Macrothink Institute™

Journal of Agricultural Studies
ISSN 2166-0379
2013, Vol. 1, No. 2

Serological Detection of *Tobacco Mosaic Virus* and *Cucumber Mosaic Virus* Infecting Tomato (*Solanum Lycopersicum*) using a Lateral Flow Immunoassay Technique

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Macrothink Institute™

Journal of Agricultural Studies
ISSN 2166-0379
2013, Vol. 1, No. 2

Received: May 29, 2013 Accepted: June 15, 2013

doi:10.5296/jas.v1i2.3768 URL: http://dx.doi.org/10.5296/jas.v1i2.3768

Abstract

A study was conducted to determine the prevalence of *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV) in some major tomato growing areas in Ghana as part of a comprehensive strategy for the management of viral diseases on tomato. The lateral flow immunoassay technique (the immunostrip test of Agdia Inc.) was used to assay for TMV and CMV in samples with symptoms of virus infection collected from some major tomato growing areas in Ghana; Vea, Tono and Pwalugu (Upper East region-UE), Agogo and Akumadan (Ashanti region-AR) and Tanoso and Tuobodom (Brong-Ahafo region-BA) in 2011 and 2012. In the UE, TMV and CMV were both detected at Vea, Tono and Pwalugu. In the AR, CMV and TMV were both detected at Akumadan while only CMV was detected at Agogo. In BA, TMV and CMV were both detected at Tanoso and Tuobodom. Field incidence of TMV was higher in the UE which had higher average daily temperatures. The serological identification of TMV in the three regions is the first of this virus in Ghana. This is also the first report of the use of this lateral flow immunoassay technique to detect these viruses in the country. The identification of TMV and CMV in Ghana gives an indication of the need to focus efforts for virus disease management on these virus in addition to the already known begomoviruses like *Tomato yellow leaf curl Mali* virus, *Tomato leaf curl Kumasi* virus and *Tomato leaf curl Ghana* virus.

Keywords: Diagnostics, Immunostrip, Incidence, Severity, Tomato

1. Introduction

In Ghana, tomato is a very popular and an important vegetable crop which is consumed on nearly daily basis in every Ghanaian household (Wolff *et al*., 1999; Horna *et al*., 2006; Osei *et al*., 2010). The total land area utilized for tomato production in Ghana grew from 28,000 ha in 1996 to 37,000 ha in 2000, an increase of 30% (http:/www.gipc.org). Despite this development, local production is not able to meet the domestic demand and tomatoes are often imported. This can be attributed to a number of constraints including pest and diseases. One of the most important biotic constraints affecting tomato production is virus infection. It is well established that a number of whitefly-transmitted begomoviruses cause important diseases of tomato in Ghana and neighbouring countries, including tomato yellow leaf curl caused by *Tomato yellow leaf curl Mali* virus and tomato leaf curl caused by *Tomato yellow leaf curl Kumasi* virus and *Tomato leaf curl Ghana* virus (Horna *et al*., 2006; Osei *et al*., 2008; Osei *et al*., 2010; Kon & Gilbertson, 2011). However, based on the observation of mosaic, mottling and shoe-string symptoms in leaves of tomato plants, it is suspected that other tomato-infecting viruses, such as including cucumoviruses, potyviruses and tobamoviruses occur in most of the important tomato growing areas in Ghana but these have not been studied.

Two important viruses that occur in many tomato growing regions of the world are *Tobacco*

mosaic virus (TMV)/*Tomato mosaic virus* (ToMV) and *Cucumber mosaic virus* (CMV). CMV is a plant pathogenic virus in the family Bromoviridae and is the type member of the plant virus *Cucumovirus* genus. This virus has a worldwide distribution and a very wide host range. TMV/ToMV are a closely related virus that belong to the family Virgaviridae and is the type member of the plant virus genus Tobamovirus; hereafter we will refer to these viruses collectively as TMV (ICTV, 2012). Substantial losses to greenhouse and field produced tomatoes can be caused by TMV (ICTV, 2012). The symptoms induced in tomato by TMV vary greatly depending upon the variety, virus strain, and time of infection, light intensity and temperature. Symptoms appear as light and dark green mottled areas. Leaves of infected plants are often small, curled and puckered (Zitter, 1984; Sikora, 1998; Averre & Gooding, 2000). Plants infected early in their development are stunted. TMV is a stable, persistent virus most commonly introduced into plants through small wounds caused by physical contact (mechanical transmission) (Pfleger & Zeyen, 2008). The disease can reduce size and number of fruits produced; the fruit do not show malformations, however, occasionally mottling, bronzing and internal browning occurs (Zitter, 1984; Sikora, 1998; Averre & Gooding, 2000). The most important sources of inoculums for TMV are contaminated leaf and root debris and seed (Sikora, 1998).

