Journal of Agriculture and Environment Vol. 18 No. 1, 2022: 71-82
ISSN: 1595-465X (Print) 2695-236X (Online)

EVALUATION OF EIGHT TOMATO (*Solanum lycopersicum* L.) VARIETIES FOR RESISTANCE TO BACTERIAL SPOT INDUCED BY *Xanthomonas perforans*

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ABSTRACT

The study was conducted to evaluate eight tomato varieties for their genetic resistance to bacterial spot, caused by *Xanthomonas perforans*(McCulloch &Pirone)with a view to identifying those which may be used for genetic improvement. In 2021, ten seeds each of the varieties were sown per pot and later thinned to two in the screen house. At 35 days after sowing (DAS), seedlings were inoculated with the suspension *X. perforans*. Plants treated with sterile distilled water served as control. Treatments consisting of the eight inoculated tomato varieties were arranged in a completely randomized design with four repetitions. Three days after inoculation (DAI), records were taken on symptoms appearance and subsequently at 48 hours interval until 31 DAI. Disease severity on leaves was assessed at each interval and between 70-80 DAS, disease incidence and severity on fruit was evaluated. Disease progress curve was drawn and area under disease progress curve was calculated. Records on the severity on leaves showed significant difference ($P<0.05$) among the varieties evaluated in this study with the highest (38.9%) and the least (25%) on Tima and RomaVF respectively. The highest incidence and severity on fruits (100%, 32.5%) were recorded on Tima variety, which were significantly higher than those recorded on other varieties ($P<0.05$). The least incidence and severity (15.0%, 12.5%) on fruit were recorded on Roma VF. Area under disease progress curve showed that Tima recorded the highest AUDPC (765), followed by UC82B (756) while the least was recorded on RomaVF variety (501). The study revealed that the tomato varieties evaluated varied in their responses to bacterial spot, with Roma VF been the least susceptible, none of the varieties was resistant to the disease.

Keywords: Resistance; RomaVF; Tima; *Xanthomonas perforans*; AUDPC

INTRODUCTION

Tomato (*Solanum lycopersicum)*, a member of the family Solanaceae is one of the basic vegetable crops of major economic importance that ranks fourth among the leading world fresh vegetables after carrots, lettuce, and onions (FAOSTAT, 2019). Tomato is grown mostly for its edible tender and compression-sensitive fruits which can be consumed fresh in salads, cooked in sauces, soup and meat/fish dishes (Babarinsa and Ige, 2014). It also serves as raw materials for food industries by processing into value added products like juices, paste,

ketchup and canned products (Ajagbe *et al*., 2014). It is a great source of vitamin C, K, potassium and folate. It is also a major dietary source of the antioxidant lycopene, which is linked to many health benefits, including reduced risk of heart disease and cancer (Bjarnadottir, 2019). Tomato is a major food ingredient utilized by nearly every household in Nigeria, thus constituting an important component of the national food security. In 2019, over 180 million tonnes of tomatoes were produced globally, and Nigeria accounted for only 2 % (about 4 million) of the world total (FAOSTAT, 2019).

Pests and diseases pose significant threats to quality and yield of tomato in Nigeria, thereby preventing the yield potential of this essential food item from being realized. Bacterial spot was reported as one of the diseases causing devastating yield loss of tomato in Northwest Nigeria where the disease was found to be induced by *Xanthomonas perforans.* (Jibrin *et al.*, 2015; 2018). This disease has been reported to account for up to 52 % loss of fruit weight of tomato (Jones *et al*., 1986). Management strategies rely on the use of various chemical control and sanitary measures to minimize pathogen spread through contaminated seed. However, these strategies have been challenged by the emergence of tolerant strains among the bacterial population, as well as adverse effect the use of chemicals pose on human, animal, and environment (Potnis *et al*., 2015). Resistance in the host plant has been reported to be the most effective and eco-friendly means of managing this disease (Yu *et al.*, 1995; Blancard, 1997). But reports on the response of the available tomato varieties to bacterial spot are still inadequate.Therefore, this study aimed to evaluate some tomato varieties for genetic resistance to bacterial spot disease to identify tomatoes that can be used as breeding lines for genetic improvement.

