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Evaluation of Tomato (*Lycopersicon lycopersicum* (L.*)* Genotypes for their Reaction to *Tomato mosaic virus* (ToMV) Infection in Kebbi State, Nigeria

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Abstract

***Tomato mosaic virus* (ToMV) is among the most devastating viral diseases of tomato worldwide and it can cause yield losses of up to 40%. One of the effective managements of plant viral diseases is the use of resistant genotypes. This research was conducted to determine the reaction of tomato genotypes to *Tomato mosaic virus* (ToMV) infection. The experiment consists of five tomato genotypes which were evaluated for resistance to ToMV infection under screen-house conditions using Completely Randomized Design (CRD) with three replicates. The treatments were Dantaya, Danwarai, Dan-Niko, Dan-Heka, and Hybrid Platinum, which are the five most preferred farmers varieties in Kebbi State. Prior to mechanical inoculation, ToMV-infected leaf samples used as source of inoculum were tested ToMV positive using Direct Antibody Coating-Enzyme Linked Immunoassay (DAC-ELISA). Thirty-six (36) seedlings of each genotype were transplanted into plastic pots containing compost and seedlings recovery were recorded two weeks after transplanting (WAT) before inoculation. Hybrid Platinum has low establishment percentage (72.22%), compared to Dan-Heka and Dan-Niko with both 100%. All the genotypes evaluated were infected when inoculated with ToMV but with significant variation (*P* = 0.05) in their reaction to ToMV infection. Dantaya recorded the highest percent disease incidence of 50.00% while, Platinum recorded the lowest percent disease incidence of 31.94%. Three genotypes were susceptible to ToMV while, two were resistant to ToMV. The high incidence of ToMV found on farmers preferred tomato genotypes in Kebbi State is of great management concern. Also, this has implication for tomato productivity and yield in the state.**

Key words: Genotypes, Infection, Resistance, ToMV, Kebbi State
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Introduction

Tomato *(Lycopersicon lycopersicum* L.) plant, is a vegetable crop, belongs to family *Solanaceae* and second most important vegetable crop after potato. It is commonly cultivated in many parts of the world (Manibhushan, 2005). Tomato is an important vegetable crop in Nigeria and about 3,693,722 tons of fresh tomato are produced in the country annually (FAO, 2020). Tomato is ranked first among fruit vegetable globally and it serve as the main raw material for many vegetable processing industries (FAO, 2010; Pirrello et al., 2014). In major tomato producing countries, the fruit is commonly marketed fresh and used in making ketchup, juice, paste and soups. Tomato fruits are used in pharmaceutical industries to produce different vaccines (Ahmad et al., 2012). It is also used in production of therapeutic molecules for treatment of

Alzheimer´s disease, hepatitis B, C and cancers (Chen et al., 2009). Tomato plant had been used as the model species for fleshy fruit because of its short life cycle, high multiplication rate, self-pollination, and a wide range of environmental adoptability (Chevalier et al., 2011).

Despite the important of the crop, biotic factors, especially diseases caused by viruses are the major constraints to tomato production in Nigeria. About 130 viruses have been reported to infect tomato worldwide and they can cause up to 90% loss (Hanssen et al., 2010). *Tomato mosaic virus* (ToMV) is among the most important viruses associated with tomato. Symptoms expressed by infected tomato plants are mosaic pattern on leaves, curling and distortion of leaves, with internal browning and uneven ripening of fruits (Najeebullah et al., 2017). One of the effective managements of plant viral diseases is the use of resistant genotypes (Strange and Scott, 2005). Naturally, some crops genotypes are found to be resistant to plant diseases especially those caused by viruses. Kebbi State is one of the highest tomatoes producing states in Nigeria. Whether tomato genotypes grown commercially in Kebbi State are resistant to ToMV infection are not known. Knowing the relative importance of the use of resistant varieties to manage viral diseases, this research was conducted to screen tomato genotypes commercially grown in Kebbi State for resistance to ToMV infection. To the best of our knowledge, this is the first research to report screening of tomato genotypes for resistance to ToMV infection in Kebbi State.