CMV can occur wherever tomatoes are grown. CMV is usually introduced to cultivated tomatoes by aphids (Zitter, 1984) in a non persistent manner. The virus is not seed-borne in tomato (Zitter & Murphy, 2009). Tomatoes infected with CMV are often stunted and bushy and may have distorted and malformed leaves. Leaves may appear mottled but the distinctive symptom of CMV infection of tomato is shoestring of leaf blades although TMV can also cause these symptoms in tomato. The upper leaves can show symptoms while those in the midsection of the plant appear normal. Severely affected plants produce few fruits which are usually small (Sikora, 1998; Zitter, 1984; Averre & Gooding, 2000).

Typically serological methods like enzyme linked immunosorbent assay (ELISA) have been successfully used for the large scale detection and diagnosis of plant viral diseases (Clark & Adams, 1977; Francki *et al*., 1979; Hsu *et al*., 2000; Flegg &Clark, 1979). Another emerging serological diagnostic method is the lateral flow test, eg. the immunostrip test by Agdia Inc(AgDia., Elhart, Indiana, USA). Though it is relatively expensive, it is a rapid means of screening crops for the presence of pathogens especially plant viruses. Results are obtained in (< 30 minutes) making them ideal for diagnostics in the field or greenhouse.

In Ghana and other countries of West Africa, numerous studies are being undertaken on begomovirus diagnostics and management; however, relatively little work has been done on other viruses including cucumoviruses and tobamoviruses. The purpose of this study was to utilize the lateral flow test to determine whether CMV and TMV were infecting tomatoes in Ghana and to gain insight into their prevalence in different tomato growing regions of Ghana. Results from this study will contribute to development of a comprehensive package for the management of viral diseases of tomato in Ghana.

2. Materials and Methods

2.1 Field Survey

In 2011 and 2012, viral disease surveys of tomato were conducted in three important tomato growing areas in the Upper East region of Ghana (Guinea savannah agro-ecological zone) Vea irrigation site at Bolgatanga, Tono irrigation site and Pwalugu which are located between longitude 00^0 and 10^0 West and latitudes 100^0 30^0N and 110^0N with average daily temperatures of 30^0C (www.modernghana.com/GhanaHome/regions/uppereast.asp?menu_id=6). Two locations each were also surveyed, in the Ashanti region (forest ecological zone), Agogo, Akumadan (Ashanti region), lying between longitude 0.15^0W and 2.25^0W and latitudes 5.50^0N and 7.46^0N, with average daily temperatures of 27^0C (www.modernghana.com/GhanaHome/regions/ashanti.asp?menu_id=6); and the Brong Ahafo region (forest-transition ecological zone), Tuobodom and Tanoso located between longitude$7^0$45'0"N, 1^0 30'0"E with average daily temperatures of 23.9^0C (www.ghanahealthservice.org/region.php?dd=6&region=Brong%20Ahafo%20region).

All the three regions were surveyed to assess the incidence of virus disease symptoms typical of TMV and CMV (stunted and distorted growth and mosaic, mottle and shoe-string symptoms of leaves). At each location, two to four farms were sampled and representative samples showing these viral symptoms were collected and kept in an ice chest. The main tomato cultivars grown at these locations were Petomech and Petofake. The infected samples were sent to the laboratory at CSIR-Crops Research Institute, Fumesua for testing for CMV and TMV using Agdia immunostrips (a lateral flow immunoassay technique).

Field incidence and severity of viral disease symptoms was also determined based on visual assessment of disease symptoms. Here, in all the locations assessed, the numbers of plants showing virus symptoms were recorded. Severity scores were based on 0-4 scale where 0 represented apparently no symptoms, 1- slight infection, 2 – moderate infection and 3 – severe infection and 4- very severe infection. However, here it is important to note that it is not possible to determine which viruses are involved based upon disease symptoms alone.

2.2 Serological Analysis Using Agdia Immunostrips for Detection of TMV and CMV

Sample extraction bags containing SEB1 buffer (Agdia Co., used for both CMV and TMV) were cut open with scissors along the top of the labels (Fig.1). A piece of tissue (approximately 2-3 cm^2) from a diseased leaf sample was inserted between the mesh linings near the bottom of the sample extraction bag (Fig. 2) and sap from the sample was extracted by rubbing over the tissue between the mesh linings with a blunt object (Fig. 3). The sap extracted had a light green to brown colour. The immunostrip from Agdia Co. was then inserted into the channel portion of the buffer bag and allowed to remain in the extract for about 30 minutes (Fig.4). Samples that tested positive had two purple lines corresponding to the test and control lines whereas those that were negative had only the control lines appearing (Fig. 5).

