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Full Length Article

Occurrence and Distribution of *Tomato Aspermy Virus* (*Cucumovirus*) Infecting Irrigated Tomato (*Solanum lycopersicum*) in Sudan Savanna, Nigeria

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Abstract

Tomato aspermy virus (TAV) is one of the important plant viruses limiting the gainful production of fruits and vegetables globally. The present study presents the incidence and spread of TAV infecting tomato plants in Nigeria's Sudan savanna region, including Jigawa, Gombe, and Kano states. Three farms from 3 leading tomato cultivating Local Government Areas (LGAs) of each state were surveyed during the 2017 and 2018 dry farming seasons. Forty symptomatic and asymptomatic tomato leaf samples from each farm (n = 2160) were collected in five quadrants measuring 4 m × 4 m and tested against TAV using a double-antibody sandwich enzyme-linked immunosorbent serological assay (DAS-ELISA). The results indicated that TAV was detected in all the states surveyed but with significant ($P \leq 0.05$) variation in distribution. TAV incidence was found to be significantly higher ($P \leq 0.05$) in Akko (16%) and Kaltungo LGAs (15.9%) in Gombe state. In Jigawa state, Kazaure LGA had the highest virus incidence (55.2%). The highest virus incidence of 33.6% was recorded at Kura LGA in Kano state. This is the first report of TAV on tomato crops in the surveyed states, with Jigawa state recording the highest ($P \leq 0.05$) incidence (42.2%) followed by Kano (18.3%). In comparison, Gombe had the least virus incidence (15%). This finding suggests further studies on the molecular characterization of TAV to determine its strains and association with other isolates reported elsewhere. To ensure profitable production, it is recommended that awareness and effective management practices of the virus be initiated for tomato farmers in the region.

Keywords: Bromoviridae; Detection; Nigeria; Plant virus; Prevalence; Spread

Introduction

Tomato aspermy virus (TAV; family *Bromoviridae*, genus *Cucumovirus*) is a tripartite positive-sense single-stranded genomic RNA virus (Inoue *et al.* 2018), which occurs globally with a wide host range infecting vegetable and ornamental crops of high economic value causing a significant reduction in quantity and quality of produce (Maddahian *et al.* 2017). It is one of the important viruses constraining the profitable production of tomato crops (Massumi *et al.* 2009; Abraham *et al.* 2019a). Under severe infection by TAV, tomato plants express characteristic symptoms such as mottling, necrosis, deformation of leaves, stunted growth, and several axillary buds proliferation making the foliage have a bushy appearance with significant fruit set reduction and production of malformed, small-sized, and seedless fruits (ICTVdB Management 2006; Blancard 2012). Symptoms of infection by TAV are similar to symptoms due to nutritional deficiencies. For instance, leaf chlorosis, mottling, necrosis, and deformation are symptoms also expressed by plants due to nitrogen, magnesium, zinc/manganese, and boron deficiencies respectively, thereby making it difficult for most tomato farmers to accurately distinguish between these two factors in the fields. However, symptom expressions due to TAV infection are typically observed on susceptible cultivars or species usually from the vegetative growth stage with varying but progressive degrees of severity and times of appearance among the infected crops. Symptoms are observed on the whole foliage of the infected plants at random locations in the fields and the incidence may keep increasing if no control measures are initiated against the vector of the virus. Moreover, TAV-infected plants do not recover even after the application of the right dosage of pesticide or fertilizer. On the other hand, nutritional deficiency symptoms are typically expressed uniformly across the crops in the fields irrespective of the cultivar or species and their growth stages. Depending on the deficient