Materials and Methods

Tomato genotypes

Four tomato genotypes (Dan-Heka, Danwarai, Dantaya and Dan-Niko) were obtained from farmers field and one (Hybrid Platinum 708) from registered seed vendors in Kebbi State. Seedlings were raised under nursery conditions which were later transplanted into plastic pots containing compost. Uniform and healthy seedlings of 30 days old were transplanted into plastic pots filled with the sterilized compost soil and were allowed to establish at 28 ± 2^{o}C, Relative Humidity (RH) 50-60% prior to virus mechanical inoculation. The experimental plants were transplanted and maintained under insect-proofed screen-house conditions.

Source of ToMV isolates.

A survey of tomato fields was conducted in tomato growing areas of Aliero, Jega and Mayama Local Government Areas (LGAs) of Kebbi State. Infected tomato plants showing typical symptoms of tomato mosaic virus (ToMV) were sampled and leaf samples were collected. Direct Antibody Coating Enzyme Linked Immunosorbent Assay (DAC-ELISA) was used for confirmation of the virus in the symptomatic leaf samples collected. After DAC-ELISA, positive leaf samples were then used as source of ToMV isolates in screening experiment for mechanical inoculation of the tomato experimental plants.

Direct Antibody Coating Enzyme Linked Immunosorbent Assay (DAC-ELISA) Procedure

Direct Antibody Coating Enzyme Linked Immunosorbent Assay (DAC-ELISA) was used to detect ToMV in infected tomato samples collected during survey. Tomato leaf samples were macerated in a carbonate coating buffer (1:10 w/v) using plastic bags and wells of microplates were loaded with 100 µl of the extract. The loaded microplates were incubated overnight at 4°C under humid condition. 100 µl of washing buffer was pipetted in each well after incubation and washed three times in each step. Specific virus antiserum was diluted at 1:2000 ratio in antibody buffer and 100 µl were loaded to each well. Microplates were incubated for 2 hours at 37°C. Coat anti-rabbit conjugate was diluted in antibody buffer at 1:2000 and 100 µl was loaded in each well and incubated for 2 hours at 37°C. Substrate buffer of 1 mg/ml was used to dissolve P-nitrophenyl phosphate (PNPP) and 100 µl of the solution was loaded per well. Aluminium foil was used to cover plates which were then incubated at room temperature in dark. Spectrophotometer reading of the absorbance of each sample at 405 nm was taken using the ELISA spectrophotometer (BIO RAD Microplate Reader, iMark).

Treatments and experimental design

The treatments were five (5) tomato genotypes, which were designated as VI, V2, V3, V4 and V5 and were laid out in a Complete Randomized Design (CRD) and replicated three times. The experimental plants were mechanically inoculated with virus inoculum and maintained in an insect-proofed screen-house for symptom development.

Mechanical inoculations of the experimental plants

Virus transmission was achieved by mechanical inoculation (sap inoculation) method of Mohammed et al, (2012) where 0.06 M potassium phosphate buffer was prepared (80.2 mL of 0.6 M K HPO + 19.8 mL of 0.6 M KH PO + 900 ml of SDW) and pH was adjusted to 7.4 and autoclaved. ToMV infected leaf materials were triturated in phosphate buffer (0.01 M, pH 7.4) with the help of sterilized pestle and mortar. Sterilized double layered cloth was used to filter solution to remove leaf debris. Prior to inoculation, leaves of the experimental plants were dusted with fine 600 mesh carborundum powder and the infective plant sap were applied gently using a cotton wool pad stroking from petiole to the leaf tip. Using washing bottle, inoculated leaves was rinsed thoroughly 10 minutes after inoculation and the experimental plants were kept for symptom development and appearance. To ensure virus transmission, experimental plants were re-inoculated after 48 hours. Plants inoculated with phosphate buffer alone were used as controls for the experiment. Prior to inoculation, the experimental plants were kept in a dark room for 24 hours.

