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FIRST REPORT OF *TOMATO BUSHY STUNT VIRUS* (*TOMBUSVIRUS)* INFECTING IRRIGATED TOMATO (*Solanum lycopersicum* [L.]) IN NORTHERN NIGERIA

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SUMMARY

The detection of *Tomato bushy stunt virus* (TBSV) infecting tomato plants in Sudan savanna region (Gombe, Jigawa and Kano) of Nigeria is reported in this study. In 2017 and 2018 dry seasons, three farms each from three major tomato producing Local Government Areas (LGAs) in each state were surveyed. Forty symptomatic and asymptomatic tomato leaf samples per farm (n=2160) were collected in five quadrants measuring 4m x 4m in size and indexed against TBSV using double antibody sandwich enzyme-linked immunosorbent serological assay. The results obtained indicated that TBSV occurred in all the states surveyed. In Gombe, TBSV incidence was found to be significantly higher (*P =0.05*) in Yamaltu-Deba LGA (16.0 %) followed by Akko (14.0 %) while Kaltungo had the lowest incidence of 12.1%. Kura LGA had the highest virus incidence (18.0 %) followed by Garun Mallam (10.7%) while the least incidence (6.5 %) was recorded at Bagwai in Kano State. In Jigawa, the highest virus incidence of 13.0 % was recorded at Kirikasama LGA followed by Kazaure (9.5%) while Hadejia had the least incidence of 5.2%. Among the States surveyed for TBSV, Gombe had the highest (*P = 0.05*) virus incidence (14.0 %) followed by Kano (11.7 %) while the least incidence (9.2%) was recorded in Jigawa. This is the first report of TBSV on tomato in northern Nigeria. Awareness programs need to be organized for tomato farmers on the incidence and management of the virus disease.

Keywords: *Tomato bushy stunt virus*, detection, tomato, DAS-ELISA, Nigeria.

Tomato bushy stunt virus (TBSV), type member of the genus *Tombusvirus: Tombusviridae*), is a unipartite, icosahedral (T= 3) single-stranded positive sense RNA virus with 30 nm in diameter (5; 21; 30). It has a wide host range but principally attacks economically important solanaceous crops (23) causing several epidemic disease outbreaks in *Solanum lycopersicum* (6; 10; 12; 17), *Capsicum annuum* (6), and *Solanum melongena* L. (6; 17). There is no known biological vector of TBSV, although virus incidence is often associated with the soil and irrigation water (22). The virus is also transmitted either naturally through infected seeds, pollen, and propagative materials or mechanically by the use of contaminated cutting tools (9; 18; 23). Leaves on plants infected with *Tomato bushy stunt virus* are small in size, cupped, and curled downward. The youngest leaves are twisted and exhibit apical necrosis. Necrosis may develop, killing the young shoot. A proliferation of lateral shoots leads to an overall stunting and bushy appearance. Lower leaves are chlorotic with a purple tinge and twisted over or may be completely reversed (reviewed by Abraham *et al.* (2)). TBSV has been reported to be damaging to tomato both in the field and greenhouses (13). Tomato fruit yield is significantly reduced if virus infection occurs early in the season. Yields are reduced as fruits become smaller and show chlorotic rings and blotches that lower the economic value of the crop (3; 4; 17; 30). Yield losses as high as 80% due to TBSV attack in tomato have been reported (12).TBSV is widespread and causes economically important diseases in several horticultural crops (22) in both tomato fields and greenhouses in South America (25), California (12), Morocco (10), Tunisia (6) and Egypt (13). In Nigeria however, limited information exits on the occurrence and spread of the virus in the northern part of the country where the bulk of tomato production lies. Hence, we report the occurrence and distribution of TBSV in northern Nigeria.