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nutrient, symptoms may either be expressed at the lower (mobile nutrients: N, P, K and Mg) or upper (immobile nutrients: Calcium, Boron, Iron, Zinc, Sulphur) parts of the foliage with no horizontal spread in time (Tjosvold and Koike 2015). In nature, TAV is principally transmitted by over 22 species of aphids in a nonpersistent manner (Palukaitis and García-Arenal 2003; Blancard 2012), but transmission by dodder, infected plant sap (Brunt 1996), and through seeds of *Phaseolus vulgaris* and *Stellaria media* (Sastry 2013) have also been reported. Bello (2017) has earlier reported TAV incidence of 27 and 28% in Sokoto and Zamfara states, respectively, on irrigated tomato plants in northwestern Nigeria. Considering the paucity of information on the current status of TAV in other major and leading commercial tomato-producing states in the country, and the significant yield losses incurred in tomato production due to *Tomato aspermy virus* disease (Nava *et al.* 1997; ICTVdB Management 2006; Hajiabadi *et al.* 2012) on the resource-poor farmers who dominate tomato cultivation as their sole means of livelihood in Nigeria. The present study was initiated, to detect the incidence and spread of TAV in three states (Gombe, Jigawa and, Kano states) in the Sudan savanna ecological zone of Nigeria.

Materials and Methods

Field survey and sample collection

A field survey and sampling of farmers' fields were conducted to ascertain the incidence and spread of TAV infecting irrigated tomato plants in three states (Gombe, Jigawa, and Kano) in the Sudan savanna region of Nigeria during the 2017 and 2018 dry seasons. In each state, three leading tomato-producing Local Government Areas (Gombe: Kaltungo, Akko, and Yamaltu-Deba LGAs; Kano: Kura, Garun Mallam, and Bagwai LGAs while in Jigawa: Kirikasama, Kazaure, Hadejia, and LGAs) were selected from which three farms each were surveyed. Forty symptomatic and asymptomatic tomato leaf samples from each farm (n = 2160) were collected in five quadrants (with each at the four corners and center of the farm) measuring 4 m × 4 m as described by Kashina *et al.* (2002). Some important Information on each farm surveyed was recorded by using a questionnaire. Each sample collected was packaged in polythene bags, labelled and kept at 4°C before diagnoses.

Serological assay

DAS-ELISA kits specified for TAV detection were obtained from the Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen Gmbh (Braunschweig, Germany) used to index tomato leaf samples against TAV incidence. The procedure described by Clark and Adams (1977) for determining the antigen-antibody reactions in ELISA was followed in this study. The Uniequip ELISA plate reader (Martinseed, Germany) set at 405 nm wavelength was used to measure the optical density of wells of the microtiter plates after 1 h. The values of the test samples were rated positively when measured to be two times the value of the negative control (check), as described by Abraham *et al.* (2020). Mean virus incidence (%) for the two years was computed as the number of positive samples detected expressed as a percentage of the total number of samples examined per farm.

Data analysis

The variation in the data collected on the incidence of TAV was analyzed, and the differences in their means were declared significant at a 5% level of probability using the standard error of means as described by Gomez and Gomez (1984). SAS statistical software package was used for the analysis.

Results

The results in Tables 1–3 showed some information on field-grown tomato cultivation in Gombe, Jigawa, and Kano states. It was observed that tomato crops were cultivated on an average of 0.8ha, 1.3 ha, and 1.2 ha farm sizes in Gombe, Jigawa, and Kano states respectively. UTC and Roma VF were the varieties observed to be cultivated in all the surveyed states. *Syria* was the most common (78%) tomato variety in Gombe while UTC was the dominant variety cultivated in Jigawa (67%) and Kano (56%). Duration of tomato cultivation by farmers varied among the surveyed states where an average of 19, 14 and 21 years in Gombe, Kano, and Jigawa respectively. A total of 67% of farmers in Kano sourced their seeds/seedlings from uncertified market vendors and fruits of the previous season while 78% of farmers in Gombe and Jigawa sourced their seeds solely from harvested tomato fruits of the previous season. Chlorosis, leaf curl, mottling, necrosis, mosaic, stunting, and twisting were the virus disease symptoms commonly observed on tomato crops across all the three surveyed states (Fig. 1). It was observed that 78% of the surveyed tomato farms were weedy while 67% of farms in Kano and Jigawa were found to be weedy. Tomato farms surveyed were averagely surrounded by 100, 89, and 67% of other tomato farms in Kano, Jigawa, and Gombe states respectively. As at the time of the survey 67% of the tomato crops were at the fruiting stage in Jigawa while 56% of tomato farms were at the vegetative growth stage in Gombe and Kano. We also observed that 56% of tomato farms surveyed were either intercropped or mixed with Pepper in Gombe and Jigawa as against 22% in Kano state. The results obtained indicated that TAV was detected in all the states surveyed but with significant ($P \leq 0.05$) variation in distribution. TAV incidence was found to be significantly higher ($P \leq 0.05$) in Akko (16%) and Kaltungo LGAs (15.9%) than was recorded in Yamaltu-Deba (13.1%) in