MATERIALS AND METHODS

Experimental site

The study was conducted in the screen house of the Department of Crop Protection, Faculty of Agriculture Ahmadu Bello University, Samaru, Zaria, situated at 11º11′ N; 7º38′ E and 686 m above sea level in the Northern Guinea Savannah ecological zone of Nigeria. The mean annual rainfall of the study area is 986.5 mm, concentrated between May and October with a peak in August. The mean annual temperature of the area is 24˚C, with minimum of 18˚C and maximum 31˚C in January and April respectively (IAR, 2021).

Preparation of Tomato Plants and Inoculation

Seeds of eight tomato varieties (Plate I) namely: Samaru Local, Datterino (Tubeless), Roma VF, Cherokee (Bekin-iri), UC82B, Rio-Grande, Tima and Tropimech were sourced from agro seed dealers in Zaria and environs. These were sown in plastic pots of 20 cm diameter filled with heat sterilized soil. Ten seeds were sown per pot and later thinned to two plants. *Xanthomonas perforans* strain (NI7, with Genbank accession numbers: KJ938615.1, KJ938639.1, KJ938632.1, KJ938605.1, KJ938598.1, KJ938591.1, KJ938584.1) was obtained from the Bacteriology laboratory of the Department of Crop Protection, Ahmadu Bello University, Zaria. The bacterial culture was revived on nutrient (NA) medium and the bacterial concentration was determined by an optical density reading, OD_{600nm} = 0.1 as 1 x 10^8 Colony Forming Units per milliliter (CFU/ml). Performed titer plates were used to confirm CFU/ml density. At 35 days after sowing (DAS), seedlings were inoculated by spraying the abaxial and adaxial leaf surfaces with the bacterial suspension to runoff, using

a hand-held sprayer (Plate II). Treatments consisting of the eight inoculated tomato varieties and the untreated control were arranged in a completely randomized design (CRD) in the screen house with four repetitions. The plants were thereafter covered with polyethene sheets for 24 hours to ensure post inoculation period of high humidity for disease development.

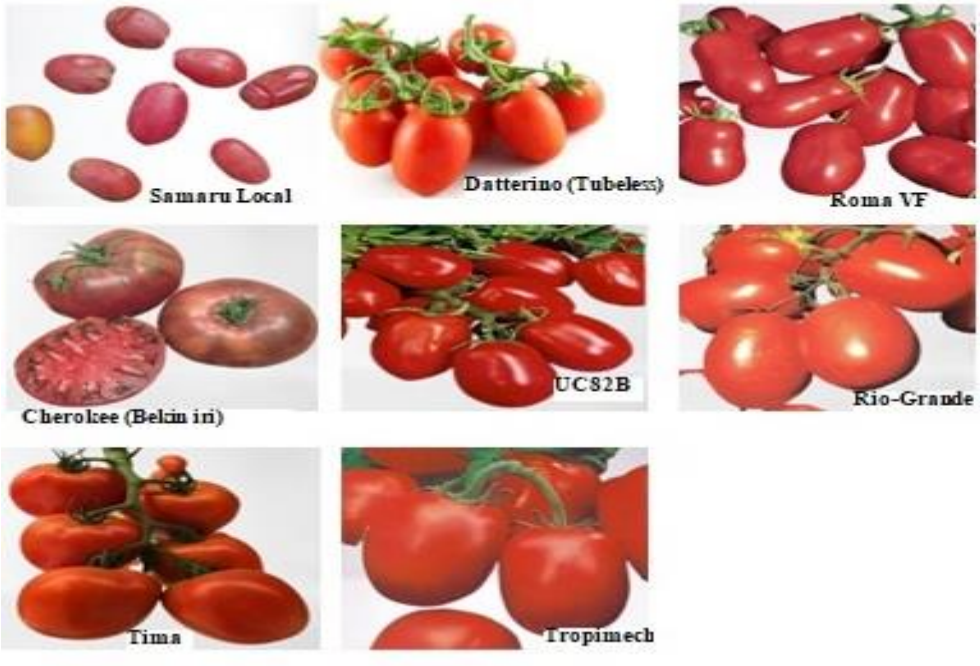

Plate I: Eight tomato varieties

Plate II: Inoculation of tomato plants

Evaluation of some Tomato Varieties for Resistance to Bacterial Spot

Three days after inoculation, plants were observed for symptoms appearance, records were taken and subsequently at 48 hours interval for 15 record days (i.e., 31 days after inoculation). Disease severity was assessed on 15 leaves per plant by scoring individual leaf on a modified disease scale of 0-6 (Horsfall and Barret, 1945) below: 0 = no disease symptoms visible on the plant;
1=1-15 % of plant affected;
2 = 16-30 % of plant affected;
3 = 31-45 % of plant affected;
4 = 46-60 % of plant affected;
5 = 61-75 % of plant affected
6 = above 75 % of plant affected.

