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SEROLOGICAL REACTIVITY OF OKRA LEAF CURL DISEASE TO AFRICAN CASSAVA MOSAIC VIRUS (ACMV) AND TOMATO YELLOW LEAF CURL VIRUS (TYLCV) ANTISERA IN SUDAN SAVANNA ZONE OF NIGERIA

[1]Zubairu, M. and [2]Alkali, G.

[1]Department of Agricultural Technology, College of Agriculture, Maiduguri, Borno State, Nigeria.

[2]Department of Crop Protection, Faculty of Agriculture, University of Maiduguri, Borno State, Nigeria.

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ABSTRACT

A survey was conducted in Maiduguri during the 2016 cropping season to identify the serotypes and distribution of Okra Leaf Curl Disease on okra plants to cover 30 major okra growing areas in Maiduguri. The confirmation of serotypes of the virus was done using African cassava mosaic virus (ACMV) and Tomato Yellow Leaf Curl Virus (TYLCV) antibodies obtained from Deutche Sammlang von Mikroorganism and Zeckulturan (DSMZ) Brauschweig, Germany. The reactivity of 30 different OLCV isolates after tests with antisera with TAS ELISA distinguished four serological profiles; those showed positive reaction with the two antisera were designated as serotype SRP A, the second serological profile included isolates that showed positive reaction with only TYLCV antisera, and were designated as serotypes SRP B, the third serological profile was isolate which showed positive reaction with only ACMV antisera and were designated as serotype C. The fourth serological profile included those isolates which had negative reaction with the two antisera (TYLCV and ACMV), and were designated as serotype SRP D. Based on the results of the study, various serotypes exists and begomovirus occurring on tomato and cassava in neighboring farms also infects okra in Maiduguri. Such information could eventually be utilized in the development of integrated begomovirus management packages in crop rotation, inter cropping and insect vector control. Further new trend of other possible management solutions for begomovirus which are worth trying in developing countries like Nigeria such as knowledge of these serotypes of the virus to provide a sound basis for understanding the present status, distribution and mixed infection structure of begomoviruses in its different hosts across the important okra and other vegetables growing areas in Nigeria.

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Keywords: Okra, Okra Leaf Curl Disease, Tomato Yellow Leaf Curl Disease, Africa Cassava Mosaic Virus, Serotypes

INTRODUCTION

The wide diversity among begomoviruses associated with mixed infections is supposedly assisting in recombination and false recombination events leading to the frequent emergence of novel begomoviruses, having devastating effect on the okra (Padidam *et al*, 1999). This recombination has played a significant role in the evolution of Gemini viruses (Seal *et al*., 2006) including origin of Okra Enation Yellow Leaf Curl virus (OELCuV) as the sequence making up OELCuV have originated from malvaceous begomoviruses; Cotton Leaf Curl Bangaluru Virus (CLCuBaV), Mesta Yellow Vein Mosaic Virus (MeYVMV), and Bhendi Yellow Vein Mosaic Virus (BYVMV) (Venkataravanappa *et al*., 2015). OELCuD may be caused by OELCuV in association with at least two distinct beta satellite (OLCuB) (Venkataravanappa *et al*., 2015).This recombination of okra leaf curl beta satellites observed in Hydrabad India showed six major and minor recombination with BYVMV, hot-spots and break points (Sohrab *et al*., 2015). Such recombination was also observed in southern India where YVMV and OLCV disease of okra show either leaf curl or yellow vein mosaic symptoms, (Sohrab *et al*., 2013) due to the emergence of new viral strains that results to the recombination and pseudo-recombination.

Serological relationship among different whitefly transmitted geminiviruses do exists that these viruses react with a various MAbs raised against any of the members belonging to the geminiviruses in their identification or differentiation between isolates of the same virus (Al-shahwan, 2001). Similar relationship was reported to occur among whitefly transmitted geminiviruses (Sequeira and Harrison, 1982; Cohen *et al*, 1983; Thomas *et al*, 1986, Roberts *et al*, 1984; Hamilton *et al*, 1984, Howarth *et al*, 1985; Harrison *et al*, 1991 and Swanson and Harrison, 1993). Therefore, antisera homologous to one whitefly transmitted geminivirus were used by Roberts *et al*, (1984); Harrsison *et al*, (1991) & Ghanem, (2003) to detect other geminiviruses.