Table 1: Some cropping information and symptoms of virus diseases of the surveyed locations 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	ʃSurrounding Crops	ΨCrop growth Stage	ƒCropping Pattern
Akko	Gadawo	N10^{0}02.919, E011^{0}16.876	0.526	UTC/Syria	20 years	PS	C, LC, M, S, Mo	W	P, T, Ok	V	MC with O and P
	Kembu-GinginGada	N10^{0}02.916, E011^{0}17.169	1.420	Syria	25 years	MV	C, S, LC, N, M,	W	Ok, T	F	MC with O
	Kembu	N10^{0}02.353, E011^{0}17.763	0.427	Syria/ Tandino	> 60 years	PS	C, S, LC, M,	WD	T, P, W	V	SC: rotated with W and P
Kaltungo	Gujuba	N09^{0}58.008, E011^{0}18.352	0.103	Syria	4 years	MV	N, C, LC, M, Mo	W	P, M, C	F	SC: rotated with P and M
	Awak	N09^{0}55.666, E011^{0}26.922	1.23	Roma VF	8 years	PS	C, LC, M, S, N	WD	T, S	V	MC with C
	Dogon ruwa	N09^{0}57.870, E011^{0}28.399	1.51	Tandino	7 years	PS	N, C, LC, M, T	W	T, Ok, O, M	V	MC with O and P
Yamaltu-Deba	Dadinkowa	N10^{0}17.802, E011^{0}30.606	0.442	Syria	5 years	PS	C,T, S, LC, M,	W	SM, M	V	MC with M and S
	FCHTRF	N10^{0}18.159, E011^{0}31.148	0.340	Syria	15 years	PS	C, LC, M, S, T	W	Ok, P	F	MC with O
	Kwadon	N10^{0}16.147, E011^{0}31.181	1.12	Syria	30 years	PS	C, LC, M, S, T, N	WD	T, O, M	F	MC with M

†PS= Previous season; MV = Market vendors. ‡C = chlorosis; LC = Leaf curl; M = Mosaic; N = Necrosis; S = Stunting; T = Twisting; Mo = Mottling. *W = Weedy; WD = Weeded. ʃP = Pepper; T = Tomato; OK = Okra; W = Water melon; M = Maize; C = Chocories; S = Sugarcane; SM = Sweet melon; O = Onion. ΨV = Vegetative; F = Flowering. ƒMC = Mixed cropping; SC = Sole cropping; O = Okra; P = Pepper; W = Water melon; C = Cucumber; S = Sweet melon; M = Maize
Source: Field Survey (2017 and 2018)

Table 2: Some cropping information and symptoms of virus diseases of the surveyed locations 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	ʃSurrounding Crops	ΨCrop growth Stage	ƒCropping Pattern
Hadejia	Mai Alkama	N12^{0}26.120, E 10^{0}35. 200	1.1024	Tandino	25 years	PS	C, N, LC, S,	W	T, O	V	MC with P
	Hadejia	N12^{0}26.379, E 10^{0}01. 173	0.620	UTC	6 years	PS	LC, M, N, Mo	W	P, T	V	SC: rotated with P and O
	Yayari	N12^{0}26.133, E10^{0}02.387	3.510	UTC	30 years	PS	C, Mo, LC, M,	WD	P, T	F	MC with OK and P
Kazaure	Dabaza	N12^{0}37.924, E008^{0}33.248	1.376	UTC	8 years	SC	S, C, Mo, N, M,	W	T, C, P	F	SC: rotated with P
	Dan Dutsi-Sadua	N12^{0}36.400, E008^{0}33.966	1.571	UTC (Graptor)	25 years	SC	C, Mo, S, LC, M, N	W	T, P	F	MC with C, OK, M
	Kurfi	N12^{0}36.670, E008^{0}35.076	0.610	Roma VF	10 years	PS	C, LC, Mo, M, N	W	M, T	F	SC: rotated with P
Kirikasama	Tarabu	N12^{0}30.646, E010^{0}10.584	1.735	UTC	25 years	PS	N, C, LC, Mo, S, T	WD	T, P	F	SC: rotated with P
	Tarabu-Kumoyo	N12^{0}30.566, E010^{0}09.693	0.834	UTC	30 years	PS	C, S, M, T, LC, N	W	M	Fw	MC with R and M
	Marma-Giryo	N12^{0}39.730, E010^{0}21.530	0.231	Roma VF	>30 years	PS	C, Mo, LC, M, S, N	W	R, T, M	F	MC with M

