coast and Kilimanjaro could form another serotype, while the isolate from Iringa is a different serotype. On the other hand, reaction with SCRI 17 groups the isolates in two serotypes, the Dodoma isolate alone, and another that groups the other five isolates together. It is recommended that other procedures such as DNA-DNA hybridization assays, polymerase chain reaction, restriction fragment length polymorphisms and sequencing can be combined with the use of monoclonal antisera for the detection and prediction or inference of Tomato yellow leaf curl disease (TYLCD) virus relationships at the quasi-species or strain levels in Tanzania.

Key words: TYLCD, begomoviruses, serological detection, monoclonal antibodies, Tanzania

*Corresponding address:
Department of Crop Protection, Institute for Agricultural Research, Ahmadu Bello University, P.M.B. 1044, Zaria, Nigeria, bkashina@yahoo.com

INTRODUCTION

Tomato, *Lycopersicon lycopersicum* Karst, is one of the most important vegetables cultivated in most regions of the world second only to potatoes, but ranked first in Tanzania (AVRDC 1993). In Tanzania, there has been progressive increase in acreage over the years without commensurate increase in tomato fruit yields (Nono-Womdim *et al*. 1996; Kashina 2003). This has been attributed to many factors, including pests and diseases. Viral diseases account for much of the economic losses in tomato fruit yield. Tomato yellow leaf curl disease (TYLCD) is one of the most widespread and economically important viral diseases worldwide. The disease is caused by *Tomato yellow leaf curl virus* (TYLCV) (Al-Musa 1982; Navot *et al*. 1991). TYLCV is a major problem of tomato production in many tropical and subtropical regions (Makkouk and Laterrot 1983). It is a member of the genus *Begomovirus*, one of four genera (*Mastrevirus*, *Curtovirus, Topocuvirus,* and *Begomovirus*), which make up the *Geminiviridae* family. Subdivision into genera is based on genome structure, plant host and insect vector (Fauquet *et al*. 2000). It has been documented that several different begomoviruses, depending on the geographical areas of cultivation, affect tomatoes grown in tropical and subtropical regions (Fauquet *et al*. 2000; Fauquet and Stanley 2005).

In Tanzania the disease was first detected in Morogoro (Czosnek *et al*. 1990). Of the four viruses so far reported on tomatoes in Tanzania, *Tomato yellow leaf curl Tanzania virus* (TYLCTZV) (Fauquet *et al*. 2000) is the most widespread and economically important (AVRDC 1993; Nono-Womdim *et al*. 1996; Kashina *et al*. 2003a), with the highest incidence occurring during the hottest periods of the year, when tomato yield losses can be as high as 100 %.

The identification of whitefly-transmitted geminiviruses by traditional virological methods has proven difficult. Many of the economically important whitefly-transmitted geminiviruses including TYLCV, are generally, not mechanically transmissible from tomato to tomato, except in very few cases (Abdel-Salam 1990). Therefore, the use of definitive bioassay hosts for virus identification, evaluation of host range and other biological properties has been very difficult in many cases. Another difficulty is the production of virus-specific antiserum which has been attributed to the instability of physical and chemical properties of virus particles during purification, poor immunogenicity of virions, and antigenic indistinctiveness of their capsid proteins with available polyclonal and most monoclonal antisera preparations (Roberts *et al*. 1984). More reliable procedures that have been used for the detection and identification of whitefly-transmitted geminiviruses, and to predict or infer virus relationships at the quasi-species or strain levels, are DNA-DNA hybridization assays (Roberts *et al*. 1984).

Tomato leaf samples showing typical TYLCD symptoms collected from different regions in Tanzania could not provide conclusive evidence as to the true identity of the virus by genetic methods (Nono-Womdim *et al*. 1996). Czosnek *et al*. (1990) identified TYLCV in Morogoro, Tanzania, using nucleic acid probes. Chiang *et al*. (1997) reported the nucleotide sequence of a distinct geminivirus, *Tomato leaf curl Tanzania virus*. Shi *et al*. (2006) identified a distinct begomovirus from Arusha, Tanzania for which the name *Tomato leaf curl Arusha virus* is proposed. It is generally thought that many viruses/strains may be responsible for the symptoms observed on tomatoes in Tanzania or, that distinct virus strains are involved. *Tomato yellow leaf curl virus* is the first reported whitefly-transmitted geminivirus that possesses a single genomic

component (Navot *et al*. 1991). However, it is unusually heterogeneous with some isolates having two genomic components (Rochester *et al*. 1994) and a high degree of sequence diversity, greater than normally found among isolates of other viruses, has been reported among genomes of TYLCV strains (Navot *et al*. 1991; Pico *et al*. 1996).