MATERIALS AND METHODS

Field survey and sampling

Field survey and sampling was conducted to determine the occurrence and spread of TBSV infecting tomato plants in Sudan savanna region (Gombe, Jigawa and Kano States) of Nigeria during the 2017 and 2018 dry seasons. These three States are among the major and leading commercial tomato producing states in Nigeria (11). Three farms each from three major tomato producing local government areas (LGAs) of each State (Gombe: Akko, Kaltungo and Yamaltu-Deba LGAs; Jigawa: Hadeja, Kirikasama and Kazaure LGAs while in Kano: Garun Mallam, Bagwai and Kura

LGAs) were surveyed. Forty symptomatic and asymptomatic tomato leaf samples from each farm (n=2160) were collected in a $4m^2$ x $4m^2$ sized-quadrant set at the four corners and one at the centre of each field (14) and indexed against TBSV using double antibody sandwich enzyme-linked immunosorbent serological assay (DAS-ELISA). Information on the coordinates, size, surrounding crops and some cultural practices of each field were documented (Tables 1, 2, 3). Collected samples were individually wrapped in polyethylene bags, labeled, stored in an ice chest and transported to the Virology Laboratory of the Department of Crop Protection, Ahmadu Bello University Zaria for analyses. Samples were stored at 4 °C prior to diagnosis.

Serological assay

Serological tests were conducted for the detection of TBSV in the collected tomato leaf samples using the DAS-ELISA as specified by the supplier (Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und ZellkulturenGmbh, Braunschweig, Germany) of the detection kits. The antigen–antibody reactions were detected and optical density (OD) of each well was measured after 1 h using an ELISA plate reader Uniequip (Martinseed, Germany) at a wavelength of 405 nm (7). Sample values at least twice that of the negative control (check) were rated positive (16). Average virus incidence (%) for the two years was calculated using the formula:

$$\text{Virus incidence (\%)} = \frac{\text{Number of positive samples/farm}}{\text{Total number of samples examined/farm}} \times 100$$

RESULTS

The results obtained in Gombe State showed that TBSV incidence was significantly higher (*P = 0.05*) in Yamaltu-Deba LGA (16 %) followed by Akko (14.0 %) while Kaltungo had the lowest incidence of 12.1% (Fig. 1). Kura LGA had the highest virus incidence (18.0 %) followed by Garun Mallam (10.7 %) while the least incidence (6.5 %) was recorded at Bagwai in Kano State (Fig. 2). In Jigawa, the highest virus incidence of 13.0 % was recorded at Kirikasama LGA followed by Kazaure (9.5%) while Hadejia had the least incidence of 5.2 % (Fig. 3). Among all the States surveyed for TBSV, Gombe had the highest *(P = 0.05)* virus incidence (14.0 %) followed by Kano (11.7 %) while the least incidence (9.2 %) was recorded in Jigawa (Figure 4).

Table 1: Symptoms of virus diseases and cropping information of the locations surveyed in Gombe State during the 2017 and 2018 dry seasons.

LGA	Location	Coordinates	Farm size (Ha)	Variety of tomato	Duration of cultivation	Source of seed	Symptoms observed	Sanitary condition	Surrounding crops	Crop growth Stage	Cropping pattern
Akko	Gadawo	N10^{0}02.919, E011^{0}16.876	0.526	UTC/Syria	20 years	Previous season	C, LC, M, S	Weedy	Pepper, Tomato, Okra	Vegetative	Mixed cropping with Okra, pepper
	Kembu-Gingin Gada	N10^{0}02.916, E011^{0}17.169	1.420	Syria	25 years	Market vendors	C, S, LC, N, M,	Weedy	Okra, tomato	Flowering	Mixed cropping with Okra
	Kembu	N10^{0}02.353, E011^{0}17.763	0.427	Syria/ Tandino	>60 years	Previous season	C, S, LC, M,	Weeded	Tomato, pepper, watermelon	Vegetative	Sole cropping: rotate with water melon and pepper
Kaltungo	Gujuba	N09^{0}58.008, E011^{0}18.352	0.103	Syria	4 years	Market vendors	N, C, LC, M,	Weedy	Pepper, maize, Chocories	Flowering	Sole cropping: rotate with pepper and maize
	Awak	N09^{0}55.666, E011^{0}26.922	1.23	Roma VF	8 years	Previous season	C, LC, M, S, N	Weeded	Tomato, sugarcane	Vegetative	Mixed cropping with cucumber
	Dogon ruwa	N09^{0}57.870, E011^{0}28.399	1.51	Tandino	7 years	Previous season	N, C, LC, M, T	Weedy	Tomato, Okra, Onion, Maize	Vegetative	Mixed cropping with okra, pepper
Yamaltu-Deba	Dadinkowa	N10^{0}17.802, E011^{0}30.606	0.442	Syria	5years	Previous season	C,T, S, LC, M,	Weedy	Sweet melon, maize	Vegetative	Mixed cropping with maize and sweet melon
	FCHTRF	N10^{0}18.159, E011^{0}31.148	0.340	Syria	15years	Previous season	C, LC, M, S, T	Weedy	Okra, pepper	Flowering	Mixed cropping with okra
	Kwadon	N10^{0}16.147, E011^{0}31.181	1.12	Syria	30 years	Previous season	C, LC, M, S, T, N	Weeded	Tomato, onions, maize	Flowering	Mixed cropping with maize

