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SHORT COMMUNICATION

THE EMERGENCE OF BEGOMOVIRUS-INDUCED YELLOW LEAF CURL DISEASE OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) IN ETHIOPIA

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ABSTRACT: A systematic survey was carried out to determine the incidence and distribution of tomato virus diseases in Ethiopia. Fifty seven tomato fields were inspected, 570 plant samples were collected and tested by DAS-ELISA against eight tomato infecting viruses. The presence of a *Tomato yellow leaf curl virus* (TYLCV) in selected symptomatic samples was confirmed by immunocapture-polymerase chain reaction (IC-PCR). The data showed that TYLCV and *Tomato mosaic virus* (ToMV) were the two widespread viruses detected in 24.6% and 47.37% of the fields, respectively; whereas *Tomato mild mottle virus* (TMMV) followed by *Potato virus Y* (PVY) were detected only in few samples or completely absent in most fields in the other parts of southern and eastern parts of the country. All tomato samples were negative for *Tomato spotted wilt virus* (TSWV), *Tomato ring spot virus* (ToRSV), *Tobacco mosaic virus* (TMV) and *Cucumber mosaic virus* (CMV). High incidence (up to 95%) and prevalence of leaf curl disease was observed in fields in the Rift Valley areas (Upper Awash, Merti, Melkasa and fields along the road Mojo to Zwai). The result indicated that tomato yellow leaf curl has emerged as a major tomato virus disease causing serious losses in the fields of Rift Valley areas.

Key words/phrases: Begomoviruses, DAS-ELISA, IC-PCR, Tomato.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetables in Ethiopia. However, farmers get lower yield mainly due to diseases, pests and sub-optimal fertilization (Tesfaye Balemi, 2008). Diseases caused by viruses are among the major factors that reduce tomato yields (Wondirad Mandefro *et al*., 2009).

Various types of viruses that infect tomato have been identified from different tomato growing areas of Ethiopia (Marchoux, 1976; Yaynu Hiskias, 1998). Mosaic and related diseases caused by *Tobacco mosaic virus* (TMV) were widespread in tomato growing areas (Agranovisky and

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Anisimoff, 1986). Yaynu Hiskias (1998) conducted survey and recorded high incidence of mosaic disease particularly in Rift Valley areas, the most important of which was the *Tomato mild mottle virus* (TMMV) followed by *Potato virus Y* (PVY) and *Tomato mosaic virus* (ToMV).

Tomato yellow leaf curl disease (TYLCD) caused by members of genus *Begomovirus* has emerged as one of the most important virus diseases of tomato worldwide in the last decades (Anupam and Malathi, 2003). The causative agent has recently been identified as *Tomato yellow leaf curl virus* (TYLCV) containing a complex number of viral species occurring in tropical and subtropical regions. The group causes severe disease with yield losses of up to 100% on economically important crops, including tomato (Fauquet *et al*., 2008).

Although symptoms resembling that of begomovirus-induced yellow leaf curl disease have been recorded in mid-1970's (Quiot, 1976), in Ethiopia, past surveys of tomato virus did not show any occurrence of the disease (Agranovisky and Anisimoff, 1986; Yaynu Hiskias, 1998). However, recent studies had showed that a disease resembling tomato yellow leaf curl disease occurred in epidemic proportion on tomato in some parts of the Rift Valley areas. The virus that was collected from a tomato field at Melkasa has been later identified as a provisional strain of *Tomato yellow leaf curl Mali virus* (TYLCMLV) under a Begomovirus group (Shih *et al*., 2006).

Apart from this effort, there is still a dearth of information on the incidence, of the begomoviruses from wider parts of tomato growing regions of Ethiopia. The main aim of this research was, therefore, to study the relative importance of viruses and viral diseases on tomato including those causing TYLCD epidemics.

MATERIALS AND METHODS

Field observation and sample collection

The survey was conducted in the Rift Valley areas of eastern Shewa (Upper Awash, Merti, Melkasa, along the road from Mojo to Zwai), in western Ethiopia (Ambo to Guder), southwestern Ethiopia (Wolliso to Jimma), southern Ethiopia (Butajira to Wolayta) and west and east Harerge. The Rift Valley areas and western Ethiopia are the major tomato production areas in the country (Tesfaye Balemi, 2008).