Disease severity (DS) was calculated using the formula below:

$$DS = \frac{\text{Sum of individual disease scores}}{Total\ number\ of\ plants\ assessed\ x\ maximum\ score}\ x\ 100$$

Between 70 and 80 DAS, disease incidence on fruit was evaluated by expressing the number of fruits containing spots as percentage of the total number fruits assessed. Disease severity was assessed by scoring individual fruits on a 0-6 scale (Jones *et al.*, 2000) below.
0 = no lesion on fruit;
1 = 1-10 spots;
2 = 11-20 spots;
3 = 21-30 spots;
4 = 31-40 spots;
5 = 41-50 spots;
6 = above 50 spots.

Disease severity on fruits was calculated as described for leaves above.

Area under disease progress curve (AUDPC) was calculated using the formula described by Madden *et al.* (2007) as follows:

$$AUDPC = \sum_{i=1}^{n-1} \frac{Y_i + Y_{i+1}}{2} \times (T_{i+1} - T_i)$$

Where:

$Y_i = Disease\ severity\ (percent)\ at\ i^{th}\ observation$
$T_i = Time\ (days) at\ i^{th}\ observation$
$n = Total\ number\ of\ observations$
$i = 1, 2, 3 \ldots\ldots 15.$

Statistical Analysis

Data on disease incidence and severity were subjected to analysis of variance (ANOVA) using version 9.0 of the statistical analysis software (SAS, 2002) and means were

separated using the new Duncan multiple range test (NDMRT) at 5% level of probability. Disease progress curve (DPC) was drawn by plotting disease severity against time (period of infection). AUDPC was represented as chart.

RESULTS

Incidence and Severities of Bacterial Spot on Tomato Varieties

Bacterial spot symptoms consisting of small, brown circular spots, which are sunken on the upper leaf surface with yellow halos and slightly raised below were observed on the inoculated plants (Plate III). Table 1 shows the severity of bacterial spot on leaves of the tomato varieties evaluated in this study. Three days after inoculation (DAI), the highest disease severity was recorded on Tima tomato variety, which was statistically similar ($P>0.05$) to the recorded on Tropimech. The disease severity recorded on both Cherokee and Datterino were significantly higher ($P<0.05$) than the severity recorded on Samaru Local, UC82B, Rio Grande and Roma VF varieties. At 5 DAI, the highest severity recorded on Tima was significantly higher ($P<0.05$) than those recorded on all other varieties. This was followed by Tropimech, which was statistically at par ($P>0.05$) with the one recorded on both Cherokee and UC82B varieties respectively. The least disease severity was recorded on Samaru Local variety. Similarly at 7 DAI, the highest severity was recorded on Tima variety but was however statistically similar ($P>0.05$) to the recorded on UC82B, Tropimech and Cherokee. The least severity was recorded on Rio Grande variety. At 9 DAI, the highest severity was recorded on Tima variety which was significantly higher ($P<0.05$) than those recorded on all other varieties. This was followed by UC82B, Tropimech and Cherokee varieties, which were significantly higher ($P<0.05$) than the severity recorded on Datterino. The least severity of 10.4 % was recorded on SamaruLocal, Rio Grande and Roma VF. At 11 and 13 DAI, the highest disease severity, 22.9 % and 25.7 % respectively were recorded on Tima, which were significantly higher ($P<0.05$) than those recorded on other varieties. The least disease severity was recorded on Roma VF. From 15 DAI to 21 DAI, the highest disease severity was recorded on Tima which was significantly higher than other varieties except Tropimech and Cherokee at 17 and 21 DAI where the severities were statistically at par. However, at 23 DAI and 25 DAI, Cherokee, Tropimech, and Datterino recorded significantly higher ($P<0.05$) disease severities than other varieties, while at the same period, Roma VF recorded the least severities. At 27 DAI to 31 DAI, the highest severities were recorded on SamaruLocal and these were statistically similar ($P>0.05$) to those recorded on UC82B, Rio-Grande and Datterino at 27 DAI but significantly higher ($P<0.05$) than other varieties.