Whitefly transmitted geminiviruses found in tomatoes in different regions have consistently different profiles, whereas those from the same region have similar profile (Harrison e*t al*., 1991; Muniyappa *et al*, 1991; Macintosh *et al*, 1992). Hong and Harrison (1995) comparing TYLCV isolates from various countries showed distinction among them by the nucleotide sequence of their coat protein gene. A culture of Cotton Leaf Curl Virus from Pakistan (CLCuV-PK), was transmitted by whiteflies *(Bemisia tabacci)* to seven plant species, including French bean, okra, tobacco and tomato, and caused vein thickening and leaf curl symptoms in the plants which were readily detected in triple antibody sandwich ELISA (TAS-ELISA) with 11 out of 31 monoclonal

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antibodies raised against the particles of three other geminiviruses: African cassava mosaic, Indian cassava mosaic and okra leaf curl viruses. Smaller differences in epitope profile were found among virus isolates from cotton *(Gossypium hirsutum)* collected from different districts in Pakistan over a 5-year period.

All whitefly transmitted geminiviruses have been found to be serologically related and their interrelationship help in their detection by ELISA (Sequeira and Harrison, 1982; Cohen *et al*, 1983) with either polyclonal or cross reacting monoclonal antibodies (Thomas *et al*., 1986). Various geminiviruses have been distinguished by the reactivity in ELISA of individual virus isolates with panels of monoclonal antibodies (MAbs) raised against purified particles of selected whitefly transmitted geminiviruses and having ranges of cross reactivity with heterologous whitefly transmitted geminivirus (Harrison *et al*, 1991; Swanson and Harrison, 1993). Hence the study therefore aims to establish the serotypes of okra leaf curl geminivirus found in other crops growing in the same agro ecological zones.

MATERIALS AND METHODS

Field Survey and Sampling

Field survey were carried out in okra fields to collect okra plants showing symptoms of leaf curl virus during 2016 cropping season at 30 locations around Maiduguri namely; Goni Kakkari, Shuwari 4, Shuwari 3, Shuwari Madinatu, Gonglong Lawanti, Gonglong Kayayya, Old Maiduguri Lambu Area, Old Maiduguri Kasan Yashi, Shuwari Power Station, Shuwari El-miskin, Farm center, Dusman, Shokari, Alizarmari, Angiudda, Khaddamari, Zabarmari, Bulamatari, Goniri, Fori, Kiriri, Kazallari, Fulatari, Unimaid, Kolori, Molai, Dalori, Gonglong Bulamari, Kazallari and Bale. The excised leaves samples were washed with sterile water dried and then sealed in plastic bags and stored at -20˚C until usage.

Determination of Sero-reactivity of Okra Leaf Curl Virus to ACMV and TYLC Antisera

The triple antibody sandwich enzyme linked immunosorbent assay (TAS ELISA) was performed as described by Clarks and Adams (1977), for the determination of the serotypes of OLCV at the plant virology laboratory, Department of Crop Protection, Ahmadu Bello University, Zaria, Nigeria.

Monoclonal antibodies of Tomato Yellow Leaf Curl Virus (TYLCV) and African Cassava Mosaic Virus (ACMV) were used. The specific antibody was diluted in a coating buffer to give a dilution ratio of 1:1000. 200µl of the solution were added to each well. The microtiter plate was then covered and incubated at 37°C for 2 hours. The plates were then washed 3 times with PBS-

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Tween using wash bottle and soak for three minutes. The plates were then blot dried by tapping upside down on tissue paper. 200 µl of 2% skim milk in PBS Tween was added to each well and incubated for 30 minutes at 37°C for blocking. Thereafter, the plate was inverted and allowed to drain. The samples were then extracted by grinding the leaves in an extraction buffer and 200 µl of the aliquots solution was added to each well and the plate was then covered and incubated overnight at 4°C. The plates were then washed 3 times and blot dried. 200 µl of the TYLCV and ACMV MAb were diluted in the ratio of 1:50 conjugate buffer into each well. The plates were then covered and incubated for 2 hours at 37°C and washed again three times with PBS-Tween. RAM-AP was then diluted in conjugate buffer in the ratio 1 µl:1000 ml and 200µl were then added to each well. The plates were then covered and incubated for 1 hour at 37°C. The plates were washed again 3 times with PBS-Tween and blot dried and 200 µl aliquots of freshly prepared substrate (1mg/ml) of para-nitrophenyl- phosphate in substrate buffer. 200 µl of the solution was added to each well and incubated at room temperature for 30 minutes. Colour change were measured with ELISA reader (Spectrophotometer) at absorbance value A.405nm were accepted as positive (+) when the reading was greater than twice the mean absorbance value of the virus free control sample. Any other value below that was considered as negative (-). The positive value were visualized when the colourless substrate p-nitrophynyl phosphate give rise to a yellow p-nitrophenol product.