Harrison and Robinson (1999) reported the use of a panel of monoclonal antibodies to establish existing genomic and antigenic relationships among naturally and geographically diverse species of begomoviruses. This paper reports the outcome of an investigation conducted to determine the serological relationship among isolates of TYLCD from tomato-growing regions in Tanzania.

MATERIALS AND METHODS

Sampling and sample collection

Surveys were conducted in 2000/2001 from Morogoro, Iringa, Kilimanjaro, Coast, Arusha and Dodoma regions of Tanzania to collect disease isolates for sero-characterization. The regions were selected based on tomato production history and disease incidence earlier reported by Nono-Womdim *et al*. (1996). Leaf samples of infected tomato plants showing typical symptoms of Tomato yellow leaf curl disease (Gafni 2003) were collected from each region as described earlier (Kashina *et al*. 2002).

Triple Antibody Sandwich-Enzyme-Linked Immunosorbent Assay Tests (TAS-ELISA)

Detection of the virus was done using commercial polyclonal and monoclonal antisera (Adgen Diagnostic Systems, Scotland). The TAS-ELISA format was adopted as described by Pico *et al*. (1999) and outlined earlier (Kashina *et al.* 2003b). Polyclonal antibody (PAb) was diluted in carbonate buffer, pH 9.6 at 1:1 000 μl (v/v) to coat for viruses and the coated plates were incubated at 37°C for 4 h. After 4-h incubation and washing with phosphate buffered saline-Tween 20 (PBS-T), pH 7.2, 100 μl of each test sample ground in extraction buffer, pH 8.5 (1:10 w/v) was added and incubated overnight at 4°C. Monoclonal antibodies, SCRI 17, SCRI 20, SCRI 23 and SCRI 33 were diluted in conjugate buffer at 1:1000 μl (v/v) and used as probe antibodies. The plates were incubated for 2 h at 37°C, washed and goat anti-mouse alkaline phosphatase (GAM-PAL) conjugate diluted in conjugate buffer (1:3 000 v/v) was added. The plates were incubated at 37°C for 1 h, washed to remove unbound conjugate and the substrate (p-Nitrophenyl phosphate) 1:1 (mg/ml) dissolved in diethanolamine buffer (pH 9.8), was added to the test wells. After incubation for 1 h, the plates were assessed visually and by the spectrophotometer (405 nm) reader. ELISA readings were rated as described by McGrath and Harrison (1995) to compare reaction strengths. Samples with absorbance values twice those of the healthy control were rated to be positive.

RESULTS

The TYLCV isolates reacted with strong signals to the polyclonal probe (PAb) for geminiviruses. The reaction of all the isolates was rated the same (4) (Table 1). When the isolates were further tested with monoclonal antibodies (MAbs) SCRI 17, SCRI 20, SCRI 23 and SCRI 33, the Dodoma and Arusha isolates were rated highest in optical density reading with MAb SCRI 20 and 23, indicating a closer relationship to the European strains of TYLCV. The remaining isolates produced lower OD values (Table 1).

Table 1. Results of triple antibody sandwich-enzyme linked immunosorbent assay tests showing absorbance values of *Tomato yellow leaf curl Tanzania virus* isolates

Virus isolate	OD reading at A_{405nm}*				
	Pab**	SCRI 17	SCRI 20	SCRI 23	SCRI 33
Arusha	4	2	4	4	2
Kilimanjaro	4	2	3	2	2
Dodoma	4	3	4	4	2
Morogoro	4	2	3	2	2
Iringa	4	2	2	3	2
Coast	4	2	3	2	2
Infected control	4	4	4	4	4
Healthy control	0	0	0	0	0

*A_{405nm} – 4 ≥ 1.8; 3 = 1.21 – 1.8; 2 = 0.61 – 1.2; 1 = 0.3 – 0.6; 0 ≤ 0.3
**Pab – Polyclonal probe for detecting geminiviruses

All the isolates rated low (2) when tested with SCRI 33. The monoclonal antibodies SCRI 20 and 23 have been used for the detection of the European strains of TYLCV, while SCRI 33 detects ACMV (McGrath and Harrison 1995).