C= chlorosis, LC= Leaf curl, M= Mosaic, N= Necrosis, S= Stunting, T= Twisting.
Source: Field Survey, (2017 and 2018).

Table 2: Symptoms of virus diseases and cropping information of the locations surveyed in Kano State during the 2017 and 2018 dry seasons.

LGA	Location	Coordinates	Farm size (Ha)	Variety of tomato	Duration of cultivation	Source of seed	Symptoms observed	Sanitary condition	Surrounding crops	Crop growth stage	Cropping pattern
Bagwai	Dabino-Center 5	N12^{0}07.394, E008^{0}13.611	0.1024	Roma VF	15 years	Seed Company	C, S, LC, M,	Weeded	Onion, Tomato, Maize	Fruiting	Mixed cropping with green peas, onions and groundnut
	Dabino-Center 4	N12^{0}07.481, E008^{0}12.699	1.720	UTC	17 years	Seed Company	C, LC, M, LC	Weedy	Tomato, Maize, Groundnut	Vegetative	Mixed cropping with maize and green peas
	Dabino-Center 3	N12^{0}07.544, E008^{0}12.729	1.050	Dan Jos	7 years	Seed Company	S, LC, T, M,	Weeded	Tomato, Cowpea, Maize	Vegetative	Mixed cropping with green peas and groundnut
Garun Mallam	Chiromawa	N11^{0}35.894, E008^{0}24.742	2.103	Roma VF	15 years	Market vendors	C, N, LC, M,	Weedy	Tomato, maize,	Vegetative	Mixed cropping with green peas
	Yantomo	N11^{0}37.594, E008^{0}24.987	0.824	UTC	>15years	Previous season	C, M, LC, S,	Weeded	Tomato, maize, green peas and cucumber	Flowering	Mixed cropping with Raddish, pumpkin and cucumber
	Kadawa	N11^{0}38.299, E008^{0}24.903	2.120	Roma VF	7 years	Market vendors	M, LC, N,	Weedy	Tomato, water melon, Maize, peas	Vegetative	Mixed cropping with green peas and maize
Kura	Butalawafadama 1	N11^{0}47.309, E008^{0}25.529	1.420	UTC (Inster)	27years	Previous season	C, LC, N, S, M	Weedy	Maize, Tomato, rice	Fruiting	Mixed Cropping with Maize, Pepper and Cabbage
	Butalawafadama 2	N11^{0}47.341, E008^{0}25.507	0.540	UTC (Inster)	10years	Market vendors	N, C, LC, T, M	Weedy	Tomato, pepper	Fruiting	Mixed cropping with Pepper and maize
	Butalawafadama 3	N11^{0}47.390, E008^{0}25.333	0.791	UTC (Inster)	15 years	Previous season	S, LC, M, C	Weedy	Tomato, maize, Cassava	Vegetative	Mixed cropping with maize and cassava,

C= Chlorosis, LC= Leaf curl, M= Mosaic, N= Necrosis, S= Stunting, T= Twisting. Source: Field Survey, (2017and 2018).
Source: Field Survey, (2017 and 2018).