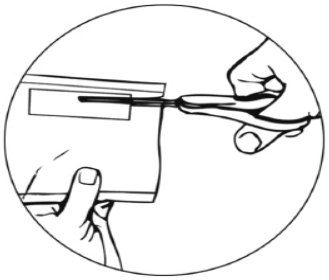

Fig.1

Fig. 3

Fig.2

Fig.4

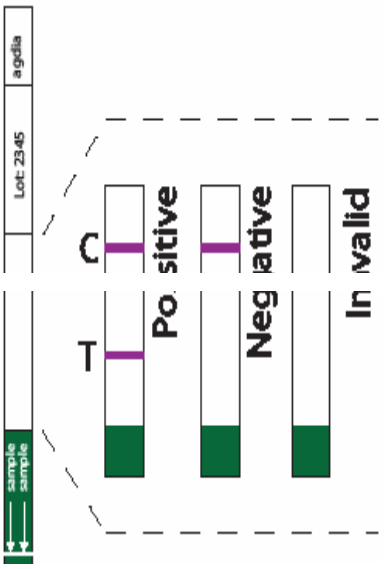

Fig. 5 Source: (Hsu, H.T. et al., 2000; Francki, R.I.B. et al., 1979; ICTVdb Management, 2006)

2.3 Statistical Analysis

The data was subjected to General Linear Model procedure using PROC GLM; SAS Institute, 2008 (Version 9). Means separation was done using the Tukey's test ($P < 0.05$). Severity data using scores were transformed to the logarithm base 10 (x+1) whiles incidence data (percentage infection) was arcsine square root transformed before analysis. Back transformed values are presented in Table 2.

3. Results and Discussion

In the Upper East region (Guinea savannah ecological zone), CMV (3/9 plants in 2011, and 4/9 in 2012) and TMV (3/9 in 2011 and 5/9 in 2012) were detected in some plants with virus symptoms from all three locations, Vea, Tono and Pwalugu (Table 1). Most plants were singly infected, although mixed infections were detected in one plant in 2011 at Tono and 2012 at Tono and Vea. In Ashanti region (forest ecological zone), in 2011, CMV (2/10) and TMV (1/10) were both detected at Akumadan (Table 1). In 2012, only CMV was detected at both Akumadan (1/7) and Agogo (8/10). Mixed infections were detected in Akumadan (1 plant) in 2012. These results are consistent with those of two other virus surveys conducted in this region in 2010 and 2012 where CMV but not TMV was detected in tomato plants showing symptoms of leaf mosaic mottle and strap leaf (2/2 samples with mosaic and strap leaf symptoms from one field in 2010 and 5/5 samples with mosaic and strap-leaf tested from one field in 2012). In the Brong-Ahafo region (forest-transition ecological zone), in 2011, TMV (2/10 plants) and CMV (3/10) were detected at Tanoso while TMV (3/10) and CMV (1/7) were detected at Tuobodom. In 2012, only CMV was detected at both Tuobodom (3/10) and Tanoso (2/7) (Table 1). Mixed infection of only one sample was detected at Tuobodom in 2012. CMV was also detected in plants from Tuobodom with mosaic, mottle and strap-leaf symptoms in the other surveys (from one sample with green-yellow mottle in 2010 and from 2/2 samples with mosaic, mottle and strap-leaf symptoms from each of two fields in 2012). The findings of CMV and TMV in three geographical regions and most locations in these regions indicate that both viruses are present in Ghana and are endemic in these tomato-growing regions. Moreover, given the severity of the symptoms of the infected plants it is likely that these viruses could cause yield losses. There is also the possibility that with time the incidence of these two viruses could increase in these areas in future, if management efforts are not implemented.

However, it is important to note that not all plants showing symptoms of virus infection were found to be infected with CMV or TMV. In both 2011 and 2012, three of nine plants showing symptoms of mosaic and mottling were negative for CMV and TMV infection. This indicates that other viruses were likely responsible for these symptoms. While it is possible that these symptoms were caused by begomovirus infection, the lack of typical begomovirus symptoms like upcurled leaves, yellowing (especially in between the veins) and vein swelling and distortion make this less likely. It is more likely that these symptoms are due to another RNA virus, such as potyvirus. Indeed similar results to these obtained in the other 2012 virus disease survey (i.e. plants with mosaic/mottle symptoms were negative for CMV and TMV symptoms) and representative plants with these symptoms were found to be infected with the potyvirus, Pepper veinal mottle virus. This is fairly common virus infecting peppers and tomatoes in West Africa, and it is possible that it is responsible for the mosaic and mottle symptoms in the tomato plants that were negative for infection by CMV and TMV. Alternatively, it is possible that there are strains of CMV or TMV not recognized by the antesera used to make the immunostrips; however to our knowledge this has not been reported. Furthermore, for some of the samples collected in 2010 and 2012 surveys, RT-PCR was used to confirm the presence of CMV; in all cases where CMV was detected with the Agdia immunostrips, CMV was detected by RT-PCR (data not shown).