Data collection

Data were collected on:

Seedlings recovery after transplanting (%): The percentage of seedlings recovery after transplanting was calculated using the following formula.

$$\text{Establishment (\%)} = \frac{Number\ of\ seedlings\ recovered\ after\ transplanting}{Total\ number\ of\ seedlings\ transplanted} \times 100$$

Screening of tomato genotypes

Five tomato genotypes were collected and evaluated against ToMV in an insect-proofed screen-house at 28±2^{0}C temperature, 12 hours photoperiod and 50-60% relative humidity. Among the five genotypes, four genotypes are local cultivars while, one genotype is an improved released variety (that is Platinum). To examine ToMV infection, the inoculated plants were observed daily to record symptom development on the inoculated leaves and the upper systemic leaves. Leaf samples were collected from inoculated tomato genotypes and tested for presence of ToMV using Direct Antigen Coating-Enzyme Linked Immunosorbent Assay (DAC-ELISA) as described by Ullah et al. (2017). Disease severity index was scored using the modified 0 to 4 disease severity indices as described by Akhtar et al. (2010) given in Table 1, where: 0 indicates no ToMV symptoms and 4 indicates severe ToMV symptoms, typical mosaic or mottling, leaf deformity, shoe-stringing, stunting with no or few unmarketable fruits setting. Data on disease incidence were taken seven times after inoculation at weekly interval to determine percent disease incidence (DI). DI was calculated as the number or proportion of diseased plants in a population (i.e., experimental plants) using a formula of Sseruwagi et al. (2004).

$$\text{Disease incidence (\%)} = \frac{Number\ of\ plants\ with\ symptoms}{Total\ number\ of\ plant\ inoculated} \times 100$$

Table 1: Disease scale for rating of tomato genotypes against ToMV

Rating	Symptoms	Severity index	Disease reaction
0	No visible disease symptoms	0	Highly resistant
1	Complete absence of symptom or mild mosaic or mottling or leaf deformity, virus can be detected in plant tissues	0.01-1.4	Resistant
2	Moderate mosaic or mottling and leaf deformity followed by minor shoe stringing	1.5-2.4	Tolerant
3	Severe mosaic or mottling and leaf deformity, shoe-stringing, minor to medium stunting with minor reduction in fruit setting but marketable fruit setting	2.5-3.4	Susceptible
4	Severe mosaic or mottling, leaf deformity, shoe-stringing, stunting with no or few unmarketable fruits setting	3.5-4.0	Highly susceptible

Data Analysis

The data generated were subjected to analysis of variance using General Linear Model (GLM) of the Statistical Analysis System package (SAS Version 9). Least Significant Difference (LSD) was used to separate treatment means (Steel et al., 1997).

Results

Seedlings recovery after transplanting

Among the genotypes screened, Hybrid Platinum 708 (improved genotype) had the lowest establishment (72.22%), Dan Heka and Dan Niko both had 100.00%, while Dantaya and Danwarai both had 90.00 and 80.00%, respectively (Table 2). The result indicates that there was significant (P = 0.05) different among the five genotypes screened in this research for their ability to recover from transplanting shock under screen-house conditions (Table 3).

Table 2: Seedlings recovery two weeks after transplanting (2WAT)

Genotypes	No. of seedlings transplanted	Percent seedlings recovery (%)
Dan Heka	36	100.00[a]
Dan Niko	36	100.00[a]
Dantaya	36	90.00[b]
Danwarai	36	80.00[c]
Hybrid Platinum	36	72.22[d]
CV	**180**	**2.22814**
LSD		**6.3196**

Means with the same letter on the column are not significantly different (P = 0.05). CV = Critical Value of t, LSD = Least Significant Difference.

Table 3: ANOVA table for establishment count

Source	DF	Sum of squares	Mean square	F Value	P value
Variety	4	1801.0667	450.267	37.31	<0.0001**
Error	10	120.6667	12.067		
Total	14	1921.733			

DF = degree of freedom, p = probability, ** = statistically significant

Symptom types observed.

Upon ToMV inoculation on experimental tomato genotypes (Fig. 1B), the plants shown typical virus symptoms with some variability in time taken for symptoms appearance, symptoms development and lesion size formation. Seven days after inoculation, small yellowish lesions were observed. Twelve days after inoculation, the yellowish lesions turned to necrotic which is one of the characteristic symptoms of ToMV. Other symptoms observed on the experimental tomato genotypes upon inoculation include light to dark green mottling on leaf, chlorosis, and stunted growth, with light to dark mottling the common symptom on tomato genotypes screened for ToMV resistance, followed by stunted growth and chlorotic spot on the leaves (Fig. 1C).