In the survey, a total of 57 tomato fields at least 10 km apart were inspected to observe and record symptoms from foliar samples. From each field, 10 samples were taken and a total of 570 samples were collected. Field disease

incidence was recorded at each field by dividing the number of symptomatic plants to the total number of plants in the field (Venkataravanappa *et al*., 2013). The collected samples were dried in silica gel, labeled and brought to Biotechnology Laboratory at Holetta Agricultural Research Centre of Ethiopian Institute of Agricultural Research (EIAR), for serological test or serological and IC-PCR tests.

Serological test for tomato viruses

Identification of viruses was undertaken using serological methods. The polyclonal antibodies and their enzyme conjugate were purchased from Bioreba, Switzerland except for those of TMMV which were kindly provided by Dr. H.J. Vetten, Julius Kuehn Institute, Germany. The standard double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used to identify eight possible viral agents that are associated with the disease according to Clark and Adams (1977); The viruses were; *Tomato yellow leaf curl virus* (TYLCV), *Tomato mosaic virus* (ToMV), *Tomato spotted wilt virus* (TSWV), *Tomato ring spot virus* (ToRSV), *Tobacco mosaic virus* (TMV), *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Tomato mild mottle virus* (TMMV).

Microtitre ELISA plates were coated with a purified immunoglobin G (IgG) diluted in coating buffer (100 µl/well) and incubated at 37°C for 2-4 hours. The antibody adsorbed on the plate was the trapping antibody. Aliquot of each test sample was prepared by grinding 20 mg of dried leaf sample in 1 ml extraction buffer, positive control and negative control were added in duplicate wells in ELISA plates (100 µl/well). The plates were incubated overnight at 4°C. Antigens (viruses) specific to the bound trapping antibody were washed to remove proteins.

The virus attached to the trapping antibody was detected by adding IgG-alkaline phosphatase in conjugate buffer (100 µl/well) and incubated at 37°C for 4 hours. In the case of freshly prepared substrate, aliquot samples were mixed with 1 mg pNPP (p-Nitrophenyl Phosphate) and dissolved in 1 ml of substrate buffer (100 µl/well). Colour development was observed after incubation of 30 to 60 minutes at room temperature. Plates were visually inspected followed by reading with a HUMAREADER PLUS, ELISA reader with a 405 nm absorbance filter. A well with absorbance value of at least twice that of the negative control wells was considered positive (Nguyen *et al*., 1990).

Immunocapture-PCR tests to amplify TYLCV DNA

Immunocapture-PCR test was conducted as a confirmatory test on randomly selected samples from TYLYV positive for serological test according to Deng *et al*. (1994). This was done without DNA extraction (LeamKhang *et al.*, 2005). A PCR tube was coated with 100 µl of TYLCV antibody (Bioreba) and washed 3 times with ELISA buffer after being incubated at 37°C for 3 hours. Then 100 µl of homogenized test sample (20 mg of dried sample leaf was homogenized in 1 ml extraction buffer) was added and incubated overnight at 4°C. The PCR tube was washed and PCR was conducted in 50 µl (total volume) mix contained 10 µl 5xTaq polymerase buffer (PCR buffer), 5 µl $MgCl_2$ (25 mM), 2.5 µl dNTPs (10 mM), 1 µl each primer (100 pmol), 0.25 µl (5 units) Taq polymerase (Promega) and 30.25 µl PCR water.

A degenerate primer pair, Begomo-146 (5'-TAA TAT TAC C(GT) G(AT) (GT) G(AGC) CC (GC) C -3') and Begomo-672 (5'-TGG AC(CT) TT(AG) CA(AT) GG(GCT) CCT TCA CA -3') derived from consensus sequences that are common to most begomoviruses was used for molecular identification of TYLCV. The reaction mix was subjected to an initial denaturation step of 3 min at 95°C, followed by 35 amplification cycles of 1 min at 95°C, 1.5 min at 52°C and 1 min at 72°C and a final extension period of 10 min at 72°C. PCR product (5 µl) was visualized in 1% agarose gel in UV light along with1 kb DNA size marker (Promega).

RESULTS

Virus diseases incidence in tomato fields

Diverse virus disease symptoms including leaf yellowing with curling, stunted growth, mosaic and mottling were observed in most of the tomato fields at UAAIE, Merti, Melkasa, and along the road Mojo to Zwai in east Shewa. However, a few fields from southwestern Ethiopia along the road from Wolliso to Jimma showed disease symptoms. Tomato plants inspected at fields along the road from Ambo to Guder, Butajira to Wolayta and Harerge were free from any virus disease symptoms.