Table 1. Detection of *Cucumber mosaic virus* and *Tobacco mosaic virus* in tomato plants with leaf mosaic and mottle symptoms collected from fields in three tomato growing regions of Ghana using lateral flow immunoassay (Agdia immunostrips)

Locations	CMV		TMV		Mixed infection (CMV & TMV)	
	2011	2012	2011	2012	2011	2012
Upper East Region						
Vea 1	-	+	+	+	-	+
Vea 2	-	-	-	+	-	-
Vea 3	-	-	-	-	-	-
Tono 1	+	-	-	+	+	-
Tono 2	-	-	-	+	-	+
Tono 3	+	+	+	+	-	-
Pwalugu 1	-	+	+	-	-	-
Pwalugu 2	+	-	-	-	-	-
Pwalugu 3	+	-	-	-	-	-
Ashanti region						
Agogo 1	-	+	-	-	-	-
Agogo 2	-	+	-	-	-	-
Agogo 3	-	-	-	-	-	-
Agogo 4	-	-	-	-	-	-
Akumadan 1	+	+	+	-	-	+
Akumadan 2	+	_	+	-	-	-
Brong-Ahafo region						
Tuobodom 1	-	+	-	-	-	-
Tuobodom 2	-	+	-	+	+	-
Tuobodom 3	-	-	-	-	-	-
Tanoso 1	-	-	-	-	-	-
Tanoso 2	+	+	+	-	-	-
Tanoso 3	+	_	+	-	-	

+ = presence - = absence

The incidence and severity of virus symptoms in tomato were increasing with time as shown in Table 2 (Incidence and severity of the two viruses were generally higher for most locations in 2012 than in 2011).

Journal of Agricultural Studies
ISSN 2166-0379
2013, Vol. 1, No. 2

Table 2. Incidence and severity of CMV and TMV at the different locations in the three tomato growing regions of Ghana

Location	CMV				TMV			
	Incidence (% infection)		Severity (scale 0-4)		Incidence (% infection)		Severity (scale 0-4)	
Upper East Region	2011	2012	2011	2012	2011	2012	2011	2012
Vea	0.00±0.00b	0.06±0.04a	0.00±0.00a	1.67±0.33a	0.01±0.01bc	0.02±0.02a	0.67±0.67ab	1.33±0.33a
Tono	0.01±0.01ab	0.13±0.11a	1.33±0.67a	1.67±0.33a	0.67±0.67bc	0.13±0.11a	0.33±0.33ab	1.33±0.67a
Pwalugu	0.02±0.01ab	0.00±0.00a	0.67±0.33a	1.00±0.58a	0.01±0.01bc	0.01±0.00a	0.33±0.33ab	1.00±0.00a
Ashanti Region								
Agogo	0.00±0.00b	0.28±0.15a	0.00±0.00a	1.97±0.99a	0.00±0.00c	-	0.00±0.00b	-
Akumadan	0.10±0.05a	0.04±0.04a	2.00±0.00a	1.25±1.25a	0.10±0.05a	-	2.00±0.00a	-
Brong-Ahafo Region								
Tuobodom	0.02±0.02ab	0.13±0.07a	0.67±0.67a	2.40±0.10a	0.67±0.02ab	0.14±0.07a	2.00±0.00a	2.25±0.25a
Tanoso	0.02±0.02ab	0.06±0.02a	0.67±0.67a	1.65±0.15a	0.07±0.02ab	-	2.00±0.00a	-
F-value	3.01	1.14	2.15	0.44	5.59	0.61	8.72	0.74
P	0.0418	0.4031	0.1111	0.8351	0.0038	0.6379	0.0004	0.5707

Note: Means with the same letter(s) are not significantly different ($P < 0.05$, Tukey's test) within columns