RESULTS

Serological profile of okra leaf curl virus isolates collected in Maiduguri

Four serological profile (SRP) were evident using two monoclonal antibodies (AS 0585, -0546/2) raised against Tomato Yellow Leaf Curl Virus (TYLCV) and (RT 0421-0421/2) raised against African Cassava Mosaic Virus (ACMV).

Table 1 show the reactivity of 30 different OLCV isolates after tests with antisera bodies in TAS ELISA. Those showed positive reaction with the two antisera were designated as serotype SRP A. the second serological profile included isolates that showed positive reaction with only TYLCV antisera, and were designated as serotypes SRP B. the third serological profile was isolate which showed positive reaction with only ACMV antisera and were designated as serotype C. The fourth serological profile included those isolates which had negative reaction with the two antisera antibodies (TYLCV and ACMV), and were designated as serotype SRP D.

Several variants of the serological profiles were distinguished by their unusual reaction with the two antisera. In SRP A, four isolates showed a strong positive reaction with TYLCV and weak positive reaction with ACMV antisera from Shuwari 4, Shuwari madinatu, Kazallari and Dalori. One isolate showed strong positive (TYLCV) and positive reaction (ACMV) with the two

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antisera from Kolori. Similarly, one isolate showed positive reaction (TYLCV) and weak positive reaction (ACMV) from Farm center. Also, one isolate showed weak positive reaction with the two antisera in serotype B from Bale. Three isolates showed strong positive reaction with TYLCV antisera from Shuwari power station, Dusman, Showkari, Alizarmari and Unimaid. Similarly, seven isolates showed weak positive reaction with TYLCV antisera from Goni kakkari, Gonglong lawanti, Bulamari, Goniri, Fori, Kiriri and Molai. In SRP C, one isolate showed weak positive reaction with ACMV antisera from Khaddamari. In serotype SRP D, seven isolates have negative reaction with both TYLCV and ACMV antisera (Table 1).

Table 1: Serological Profile of Okra Leaf Curl Virus Isolates Detected with TAS ELISA Kits DSMZ AS 0588, AS 546/2 against Tomato Yellow Leaf Curl Virus (TYLCV) and DSMZ RT 0421-042/2 against African Cassava Mosaic Virus (ACMV)

Locations	TYLCV	Absorbance Category	ACMV	Absorbance Category
Gonikakkari	0.386	+	0.254	-
Shuwari 4	1.170	+++	0.328	-
Shuwari 3	2.637	+++	0.406	+
Shuwarimadinatu	2.017	+++	0.394	+
Gonglonglawanti	1.016	+++	0.212	-
Gonglongkayayya	0.493	++	0.161	-
Old maid lambu area	0.250	-	0.132	-
Old maid kasan Yashi	1.026	+++	0.244	-
Shuwari power station	0.869	++	0.163	-
Shuwari el miskin	0.207	-	0.256	-
Farm center	0.763	++	0.517	+
Dusman	0.898	++	0.224	-
Shokari	0.628	++	0.183	-
Alizarmari	0.767	++	0.234	-
Angudda	0.246	-	0.267	-
Khaddamari	0.137	-	0.460	+
Zabarmari	0.153	-	0.127	-
Bulamatari	0.556	+	0.202	-
Goniri	0.316	+	0.183	-
Fori	0.452	+	0.320	-
Kiriri	0.327	+	0.246	-
Kazallari	2.004	+++	0.459	+
Fulatari	0.332	+	0.267	-

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Unimaid	0.789	++	0.265	-
Kolori	2.546	+++	0.753	++
Molai	0.450	+	0.331	-
Dalori	1.408	+++	0.483	+
GongulongBulamari	0.224	-	0.198	-
Kazallari	0.147	-	0.214	-
Bale	0.348	+	0.352	+
PC	(+)	+++	0.958	+++
NC	0.156	-	0.174	-

Strong Positive Reaction = +++ Positive Reaction = ++ Weak Positive Reaction = +

Negative Reaction = - PC- positive control NC – Negative control

DISCUSSION

The occurrence of the serological diversity of the OLCV isolates assessed with panel of antisera of ACMV and TYLCV in the surveyed farmers field suggested that several serotypes or epitope profile of the virus co-exist in the surveyed areas in Maiduguri. The serological similarities and mixed infection observed between isolates within the same and different locations in the surveyed areas confirm the cross reaction of the antibodies produced against one begomovirus coat protein with other begomoviruses (Nirbbay *et al*., 2010), its capability of infecting morethan 30 species in over 12 plant families and especially whitefly transmitted begomoviruses were found to be associated with cassava mosaic, tobacco leaf curl, tomato leaf curl, cotton leaf curl and okra leaf curl viruses (Konate *et al*., 1995 and Makhlouf *et al*., 2015). Similarly, Venkataravanappa *et al*., (2014) reported that sequence comparisons of begomovirus from various locations revealed the existence of different isolates in different forms also confirm the great cross infection potential of OLCV disease complexes transmitted under the natural conditions by whitefly vectors. Furthermore, Konate *et al*., (1994) reported that recombinant or mixed infection forms of begomoviruses may play a role in their diversification and adoption to new hosts and localities, resulting in many species described so far.