Accordingly, the incidence of yellow leaf curl disease symptoms ranged from 90-95% at UAAIE, 20-75% at Merti, 20-30% at Melkasa, 2-3% at fields along the road from Wolliso to Jimma and 0% (no symptom observed) to 45% at fields along the road from Mojo to Zwai (data not shown). In general, out of 47.37% of tomato fields that were infected with the diseases with the highest incidence (95%) were recorded from UAAIE;

whereas 52.63% of the tomato fields were free from any virus disease symptom (Table 1).

Table 1. Incidences of yellow leaf curl disease symptoms in tomato fields.

Surveyed areas	No of fields surveyed	Highest incidence observed (%)	No of fields with incidence of yellow leaf curl symptom				
			<1	1-5	6-20	21-50	>50
UAAIE	2	95	-	-	-	-	2
Merti	3	75	-	-	1	1	1
Awash Melkasa	3	30	-	-	2	1	-
East Shewa (Mojo to Zwai)	19	45	10	5	2	2	-
Southwest Ethiopia (Wolliso to Jimma)	5	-	-	-	-	-	-
West Shewa (Ambo to Guder)	12	-	-	-	-	-	-
Southern Ethiopia (Butajira to Wolayta)	5	-	-	-	-	-	-
Western and eastern Harerge	8	-	-	-	-	-	-
Total	**57**	**27 (47.37%)[a]**					

[a] yellow leaf curl disease symptoms observed fields

Serological virus identification

Double antibody sandwich-ELISA test and confirmatory IC-PCR test revealed the occurrence of TYLCV in 14 fields (24.6%) of the total fields, all of which are in the Rift Valley areas (Upper Awash, Merti and along the road from Mojo to Zwai). Of the total of 570 samples tested against ToMV, 102 (17.9%) samples gave positive ELISA reaction to ToMV (Table 2). Only two of the samples tested positive for PVY whereas six were positive for TMMV. Samples from all fields were negative for other tomato viruses: TSWV, ToRSV-Ch, TMV and CMV (data not shown).

Immunocapture-PCR-based detection of TYLCV

Begomovirus, specific degenerative primers for confirmation of DAS-ELISA test result of TYLCV successfully amplified the expected 520 bp products in selected samples (Fig. 1) that were ELISA-positive to TYLCV and positive control but not with the negative control. Considering the distribution of two major viruses, DAS-ELISA and confirmatory IC-PCR tests revealed that 70% of samples from UAAIE, 56.7% of samples from Merti, 40% of samples from Melkasa, 13.7% of samples from fields surveyed along the road from Mojo to Zwai were infected by TYLCV but not detected in samples from the rest of the fields. ToMV was detected from 46.8% of samples surveyed along the road from Mojo to Zwai and 23.3% of

samples from Melkasa 20% of samples from Merti and was not detected in tomato samples from the other fields. Seventy percent of samples from UAAIE, 66.7% of samples from Merti, 60% of samples from Melkasa and 47.4% of samples from fields along the road from Mojo to Zwai were infected by either or both viruses.

Table 2. The number and percentages of virus-infected samples at respective areas .

Area surveyed	F	N	Samples infected with					
			TYLCV		ToMV		Either or both viruses[a]	
			No	%	No	%	No	%
UAAIE	2	20	14	70	-	-	14	70
Merti	3	30	17	56.7	6	20	20	66.7
Melkasa	3	30	12	40	7	23.3	18	60
East Shewa, Mojo to Zwai	19	190	26	13.7	89	46.8	90	47.4
West Shewa, Ambo to Guder	12	120	(-)	(-)	(-)	(-)	(-)	(-)
Southwest Ethiopia, Wolliso to Jimma	5	50	(-)	(-)	(-)	(-)	(-)	(-)
Southern Ethiopia, Butajira to Wolayta	5	50	(-)	(-)	(-)	(-)	(-)	(-)
Western and eastern Harerge	8	80	(-)	(-)	(-)	(-)	(-)	(-)
Total	**57**	**570**	**69**	**12.1**	**102**	**17.9**	**145**	**24.9**

F - number of fields; N - number of sample collected; UAAIE - Upper Awash Agro Industry Enterprise; MARC - Melkasa Agricultural Research Center; DSAD - Dadicho Sengele Agricultural Development; (-) samples gave negative result to viral test; [a]Individual number and % of TYLCV and ToMV do not sum up because of cases of mixed infections.