Symptoms on fruits consisted of small, scab-like brown spots, which appeared slightly raised as the spots increase in size (Plate IV). Table 2 shows the incidence and severity of bacterial spot on the fruits of the tomato varieties screened in this study. The highest incidence was recorded on fruits of Tima variety, which was significantly higher ($P<0.05$) than the incidence on other varieties. This was followed by Tropimech and Cherokee, which were at par ($P>0.05$) with what was recorded on UC82B. Highest disease severity recorded on Tima variety was significantly higher ($P<0.05$) than the severities on fruits of other varieties. This was followed by UC82B, Samaru Local, Rio Grande, Tropimech and Cherokee respectively, which were statistically at par ($P>0.05$). The least incidence (15.0 %) and severity (12.5 %) were recorded on the fruits of Roma VF.

Plate III: Tomato leaves showing symptoms of bacterial spot

Plate IV: Tomato fruit showing symptoms of bacterial spot

Table 1: Severity of bacterial spot on the leaves of eight tomato varieties

Varieties	Disease severity (%) at Days after Inoculation (DAI)														
	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31
Samaru Local	2.8^{c}	4.2^{d}	9.0^{b}	10.4^{d}	16.0^{c}	18.1^{c}	21.5^{c}	22.9^{bc}	25.7^{c}	29.9^{c}	31.3^{b}	35.4^{a}	37.5^{a}	37.5^{a}	36.8^{a}
UC82B	2.8^{c}	9.7^{b}	16.0^{a}	18.1^{b}	21.5^{a}	22.9^{ab}	25.7^{b}	29.9^{a}	31.3^{b}	35.4^{ab}	38.9^{a}	35.4^{a}	36.1^{abc}	37.5^{a}	36.1^{a}
Rio-Grande	2.8^{c}	4.9^{d}	8.3^{b}	10.4^{d}	15.3^{c}	18.8^{c}	20.8^{c}	22.2^{c}	25.7^{c}	30.6^{c}	31.3^{b}	35.4^{a}	36.8^{ab}	35.4^{ab}	35.4^{ab}
Roma VF	2.8^{c}	6.3^{cd}	9.0^{b}	10.4^{d}	16.0^{c}	18.1^{c}	20.8^{c}	22.0^{bc}	25.7^{c}	25.0^{d}	20.8^{c}	20.8^{c}	20.8^{e}	20.8^{e}	20.8^{e}
Tima	10.4^{a}	16.0^{a}	18.1^{a}	21.5^{a}	22.9^{a}	25.7^{a}	29.9^{a}	31.3^{a}	35.4^{a}	38.9^{a}	31.3^{b}	29.9^{b}	28.5^{d}	29.2^{d}	27.8^{d}
Tropimech	9.0^{a}	10.4^{b}	16.0^{a}	18.1^{b}	21.5^{a}	22.9^{ab}	25.7^{b}	29.9^{a}	31.3^{b}	35.4^{ab}	35.4^{ab}	36.8^{a}	34.0^{c}	34.0^{bc}	33.3^{b}
Cherokee	5.6^{b}	9.7^{b}	16.0^{a}	18.1^{b}	21.5^{a}	22.9^{ab}	25.7^{b}	29.9^{a}	31.3^{b}	35.4^{ab}	38.9^{a}	35.4^{a}	34.7^{bc}	34.0^{bc}	33.3^{b}
Datterino	5.6^{b}	8.3^{bc}	10.4^{b}	15.3^{c}	18.8^{b}	20.8^{bc}	22.2^{c}	25.7^{b}	30.6^{b}	31.3^{bc}	35.4^{ab}	35.4^{a}	36.8^{ab}	32.0^{cd}	30.6^{c}
SE	0.66	0.90	0.96	0.75	0.82	1.09	1.05	1.00	1.28	1.32	1.28	1.40	0.80	1.02	0.83

Means followed by the same letter along the same column are not statistically different using New Duncan's Multiple Range Test at P = 0.05.