Table 3: Symptoms of virus diseases and cropping information of the locations surveyed in Jigawa State during the 2017 and 2018 dry seasons.

LGA	Location	Coordinates	Farm size (Ha)	Variety of tomato	Duration of cultivation	Source of seed	Symptoms observed	Sanitary condition	Surrounding crops	Crop growth Stage	Cropping pattern
Hadejia	Mai Alkama	N12^{0}26.120, E 10^{0}35. 200	1.1024	Tandino	25 years	Previous season	C, N, LC, S,	Weedy	Onion, tomato	Vegetative	Mixed cropping with pepper
	Hadejia	N12^{0}26.379, E 10^{0}01. 173	0.620	UTC	6 years	Previous season	LC, M, N	Weedy	Tomato, pepper	Vegetative	Sole cropping: Rotate with pepper and onion
	Yayari	N12^{0}26.133, E10^{0}02.387	3.510	UTC	30 years	Previous season	C, LC, M,	Weeded	Tomato, pepper	Fruiting	Mixed cropping with okra and pepper
Kazaure	Dabaza	N12^{0}37.924, E008^{0}33.248	1.376	UTC	8 years	Seed Company	S, C, N, M,	Weedy	Tomato, pepper, cassava	Fruiting	Sole Cropping: Rotate with pepper
	Dan Dutsi-Sadua	N12^{0}36.400, E008^{0}33.966	1.571	UTC (Graptor)	25years	Seed Company	C, S, LC, M, N	Weedy	Tomato and pepper	Fruiting	Mixed cropping with Okra, maize and cucumber
	Kurfi	N12^{0}36.670, E008^{0}35.076	0.610	Roma VF	10 years	Previous season	C, LC, M, N	Weedy	Tomato, Maize	Fruiting	Sole Cropping: Rotate with pepper
Kirikasama	Tarabu	N12^{0}30.646, E010^{0}10.584	1.735	UTC	25years	Previous season	N, C, LC, S, T	Weeded	Pepper, tomato	Fruiting	Sole Cropping: Rotate with pepper
	Tarabu-Kumoyo	N12^{0}30.566, E010^{0}09.693	0.834	UTC	30years	Previous season	C, S, M, T, LC, N	Weedy	Maize	flowering	Mixed cropping with rice and maize
	Marma Giryo	N12^{0}39.730, E010^{0}21.530	0.231	Roma VF	>30 years	Previous season	C, LC, M, S, N	Weedy	Tomato, rice, maize	Fruiting	Mixed cropping with maize

C= Chlorosis, LC= Leaf curl, M= Mosaic, N= Necrosis, S= Stunting, T= Twisting. Source: Field Survey, (2017 and 2018).

Source: Field Survey, (2017 and 2018).