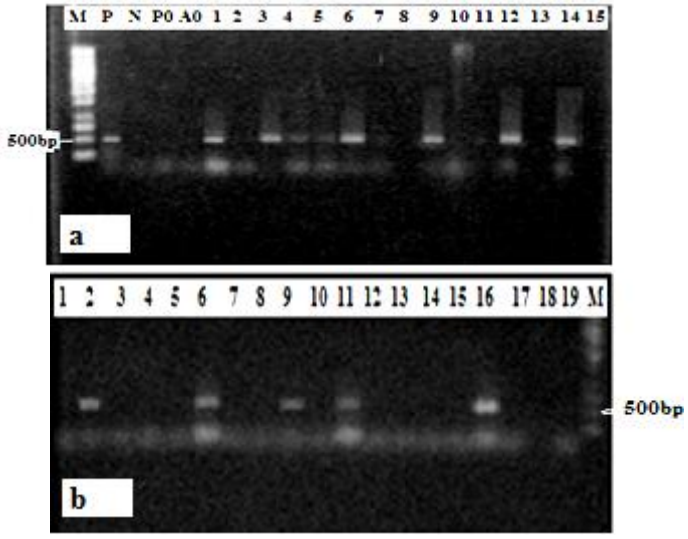

Fig. 1. Agarose gel electrophoresis showing IC-PCR products of TYLCV, using the primer combinations Begomo_146 and Begomo_672. (a) M -1kb size marker; P - positive control; N - negative control; P0 - only antibody added (no plant material); A0 - no antibody added (only a positive sample); number 1 to 15 indicate samples tested, (b) 1-19 samples tested.

DISCUSSION

This study showed that two viral agents, *Tomato yellow leaf curl virus* (TYLCV) and *Tomato mosaic virus* (ToMV) were detected amongst the different viral agents infecting tomato. The detection of TYLCV in most of the field plants corroborates with the recent epidemic of TYLCD at UAAIE and other places in the Rift Valley. Shih *et al*. (2006) revealed that the TYLCV species in Ethiopia belongs to *Tomato yellow leaf curl Mali virus* (TYLCMLV). TYLCD is currently considered as a major constraint of tomato production in these areas in the last decade. Globally, TYLCD is the most devastating tomato virus disease often causing up to 100% yield loss (Nakhla and Maxwell, 1999).

The exact reason for the sudden occurrence of TYLCD recently in epidemic proportion in Ethiopia is not clearly known so far. However, it is likely that the warmer environmental conditions together with more intensive monoculture in the Rift Valley may have provided favorable condition for the viruses and its vector white fly (*Bemisia tabaci*). The emergence of TYLCD may be associated with the global expansion of the B biotype of *B. tabaci* (Aunpam and Malathi, 2003). Hence, accurate biotyping of its population in tomato fields is important in implementing effective crop management strategies to control diseases transmitted by viruses.

Although Yaynu Hiskias (1998) reported that the incidence of ToMV was low, this study showed that its incidence has increased considerably indicating that mosaic viruses are becoming widespread together with TYLCD. The high incidence of the virus in the Rift Valley may be related to the specific cropping practices in the area including the use of irrigated water which would spread the virus (Sastry and Zitter, 2014). Furthermore, the use of virus-infected seeds for sowing and corresponding lack of crop hygiene during transplanting and other crop management operations may have aggravated the transmission from one plant to another through contact of plant parts (FAO, 2010).

More than 45% of the investigated areas i.e., all fields from western Shewa, southwestern Ethiopia, southern Ethiopia, and western and eastern Harerge did not show any viral disease symptoms of tomato. This is similar to the report of Yaynu Hiskias *et al*. (1999) that the greater number of tomato plants showing virus disease symptoms were found from the Rift Valley areas.

In economic terms, the widespread recent epidemics of TYLCD may have huge negative impact in the Rift Valley areas which provide most of the national tomato production. Furthermore, as temperature and rainfall are two of the critical factors affecting distribution and abundance of *B. tabaci* (Mohd *et al*., 2009), it is likely that the area coverage of the whitefly population and hence that of TYLCD will expand with extension of warm temperature and dry season to other tomato growing areas of Ethiopia due to factors such as global warming. This situation calls for effective management options. The control of TYLCD may not be an easy task and is based on the implementation of several management strategies, including cultural practices like crop rotation, the use of crop-free period, control of the insect vector and the use of resistant varieties (Anupam and Malathi, 2003). Hence, studies on integrated management of TYLCD based on adequate understanding of the causal virus, its whitefly vectors and other epidemiological parameters including alternate hosts and appropriate planting are important pre-requisites towards its management.

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REFERENCES

Agranovisky, A.A. and Anisimoff, B.V. (1986). Virus diseases of tomato in Ethiopia. *Acta Hort.* **190**: 115–119.