DAC-ELISA Reaction

Positive and negative reactions were determined by visual assessments with different grades of yellow colour indicating positive reactions (Fig. 1A) while, those that were negative remained colourless. The samples that were considered as positive are those with an absorbance value that was twice the values of negative control.

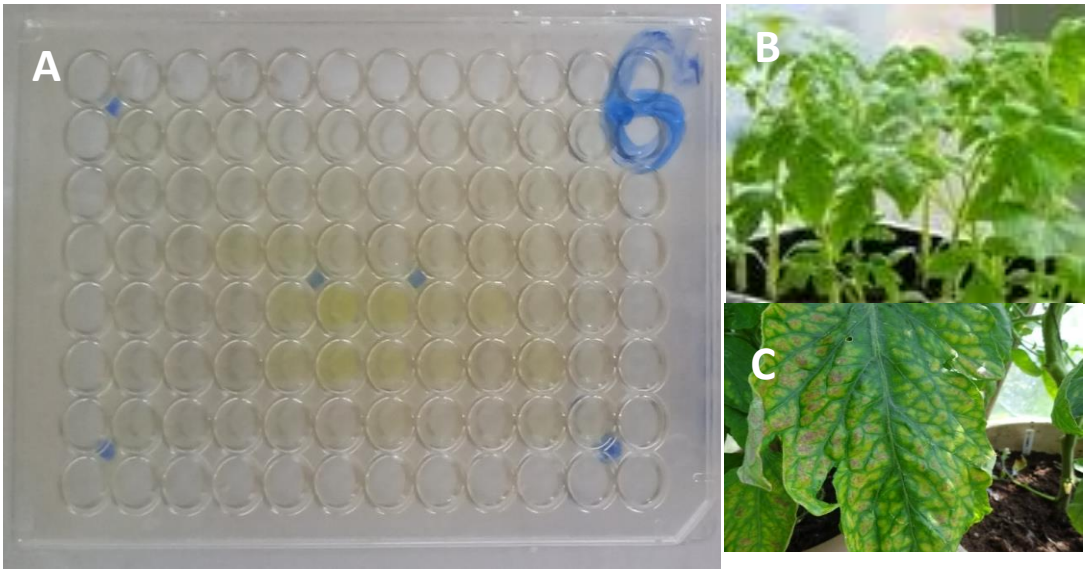

Fig 1: ELISA plate showing positive bands (yellow wells) and negative bands (colourless wells) (A), a healthy tomato seedling just after inoculation (B) and tomato leaf showing mosaic symptom (C).

Disease Incidence and Disease Severity Index

All the tomato genotypes screened for ToMV resistance were infected after mechanically inoculated with ToMV inoculums but with variation in their disease reaction. Results obtained show significant differences (P = 0.05) (Table 5) among the tomato genotypes screened for resistance to ToMV infection. Percent disease incidence ranged from 31.94 – 50.00% (Table 4) as compared to negative control (healthy tomato plants inoculated with buffer). Dantaya recoded highest percent disease incidence of 50.00%, followed by Danwarai and Dan Heka with 45.57% and 45.31%, respectively. While Hybrid Platinum recorded the lowest percent disease incidence of 31.94% (Table 4).

Disease reaction varied significantly among the genotypes. Danwarai, Dan Niko and Dan Heka were all susceptible to ToMV infection, while Dantaya and Platinum were tolerant to ToMV infection. Results of disease incidence showed significant differences, while there were no significant differences (P = 0.05) in the disease severity index among the five genotyped screened for ToMV infection (Table 6).

Table 4: Percentage disease incidence, disease severity index and disease reaction shown by five tomato genotypes against ToMV

Genotypes	**Percent disease incidence (%)**	**Severity index**	**Disease reaction**
Dantaya	50.00[a]	2.2	Tolerant
Danwarai	45.57[bb]	2.5	Susceptible
Dan Niko	45.31[b]	3.1	Susceptible
Dan Heka	35.86[c]	2.7	Susceptible
Platinum	31.94[d]	2.4	Tolerant
CV	**2.2281**	**2.2281**	
LSD	**3.6562**	**1.1881**	

Means with the same letter on the column are not significantly different ($P = 0.05$). CV = Critical Value of, LSD = Least Significant Difference.