DISCUSSION

According to documented reports (Pico *et al*. 1996), a high degree of homology in coat protein exists among related whitefly-transmitted geminiviruses. This explains why the TYLCTZV isolates reacted strongly to the geminivirus polyclonal probe. However, monoclonal antibodies are specifically produced against single coat protein epitopes. Thus, the differences in reaction to the monoclonal antibodies of TYLCV indicate that variability exists between the coat protein epitopes of TYLCV and TYLCTZV on one hand, and among the TYLCTZV isolates on the other. Only the two TYLCTZV isolates (Arusha and Dodoma) share a high sequence homology in coat protein with the European and related TYLCV isolates. The other TYLCTZV isolates are only distantly related to TYLCV. Furthermore, based on the differences in reaction, the serological relationships between the TYLCTZV isolates can be determined. For instance, the reaction with either SCRI 20 or SCRI 23 shows that the isolates from Arusha and Dodoma shared a high degree of homology, and could belong to one serotype. The other isolates from Morogoro, Coast and Kilimanjaro could form another serotype, while the isolate from Iringa is a different serotype. On the other hand, reaction with SCRI 17 groups the isolates in two serotypes, the Dodoma isolate alone, and another that groups the other five isolates together.

All the TYLCTZV isolates reacted weakly with SCRI 33, which detects ACMV, indicating that they were all distantly related to the *African cassava mosaic virus*. Testing the reaction of the TYLCTZV isolates to additional geminivirus monoclonal antibodies, including those specifically produced to TYLCTZV would yield profiles that would be useful for the identification of TYLCV strains based on coat protein sequences.

Differences in coat protein epitopes have implications for whitefly transmission of begomoviruses. It has been reported that the begomoviral coat protein is the only gene product that interacts directly with whitefly vectors during the circulative transmission of the virus (Rybicki 1994; Czosnek *et al*. 2002). Additional evidence shows that the coat protein plays a role in vector recognition and acquisition (Accoto *et al*. 1994). Furthermore, the specificity and frequency of virus transmission by the whitefly vector has been associated with the selection of variants in the coat protein for effective transmission by the vector (McGrath and Harrison 1995). All these imply that variation in the virus coat protein epitopes will influence the efficiency of transmission by the whitefly vector. This would explain the observed differences in the transmission efficiency of the TYLCTZV isolates by the vector, *B. tabaci* (Kashina *et al*. 2003b), and the variation in disease incidence and severity at different locations reported by Kashina *et al*. (2002). The European TYLCV isolates have been reported to be identical in sequence with the Eastern Mediterranean TYLCV isolates. However, this sequence identity was found to decrease with increasing geographical distance (Noris *et al*. 1994). The combined use of traditional and molecular diagnostic procedures will further elucidate the true situation of TYLCD in Tanzania with regards to available virus strains/species.

ACKNOWLEDGEMENTS

The authors are grateful to the German Academic Exchange Services for sponsoring the study and to the Tanzania Official Seed Certification Agency for making available its laboratory facilities. Dr S. Winter of the German Collections of Microorganisms (DSMZ), Germany assisted us with some monoclonal antibodies.

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POLISH SUMMARY

WŁAŚCIWOŚCI TRANSMISYJNE WIRUSA ŻÓŁTEJ KĘDZIERZAWOŚCI POMIDORA Z TANZANII

Stwierdzono, że wirus żółtej kędzierzawości pomidora infekujący rośliny pomidora w Tanzanii różni się od geminiwirusów Starego Świata. Badania podjęto w celu określenia właściwości transmisyjnych tego wirusa takich jak czas żerowania wektora na roślinie porażonej potrzebny do nabycia przez niego wirusa, czas żerowania wektora na roślinie infekowanej potrzebny do jej inokulacji, czas życia wirusa w wektorze, inokulacja mechaniczna oraz przenoszenie przez nasiona lub przeszczepianie. Otrzymane wyniki wykazały, że wirus jest przenoszony przez *Bemisia tabaci* Genn., nie jest natomiast przenoszony mechanicznie ani przez nasiona. Minimalne czasy nabycia wirusa przez wektora i inokulacji rośliny wynosiły 30 minut. Zatem właściwości czynnika powodującego objawy żółtej kędzierzawości na pomidorach z różnych regionów Tanzanii są podobne i nie odbiegają od właściwości garunków *Begomowirus* badanych gdzie indziej.