Anupam, V. and Malathi, V.G. (2003). Emerging geminiviruses problems: A serious threat to crop production. *Ann. Appl. Biol*. **142**: 145–146.

Clark, M.F. and Adams, A.N. (1977). Characteristics of the micro plate method of enzyme linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol*. **34**: 475–483.

Deng, D., Grarth, P.F., Robinsen, D.J. and Harrison, B.D. (1994). Detection and differentiation of whitefly-transmitted geminivirus in plants and vector insects by polymerase chain reaction with degenerate primers. *Ann. Appl. Biol*. **125**: 327–336.

FAO (2010). Quality declared planting material: Protocols and standards for vegetative propagated crops. Food and Agriculture Organization of the United Nations, Rome.

Fauquet, C.M., Briddon, R.W., Brown, J.K., Moriones, E., Stanley, J., Zerbini, M. and Zhou, X. (2008). Geminivirus strain demarcation and nomenclature. *Arch. Virol*. **153**: 783–821.

LeamKhang, S., Riangwong, L. and Chatchawankanphanch, O. (2005). Detection of TYLCTV by PCR without DNA extraction. *Mol. Biotechnol.* **31**: 567–578.

Marchoux, G. (1976). Virology problems in Ethiopia with special reference to Solanaceae and the central south eastern provinces. Account of a Mission to Awassa Experimental Station. Awassa, Ethiopia.

Mohd, Z., Fauziah, I., Fairuz, K., Mohd, M.S. and Jamaludin, B. (2009). Population ecology of whitefly, *Bemisia tabaci*, (Homoptera: Aleyrodidae) on Brinjal. *J. Agri. Sci*. **1**(1): 1–32.

Nakhla, M.K. and Maxwell, D.P. (1999). Epidemiology and management of tomato yellow leaf curl disease. In: **Plant Virus Disease Control**, pp. 565–583 (Hadidi, A., Khetarpal, R.K. and Koganzawa, H., eds.). The American Phytopathological Society, USA.

Nguyen, V.K., Rihn, B., Heckel, C., Bisseret, F., Girardot, R. and Monteil, H. (1990). Enzyme immunoassay (ELISA) for detection of *Clostridium difficile* toxin B in specimens of feaces. *J. Med. Microbiol*. **31**: 251–257.

Quiot, B. (1976). Virus diseases of market garden crops. Account of a Mission to Ethiopia. Awassa Experimental Station. Awassa, Ethiopia.

Sastry, K.S and Zitter, T.A. (2014). **Plant Virus and Viroid Diseases in the Tropics**. **Volume 2, Epidemiology and Management**. Springer Science + Business Media, London.

Shih, S.L., Green, S.K., Tsai, W.S., Lee, L.M. and Wang, J.T. (2006). First report of begomoviruses associated with tomato yellow leaf curl disease in Ethiopia. *Plant Dis*. **90**(7): 974–979.

Tesfaye Balemi (2008). Response of tomato cultivars differing in growth habit to nitrogen and phosphorus fertilizers and spacing on vertisol in Ethiopia. *Acta Agri*. **91**: 103 – 119.

Venkataravanappa, V., Reddy, C. and Reddy, M.K. (2013). Begomovirus characterization and development of phenotypic and DNA-based diagnostics for screening of okra genotype resistance against *Bhendi yellow vein mosaic virus*. *Biotech*. **3**(6): 461–470.

Wondirad Mandefro, Eshetu Ahmed, Mohammed Yesuf, Alemu Lencho, Yaynu Hiskias, Meki Shehabu, Fekede Abebe, Temam Hussien and Adane Abraham (2009). Review of vegetable diseases research in Ethiopia. In: **Increasing Crop Production through Improved Plant Protection**, pp. 203–230 (Abraham Taddesse, ed.). Proceedings of the 14th Annual Conference of the Plant Protection Society of Ethiopia (PPSE), EIAR, Addis Ababa, Ethiopia.

Yaynu Hiskias (1998). **Characterization of Potyvirus Isolates from Hot Pepper and Tomato in Ethiopia**. Doctoral dissertation. Faculty of Agricultural Science, George-August University, Gottingen, Germany.

Yaynu Hiskias, Lesemann, D.E. and Vetten, H.J. (1999). Occurrence, distribution and economic importance of potyviruses infecting hot pepper and tomato in the major growing areas of Ethiopia. *J. Phytopathol*. **147**: 5–11.