Table 5: ANOVA table for disease incidence

Source	DF	Sum of squares	Mean square	F Value	P value
Variety	4	678.932	169.7331	42.02	<0.0001**
Error	10	40.390	4.039		
Total	14	719.323			

DF = degree of freedom, p = probability, ** = statistically significant

Table 6: ANOVA table for disease severity

Source	DF	Sum of squares	Mean square	F Value	P value
Variety	4	1.331	0.333	0.78	0.5629^{NS}
Error	10	4.265	0.427		
Total	14	5.597			

DF = degree of freedom, p = probability, NS = not significant

Discussion

Since reduction in the yield of tomato due to the infection of ToMV is usually up to 40% and may cause fluctuation in the price of the crop. Management measure is inevitable in the production of tomato, and this calls on the government and other relevant agencies to advocate the use of resistance varieties to farmers as a foremost management strategy. Plant breeders could develop or improve more tomato cultivars for ToMV resistance which is achievable using maker-assisted selection (MAS) to prevent the virus vector: whitefly (*Bemisia tabaci*) from infecting the plants.

According to Alishiri et al. (2013), viral diseases have been ranked as the most important constraint among tomato disease, basically because of the absence of enough information on them. Therefore, the status, incidence, and severity index of viral diseases in different Agro-ecological zone have not been fully researched. An experiment was conducted to screen tomato genotypes that are commonly cultivated in Kebbi State, Nigeria against *Tomato mosaic virus* (ToMV) infection. ToMV is a member of tobamoviruses causing serious yield losses in tomato production (Alishiri et al., 2013). Using resistant genotypes as management practice in plant viral diseases had been reported as cheapest, effective, and environment friendly approach (Strange & Scott, 2005).

ToMV symptoms appeared seven to ten days after inoculation on susceptible tomato genotypes. Symptom types as observed from the five tomato genotypes screened were light to dark green mottling on the leaves, stunted growth, and chlorosis were observed on leaves of the tomato plant when inoculated with ToMV. Light to dark green mottling is the most common symptom as observed by the researcher, followed by stunted growth and chlorotic leaf spot. Most of these symptoms were best observed at early weeks of inoculation and were generally mild and transient, which are like the symptoms described by earlier researchers (Najeebullah et al., 2017; Ayo-John & Odedara, 2017). The appearance of the symptoms at early weeks of inoculation revealed that tomato plants are more susceptible

to ToMV infection at vegetative growth stage. Dantaya and Hybrid Platinum restrict the virus to only inoculated leaves, while, in the three genotypes that are susceptible to ToMV the virus spread throughout the inoculated plants within seven to ten days after inoculation. This implies that, localized symptom of ToMV infection on tomato plants might be sign of resistance. Similar scenario has been reported by Smith & Murakishi, (1993) and Najeebullah et al. (2017). The restriction of virus to only the inoculated leaves by Dantaya and Hybrid Platinum can be attributed to tolerant traits possess by the genotypes.

All the genotypes screened were infected when inoculated with ToMV inoculum but with variation in their reaction to infection of ToMV. This is in line with the findings of Schuerger & Brown, (1997) who stated that ToMV has become an important viral disease of tomato worldwide. The result also supported the assertion of Lana & Adegbola, (1997) who stated that *Tomato mosaic virus* and *Tomato yellow virus* are the most serious viruses of tomato, as they can cause as much as 90% loss in fruit yield.

There were variations among the genotypes screened in symptom development, disease incidence and severity index. These findings are in line with those of Chitra et al. (2002), who have also reported similar variation in ToMV symptoms. Based on the percent disease incidence and mean severity index recorded from the research, it is observed that three tomato genotypes were susceptible to ToMV infection, while two were tolerant to ToMV infection. The tolerance of ToMV infection shown by the improved variety (Hybrid Platinum) as compared to the three local genotypes was in line with the works of De francq, (1989); Hansen, (1990) and Mwaulle, (1995) who found improved varieties of tomato to be of high resistance to diseases compared to local varieties. While Dantaya might be naturally tolerant to ToMV infection.

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