This study has proved that most whitefly transmitted begomoviruses are serologically related and distinguished by reactivity of individual virus isolates with panels of monoclonal antibodies raised against one begomovirus (ACMV and TYLCV) and having range of cross reactivity. Viruses occur singularly or in mixtures whereby two viruses detected in one sample. Similar results have been observed by Swanson and Harrison, (1993) that a network of antigenic relationships was revealed by reaction between OLCV antisera and other whitefly transmitted geminiviruses from six plant species in 12 countries.

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By using antibodies, it was demonstrated that several whitefly transmitted geminiviruses are serologically related (Roberts *et al*., 1984). The results of OLCV detection in field samples demonstrated that TYLCV and ACMV differed in their serological reactivity. Konate *et al*., (1995) observed the cross reactivity of MAbs by TAS-ELISA where they found four epitope profile with the characteristics of ACMV, OLCV, TYLCV and TobLCV. Similar results were found by Swanson and Harrison (1993) who detected all the OLCV isolates from West Africa. ACMV has a narrow spectrum of serological reactivity detecting only 8 serotypes out of 30 isolates with conspicuous symptoms of the virus. This probably implies the existence of fewer ACMV strains in the fields in Maiduguri possibly because cassava is not a key crop in Maiduguri and ACMV, although widely distributed in the middle belts and southern parts of Nigeria does not present such a serious problem in the area probably which was why ACMV was recorded in only few locations compared to TYLCV which was recorded in 23 locations in the surveyed areas. This explains the fact that most of the localities in the surveyed areas grow tomatoe during the dry season which mostly extended into the rainy periods confirm the great cross infection potential of TYLCV compared to ACMV to the okra plants which was usually grown side by side with tomato plants in the localities. These results established that begomoviruses occurring on tomato in the neighboring gardens also infect okra in the study areas. Such information should eventually be utilized in the development of integrated begomovirus disease management packages; such as considering alternative hosts of the virus, mixed cropping in the same field, crop rotation and other possible management solutions for begomovirus cross-infection among its range of hosts which are worth trying in developing countries like Nigeria. Konate *et al*., (1995) stated that cassava is not widely distributed in Burkina Faso thus did not pose a serious threat. The phenomenom of mixed infection is extremely important for virus evolution because mixed infections are prerequisite for the occurrence of natural recombination events which may contribute to the appearance of new begomovirus, as reported by Venkataravanappa *et al*., (2013); Padidam *et al*., (1999) and Sanz *et al*., (2000) where they found expanding diversities resulting in emergence of new virus strains and their ability to infect new hosts. The serological relationship between ACMV and OLCV in this study also corroborates with the findings of Gahnem, (2003) where he found serological relationship between OLCV-SA, SLCV and TYLCV using 1:100 cross absorbance with ACMV-PAB. Also MAbs to TYLCV succeeded to detect the presence of leaf curl virus in diseased tissue of okra plants. Similar results were reported by Makhlouf *et al*., (2015) figures out that polyclonal antiserum to TYLCV detected OLCV in cotton and okra plants. This is as a result of frequent breakdown of the okra leaf curl viral disease resistance breakdown in okra varieties. This is also in agreement with the findings of Mishra *et al*, (2017), Sanwal *et al*., (2014) where they stressed the evolution of new viral strains as the major factors responsible for the breakdown of tolerance in okra varieties.

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CONCLUSION

The sero-distribution of the pathogen in the study area show functional interaction with varying efficiencies possibly leading to new begomovirus recombination and pseudo-recombinants with component from different host crop formed which may overcome plant resistance and increase the host range of the begomoviruses already existing in the study area. This might be why okra in Maiduguri is a host to many begomovirus (TYLCV and ACMV) complexes with uncharacterized variants and/strains. Thus there is an urgent need to come up with further studies on the disease spread, host range as well as molecular characterization of begomovirus to identify the currently circulating OLCV isolates complex as well as possible emergence of new strains with their extended or alternative host characteristics in Maiduguri Sudan Savanna zone of Nigeria.

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