†PS = Previous season; SC = Seed company. ‡C = chlorosis; LC = Leaf curl; M = Mosaic; N = Necrosis; S = Stunting; T = Twisting; Mo = Mottling. *W = Weedy; WD = Weeded. ʃP = Pepper; T = Tomato; C = Cassava; M = Maize; R = Rice; O = Onion. Ψ V = Vegetative; F = Fruiting; Fw = Flowering. ƒMC = Mixed cropping; SC = Sole cropping; O = Onions; P = Pepper; C = Cucumber; OK = Okra; M = Maize; R = Rice. Source: Field Survey (2017 and 2018)

Gombe state (Fig. 2). In Jigawa state, Kazaure LGA had the highest virus incidence (55.2%), followed by Kirikasama (38.9%), while the least incidence (32.6%) was recorded at Hadejia (Fig. 2). The highest virus incidence of 33.6 % was recorded at Kura LGA, followed by Bagwai (18.1%), while Garun Mallam had the least incidence of 13.2% in Kano state (Fig. 2). Of all the states surveyed for TAV, Jigawa recorded the highest ($P \leq 0.05$) mean incidence (42.2%), followed by Kano (18.3%), while the least virus incidence (15%) was recorded in Gombe (Fig. 3).

Discussion

The present study examined the incidence and spread of TAV on irrigated tomato crops in the Sudan savannah region (Gombe, Jigawa, and Kano states) of Nigeria. TAV was detected for the first time on field-grown tomato crops in all the states surveyed. The detection of TAV naturally infecting tomato (Nava *et al.* 1997; Jafari *et al.* 2010; Bello 2017), chrysanthemum and gladiolus (Raj *et al.* 2007, 2011; Maddahian *et al*. 2017) have been reported previously from several parts of the world. This has further supported the report on the phytopathogenic and global occurrence of TAV Kafi and Ghahsareh (2009). *Senso lato*, the incidence of TAV in the region could be attributed to several factors. The study areas (Gombe, Jigawa, and Kano states) are located in sub-Saharan Africa in the tropical zone of the world is

Table 3: Some cropping information and symptoms of virus diseases of the surveyed locations 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	ʃSurrounding Crops	ΨCrop growth Stage	ǂCropping Pattern
Bagwai	DabinoCenter 5	N12°07.394, E008°13.611	0.1024	Roma VF	15 years	SC	C, S, N, LC, M,	WD	T, O, M	F	MC with O, GP and G
	DabinoCenter 4	N12°07.481, E008°12.699	1.720	UTC	17 years	SC	C, LC, M, N, LC	W	M, T, G	V	MC with GP and M
	DabinoCenter 3	N12°07.544, E008°12.729	1.050	Dan Jos	7 years	SC	S, LC, T, M, N	WD	Co, M, T	V	MC with GP and G
Garun Mallam	Chiromawa	N11°35.894, E008°24.742	2.103	Roma VF	15 years	MV	C, N, LC, M,	W	M, T	V	MC with GP
	Yantomo	N11°37.594, E008°24.987	0.824	UTC	>15years	PS	C, M, LC, S,	WD	GP, M, Cu, T	Fw	MC with RD, Pk, Cu
	Kadawa	N11°38.299, E008°24.903	2.120	Roma VF	7 years	MV	M, Mo, LC, N,	W	W, T, M, GP	V	MC with M and GP
Kura	Butalawafadama 1	N11°47.309, E008°25.529	1.420	UTC (Inster)	27 years	PS	C, LC, Mo, N, S, M	W	R, T, M	F	MC with M, P and Cb
	Butalawafadama 2	N11°47.341, E008°25.507	0.540	UTC (Inster)	10 years	MV	Mo, N, C, LC, T, M	W	P, T	F	MC with M, and P
	Butalawafadama 3	N11°47.390, E008°25.333	0.791	UTC (Inster)	15 years	PS	S, N, LC, M, C, Mo	W	M, C, T	V	MC with M and C