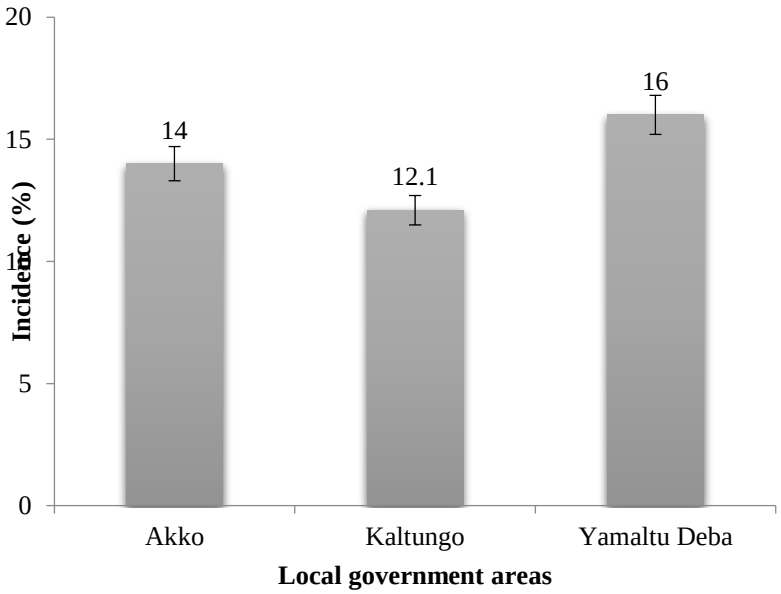

Figure 1: Incidence of *Tomato bushy stunt virus* in three local government areas of Gombe State during the 2017 and 2018 dry seasons. Bars indicate standard error of means at 5% probability level.

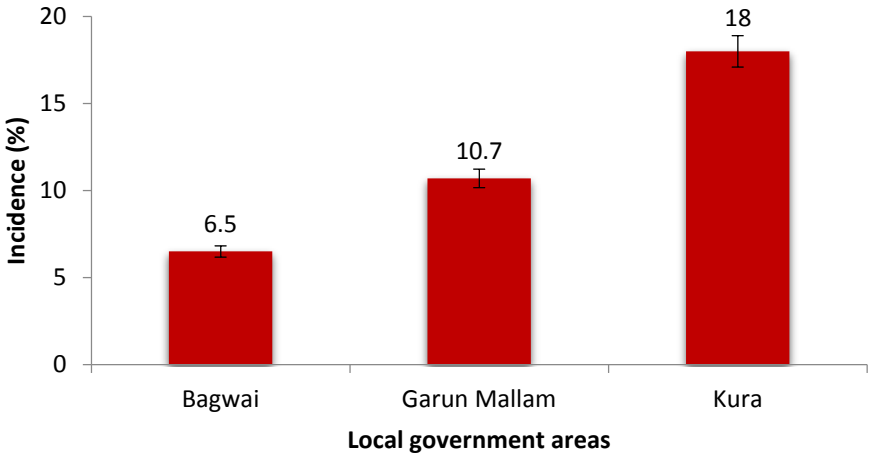

Figure 2: Incidence of *Tomato bushy stunt virus* in three local government areas of Kano State during the 2017 and 2018 dry seasons. Bars indicate standard error of means at 5% probability level.

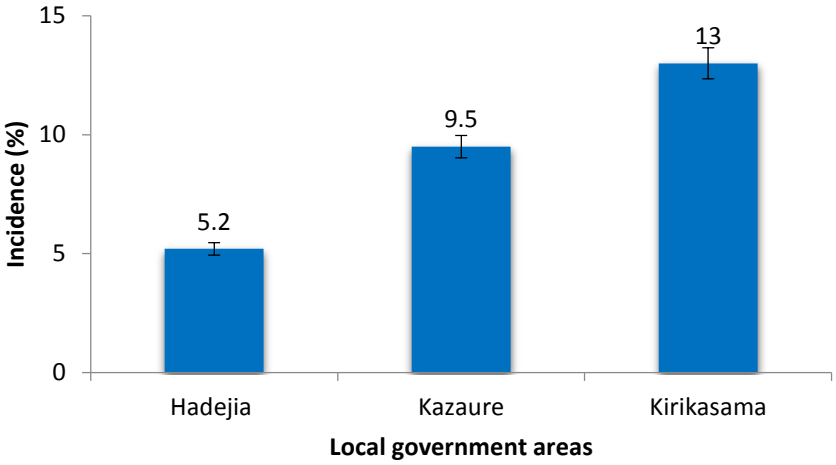

Figure 3: Incidence of *Tomato bushy stunt virus* in three local government areas of Jigawa State during the 2017 and 2018 dry seasons. Bars indicate standard error of means at 5% probability level.