Given the relatively high incidence of CMV and TMV in Ghana, it is clear that an accurate, reliable and effective method for routine diagnosis of TMV and CMV is desirable. Our results show that the Agdia immunostrip lateral flow assay proved to be very effective in the detection of these viruses in this study. The detection of the CMV and TMV in almost all the locations assessed in the three regions in the different agro ecologies showed the widespread occurrence of these two viruses in important tomato growing areas in Ghana. In some cases, mixed infections of CMV and TMV were detected indicating that both viruses can infect an individual plant, which further complicated diagnosis. Mixed infections of CMV and TMV have been previously reported by Chen *et al*., (2011). Taken together with the fact that CMV and TMV can cause similar symptoms in tomato plants, this further shows the need to have a reliable method to differentiate the viruses. This is the first report of such a serological detection of these viruses in Ghana using the lateral flow immunoassay technique and it reveals that the method can be used for routine detection of these viruses. The drawback to the routine use of this method is the relatively high cost. However, as the distribution and relative prevalence of these viruses are established and the symptoms of each virus in tomato in Ghana are better established this would reduce the number of immunostrips needed for routine detection.

In this study, fields with plants infected with viruses exhibited stunted and abnormal growth as well as various leaf symptoms including mosaic/mottle, distortion and strap leaf. It is likely that plants with these symptoms, particularly in plants infected early in growth, will experience some degree of loss. Similar observations regarding the detection of CMV and TMV in tomato were made under similar climatic conditions in Nigeria by Umeh *et al.* (2002) in a survey of diseases and insect pest and farmers' practices in the cropping of tomato. They indicated that the presence of TMV and CMV might have contributed to yield losses. CMV has also been identified as a very important virus of tomato in a neighbouring country, Togo (Dafalla, 2003). Thus the finding of these viruses in Ghana is perhaps not unexpected and further shows the importance of the viruses in West Africa.

It is interesting to note that the prevalence of TMV was highest in the Upper East region (Guinea savannah) where average daily temperatures are higher than in the Ashanti region (forest ecological zone) and Brong-Ahafo region (forest-transition zone). While it is possible that this relates to the different temperatures, it is more likely due to other factors. One possibility is the frequent use of tobacco products by the farmers (personal observation) which might have resulted in the early infection of the seedlings during handling. It has been reported by Pfleger and Zeyen (2008) and Bagley (2001) that the common source of virus inoculum for TMV includes certain tobacco products that contaminate workers hands. It has been reported (bolga.ghanadistricts.gov.gh) that the use of tobacco for marriages and funerals in the Upper East region is widespread across the region. The 2008 Demographic and Health Survey indicate that 11.4% of the people are smokers minus other forms of tobacco usage. It was also reported that the tobacco crop is freely cultivated and more or less a cash crop of the region (bolga.ghanadistricts.gov.gh).

This could increase the overall incidence of the virus in the area. It would be of interest to determine the prevalence of TMV infection in tobacco in this area. Alternatively, the higher incidence of TMV in this area could be due to planting of contaminated seed together with

certain cultural practices that favour subsequent infection of seedling and spread of virus.

CMV was identified in 17 locations in the three regions in 2011 and 2012 whereas TMV was identified in 12 locations. These results indicated that CMV is widespread and prevalent in Ghana than TMV. This was also the case of the other surveys conducted in 2010 and 2012. This can be attributed to the wide host range of CMV which includes plantain, banana, cucumber, maize, yam, peanut and soybean. This is in contrast to TMV which has a more narrow host range including tobacco and pepper. Furthermore, the widespread distribution of CMV would be favoured by the transmission of the virus by multiple species of aphids in a non-persistent manner (Carrere *et al*., 1999; de Breuil *et al*., 2005, Eni *et al*., 2008; Zitter & Murphy, 2009; Dheepa & Paranjothi, 2010, MoFA, 2011).

These differences in biological properties will impact management practices, with resistant varieties, clean seed and sanitation being approaches that can be used for TMV and only virus-free transplants and cultural practices currently available for CMV.

4. Conclusion

Serological tests are very useful as detection methods for plant-infecting viruses due to their sensitivity, easy adaptability, relatively low cost and capacity for detection of large number of samples. The development of lateral flow devices, such as immunostrips, have added the capacity for rapid detection. Here, we took the advantage of the properties of the lateral flow tests to identify CMV and TMV in tomato samples from various growing regions in Ghana. The finding that these viruses are widespread in Ghana indicates the need to focus our virus management efforts in Ghana not only on begomoviruses but also on RNA viruses like CMV and TMV.

Acknowledgements

The authors express profound gratitude to the USAID, IPM CRSP (Cooperative Agreement Number EPP-A-00-04-00016-00) for financial support of this study. We are also thankful to all the Technicians who assisted with data collection and compilation.

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