†PS = Previous season; SC = Seed company. ‡C = chlorosis; LC = Leaf curl; M = Mosaic; N = Necrosis; S = Stunting; T = Twisting; Mo = Mottling. *W = Weedy; WD = Weeded. ʃP = Pepper; T = Tomato; Co = Cowpea; C = Cassava; G = Groundnut; M = Maize; Cu = Cucumber; GP = Green peas; W = Water melon; R = Rice; O = Onion. ΨV = Vegetative; F= Fruiting; Fw = Flowering. ǂMC = Mixed cropping; O = Onions; G = Groundnut; P = Pepper; Cu = Cucumber; GP = Green peas; C = Cassava; R = Radish; M = Maize; Cb = Cabbage; Pk = Pumpkin. Source: Field Survey (2017 and 2018)

Fig. 1: Disease symptoms expression on tomato plants infected by TAV: (**A**) healthy tomato plant; (**B**) Showing chlorosis, mottling, necrosis, and deformation of leaves; (**C**) severely infected tomato plants showing reduced leaf and stem, chlorosis, necrosis, and stunted growth with a bushy appearance

characterized by arid, steppe, and high temperatures (Beck *et al.* 2018) favouring many vegetable crops pathogens, and vectors of viruses. An increase in temperature as an effect of climate change has favoured the multiplication of virus vectors such as aphids and whiteflies which in turn influences the emergence, global geographical distribution, and severity of some tomato viruses including *Tomato brown rugose fruit virus* (ToBRFV), *Tomato chlorosis virus* (ToCV); *Tomato yellow leaf curl virus* (TYLCV), *Tomato torrado virus* (ToTV), and *Pepino mosaic virus* (PepMV) as reviewed by Trebicki (2020). In addition, an adaptation of or favourable conditions for the vector of TAV in the study area could also ensure the prevalence of the virus in the region. The cultivation of tomato on small, fragmented fields in all the surveyed states was due to the poor resource status of most of the farmers. The relatively higher incidence of TAV in Jigawa state could be influenced by the predominant cultivation of the UTC tomato variety in the state which may be susceptible to the TAV compared to the *Syria* variety mainly cultivated in Gombe which had the lowest incidence of the virus. The increase in the number of emerging novel virus species and new virulent strains of known tomato viruses capable of breaking the defense of resistant tomato cultivars have been reported (Rivarez *et al.* 2021). The continued reliance of most of the farmers on seeds from the previous planting season and the purchase of seedlings from uncertified local market vendors who raised the seedlings from untreated and unprotected nurseries against insect transmitting vector (aphid species) of TAV increase the chance of seedling infection. Jones (2021) pointed out the use of virus-contaminated/infected planting materials as a major factor influencing the epidemiology of plant viruses in the fields. Contaminated or infected seeds have been reported as possible means for TAV dissemination (ICTVdB Management 2006; Sastry 2013). Common symptoms noted on tomato crops were leaf curl, mosaic, stunting, leaf mottling, twisting or malformation, necrosis, and chlorosis have earlier been reported to be