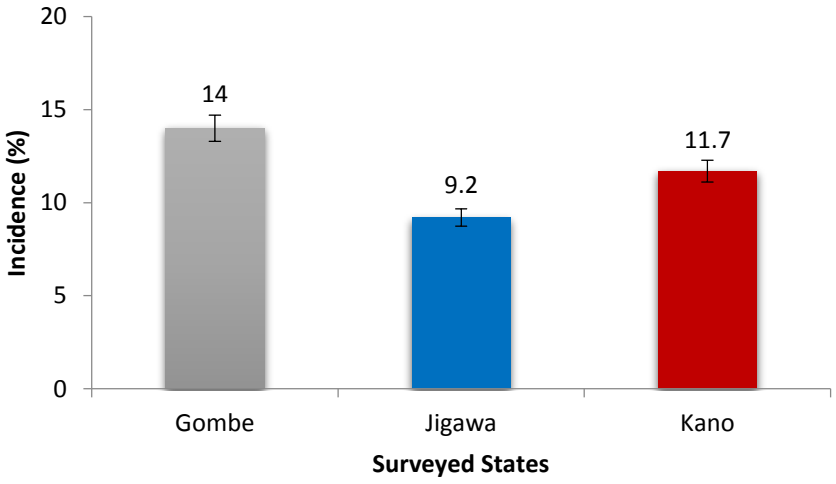

Figure 4: Incidence of *Tomato bushy stunt virus* in three States of northern Nigeria during the 2017 and 2018 dry seasons. Bars indicate standard error of means at 5% probability level.

DISCUSSION

The study reports that TBSV occurs naturally and infecting tomato plants in northern Nigeria. Reports on the occurrence of TBSV have been documented with varying incidences from different countries of the world (8; 13; 15; 24; 27). The serological detection of TBSV in all the tomato farms surveyed further confirmed that the observed disease symptoms (Plate I) were induced by the virus as earlier reported by Hafez *et al*. (13). Higher incidence of TBSV observed from three local government areas (Yamaltu Deba (Fig. 1), Kirikasama (Fig. 2), Kura (Fig. 3)) and Gombe State (Fig. 4) could be attributed to the use of TBSV borne seeds sourced from previously harvested tomato fruits (Tables 1, 2 & 3). TBSV has been reported as an important seed-borne virus in tomato (15, 22, 28) with transmission efficiency range of 50 - 65 % (29). The continuous cropping of tomato crops over a long period which maintains the cycle and favours inoculum build-up of the virus in the soil could also be a factor for the prevalence of virus incidence in the surveyed regions since TBSV has been reported to be soil-borne (27).Tomlinson and Faithful (29) and Gerik *et al*. (12) have demonstrated that transplanting of tomato seedlings in TBSV contaminated soils caused an average infection incidence of 55%. Manabayeva *et al*. (19) established the ability of soil-borne TBSV to cause systemic infections in tomato and tobacco through the roots without requiring the virus coat protein but the host defense suppressor identified as *P*19. A common practice of burying or leaving infected tomato stands in the fields by farmers in the surveyed region could further have encouraged inoculum build-up the virus in soil (1). Syria and UTC tomato varieties were commonly cultivated in all the localities with higher TBSV incidence (Tables 1, 2 & 3) which suggests that the varieties are readily susceptible to the virus attack compared to other varieties. Infections of susceptible plant hosts by TBSV have been reported to cause severe disease epidemic under field conditions (4; 6; 23). Moreover, the bulk of tomato cultivation in the surveyed areas is carried out during the dry season and solely relying on surface irrigation which has been implicated for successful transmission of TBSV in crops in the fields (26; 29).

CONCLUSION

To the best of our knowledge this is the first report of TBSV infecting field grown tomato in northern Nigeria. Gombe state had the highest incidence of the virus. Farmer's cultural practices such as source of seeds, varietal selection, cropping pattern and system, type of irrigation system adopted and farm sanitary measures could be said to be key

factors influencing the occurrence and spread of the virus in the region. Awareness programs need to be organized for tomato farmers on the incidence and integrated management of the virus.

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