Table 2: Incidence and severity of bacterial spot on fruits of some tomato varieties

Varieties	Incidence (%)	Severity (%)
Samaru Local	45.0^{c}	24.2^{bc}
UC82B	55.0^{bc}	25.8^{b}
Rio-Grande	45.0^{c}	24.2^{bc}
Roma VF	15.0^{d}	12.5^{d}
Tima	100.0^{a}	32.5^{a}
Tropimech	65.0^{b}	24.2^{bc}
Cherokee	65.0^{b}	24.2^{bc}
Datterino	50.0^{bc}	21.7^{c}
SE	5.59	0.98

Means followed by the same letter along the same column are not statistically different using New Duncan's Multiple Range Test at P = 0.05.

Disease Progress of Bacterial Spot on Tomato Varieties

Figure 1 shows the bacterial spot disease progress on the eight tomato varieties screened in this study. There was a progressive increase in disease severity across all varieties from the first day after symptoms appearance until between 19th and 25th day when the infection began to reduce. Thereafter, there was almost a constant disease severity as shown by a near flatted curve across all varieties.

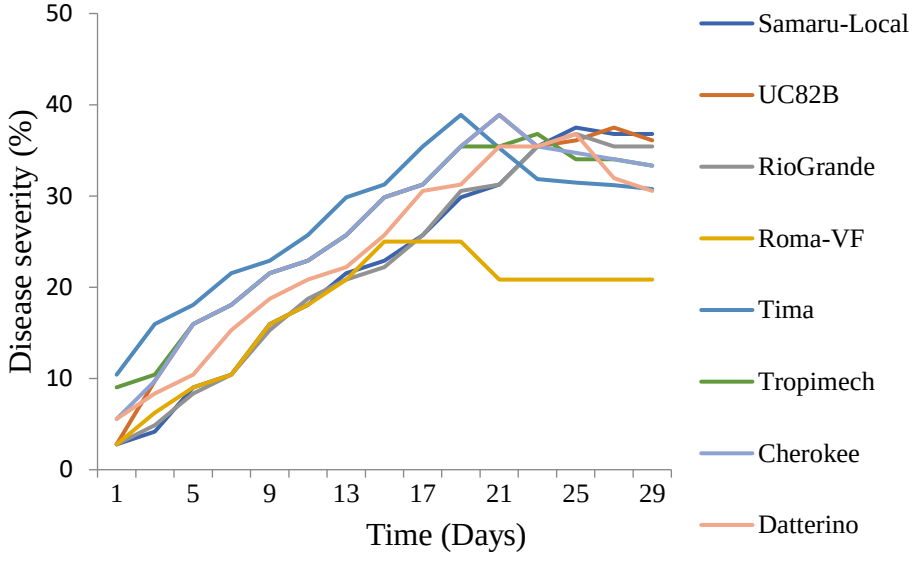

Figure 1: Bacterial spot disease progress curve on eight tomato varieties

The area under disease progress curve (AUDPC) is summarized in Figure 2. Tima recorded the highest AUDPC (765), followed by UC82B (756) and Cherokee (746). The least AUDPC was recorded on Roma VF variety (501).