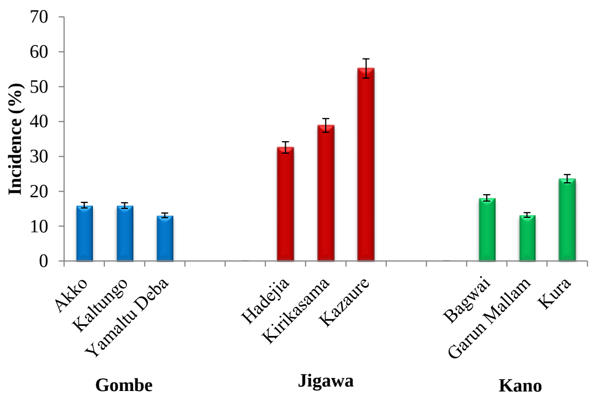

Fig. 2: Incidence of *Tomato aspermy virus* in Gombe, Jigawa and Kano states during the 2017 and 2018 dry seasons. Bars indicate the standard error of means at a 5% probability level

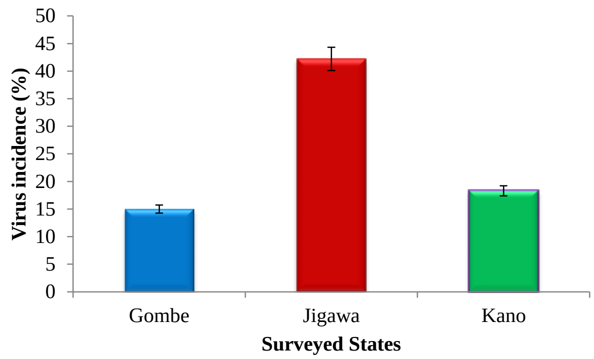

Fig. 3: Mean incidence of *Tomato aspermy virus* in Gombe, Jigawa and Kano states during the 2017 and 2018 dry seasons. Bars indicate the standard error of means at 5% probability level

associated with virus diseases (Gallitelli 2000) which were not probed in the present study. The observed disease symptoms incited by TAV as similarly reported by Bello (2017) were further affirmed by the two seasons of serological detection of the virus in all the tomato fields surveyed. Our interaction with the farmers in the course of the study revealed that the majority of the farmers are unaware of viral diseases and their effective management measures. Poor weed management is another important factor that influences virus disease spread. Most of the farms surveyed were found to be weedy which may serve as an inoculum source for the transmission of viruses to tomato plants by vectors. A total of nineteen weed species have been detected to be infected with TAV in tomato fields in northern Nigeria (Bello 2017; Abraham *et al.* 2019b). Similarly, 19 and 14 weed species were detected to be naturally infected with *Tomato yellow leaf curl virus* (TYLCV) and *Tomato ringspot virus* (ToRSV) in northern Nigeria, respectively (Abraham *et al.* 2021a, b). All-year-round cultivation of tomatoes in the study area is another significant factor that could avail an uninterrupted TAV disease circle (Bernardo *et al.* 2018). It was also observed from the farmer’s fields that tomato plants are been inter-cropped with other alternative hosts of TAV vector and the proximity of tomato fields to other surrounding vegetable fields could support vectors of viruses that may infest tomato and transmit viruses (Mazyad *et al.* 1994; Jeger 2020).

Conclusion

The incidence and spread of TAV naturally infecting tomato crops in the Sudan savannah region (Gombe, Jigawa, and Kano states) of Nigeria were established in the study. This is the first time to detect TAV on tomato crops in the surveyed states with Jigawa state recording the highest incidence. Farmers' unawareness of the virus and its management measures influenced the prevalence of the TAV in the study area. This finding suggests further studies on the molecular characterization of TAV to determine its strains and association with other isolates reported elsewhere. It is recommended that awareness and effective management practices of the virus be initiated for tomato farmers in the region to ensure profitable production.

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Author Contributions

PA, OOB, BDK and MDA planned and designed the research. PA and MPA performed the experiments. MPA analyzed the data, and PA, OOB, BDK and MDA wrote the manuscript. OOB, BDK and MDA contributed equally. All authors reviewed and approved the manuscript.

Conflicts of Interest

All the authors declare that we have no conflict of interest.

Data Availability

Data supporting the findings of this study are available in this article.

Ethics Approval

This article does not contain any studies with human participants or animals. The collection materials of the plants, complies with the relevant institutional, national, and international guidelines and legislation.

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