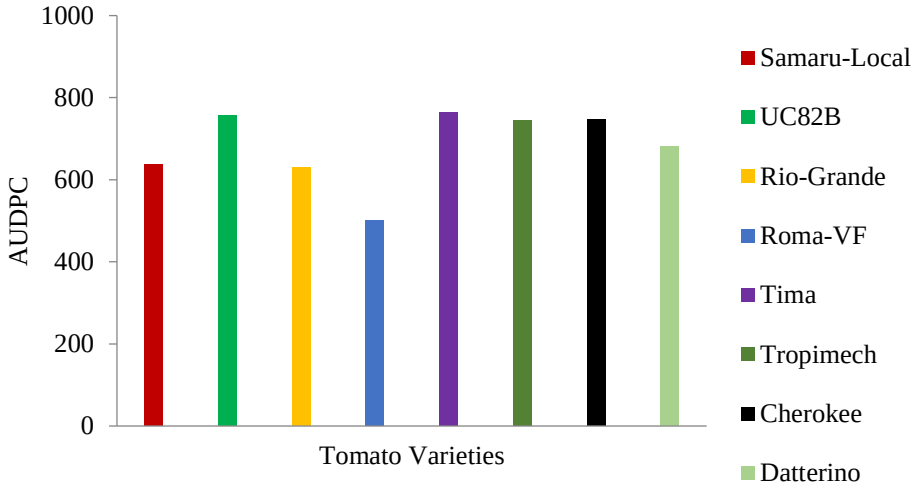

Figure 2: Area under disease progress curve

DISCUSSION

The disease incidence on fruits and severity on both leaves and fruits differed significantly among the varieties screened. These differences could be due to the differences in the genetic makeup of tomato genotypes as reported by Tokpah*et al.* (2019). The significantly higher ($P<0.05$) severity of the disease in UC82B than Roma VF and higher disease severity in Tima over Rio-Grande in this study agreed with the findings of Jimoh (2014), who reported a similar significant variation in disease severity in both dry and wet seasons trials. Though low severity of the disease was recorded on Roma VF, Rio-Grande and Samaru Local at the initial stage of infection, only Roma VF sustained such till the later stage infection on leaves as well as infection on fruits, and indication that Roma VF was the least susceptible of all the varieties evaluated in this study. This result differs from the work of Jimoh (2014), who reported that Rio-Grande was the least susceptible of all the varieties tested. However, in the same study, Roma VF was reported to have had the highest yield per plot of all the varieties evaluated, which is perhaps an indication of low severity of the disease. Tima maintained significantly higher severity than other varieties until 21 DAI when the severity began to drop. This might be due to early fruiting accompanied by shedding of leaves.

The area under disease progress curve (AUDPC) for the eight varieties also varied significantly. AUDPC has been used over the years for quantitative measurement of disease progress over time and to assess the level of disease resistance in crop cultivars (Jeger and Viljanen-Rollinson, 2001; Haynes and Weingartner, 2004). The lowest AUDPC recorded on Roma VF further confirmed it is the least susceptible of all the varieties evaluated in this study. Pandey *et al.* (2003) reported that area under disease progress curve negatively correlated to disease resistance and positively correlated to disease severity in tomato.

All the tomato varieties evaluated in this study responded differently, they were susceptible to bacterial spot. This result also corroborated with Jimoh (2014) who reported

that none of the tomato varieties evaluated was resistant to bacterial spot and bacterial speck. The *Bs2* gene, which occurs naturally in peppers, has been shown to confer resistance on pepper to bacterial spot, thus provide excellent disease control. This gene is however absent in tomatoes (Fry, 2016). Roma-VF was reported to be resistant to alternaria stem canker, fusarium wilt, fusarium wilt1, late blight, root-knot nematode, verticillium wilt and verticillium Wilt1 (Cramer, 2021). Rio Grande has been reported to be resistance to verticillium and fusarium wilt (Arim *et al.*, 2019), but susceptible to Bacterial Wilt and Stem Rot (Ambang *et al*., 2016). Similarly, Tropimech variety has also been reported to be susceptible to bacterial spot and bacterial wilt (Dossoumou *et al.*, 2021). Cherokee was reported to be resistant to bacterial speck, fusarium wilt1, fusarium wilt2, fusarium wilt3, rRoot-knot nematode, tomato spotted wilt virus and verticillium wilt (Cramer, 2021).

Genetic host resistance has been reported to be the most effective and eco-friendly method of managing this disease when sufficient genetic variation for resistance is available (Yu *et al*. 1995; Blancard, 1997). The uniqueness of resistance genes lies in the fact that they have evolved to respond to many different plant defence systems/mechanisms (Ritchie, 2007).

CONCLUSION

The study revealed that the tomato varieties evaluated varied in their response to bacterial spot. Though some of the varieties showed low disease severity at the initial stage of infection, only Roma VF sustained the low severity till the later stage of the infection, indicating that Roma VF was the least susceptible of all the varieties evaluated in this study. Tima recorded higher severity than other varieties up till 21 DAI, when the severity suddenly began to drop probably as a result of leaf shedding. None of the varieties was resistant to the disease, probably due to the absence of resistance genes such as *Bs2* in these varieties.

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