EVALUATION OF TOMATO CULTIVARS FOR RESISTANCE TO BACTERIAL WILT CAUSED BY *RALSTONIA SOLANACEARUM*

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ABSTRACT

Climate change impacts food production through altering the climatic suitability of agricultural areas for crops, pests and associated natural enemies. Bacterial wilt caused by *Ralstonia solanacearum* is a major constraint in tomato production leading to losses that amounts to 90% both in the open field and greenhouse conditions. The current methods used to manage the disease, are inadequate and pose both health and environmental risks. Host resistance has proved to be effective in management of bacterial wilt. The current study, evaluated 18 tomato varieties for resistance to *R. solanacearum* in the greenhouse. The experiment was laid out in a randomized complete block design with three replications and varieties were inoculated with 1×10^8 cfu/ml of *R. solanacearum.* Incidence of bacterial wilt was assessed eight days after inoculation, by counting the number of wilted plants to determine percentage disease incidence and disease index reactions (DI) of each cultivar. Severity was recorded using a disease rating score of 0-4 and used to determine area under disease progress curve (AUDPC). Severity and incidence of bacterial wilt was significantly different ($p < 0.001$) among the screened varieties. Six varieties including all non-hybrid varieties (Riogrande, Isisementi and Rionex) and three hybrid (F1) varieties Sifa F1, Danny F1 and Onyx F1were susceptible to bacterial wilt with disease index reaction (DI) of >30-70%. Among the F1 varieties, five varieties, Bravo F1, Kilele F1, Terminator F1, Big rock F1 and Ranger F1 had the lowest severity scores and incidences of bacterial wilt and were found to be tolerant to bacterial wilt with DI of 1-10%. Seven F1varieties were moderately tolerant and moderately susceptible with a DI of >10%-20% and >20-30% respectively. Despite the climatic risks and impacts on tomato production, tolerant cultivars can be recommended in the management of bacterial wilt for increased production and farmer incomes.

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Key words: Bacterial wilt, cultivars, disease index, screening of tomato varieties, severity

INTRODUCTION

Tomato (*Solanum lycopersicum L.,* syn. *Lycopersicon esculentum* Mill.*)* is an important vegetable grown in many areas of Kenya and other parts of East Africa (Sigei *et al.*, 2014). Tomato is used for food and most importantly as an income generating crop in peri-urban and high potential areas (Waiganjo *et al*, 2012; Mbaka *et al.*, 2013; HCDA, 2015). The nutritional composition of tomato includes vitamins A and C, potassium, fiber and lycopene and its regular usage is known to reduce the risk of cancer. The major tomato growing counties in Kenya include Kirinyaga, Kajiado, Bungoma, Meru, Kwale and Taita Taveta (Avedi *et al*, 2022). Rain fed agriculture and irrigation systems are the most practiced method of water application in tomato farming. In 2018, tomato production was 21.2 t/ha with an area harvested being 28.3 hectares yielding 599.458 tons (FAOSTAT, 2018).

Tomato farming is constrained by pests and diseases, among them bacterial wilt disease caused by *Ralstonia solanacearum*. The soil borne pathogen causes serious losses on crops due to its wide host range of more than 450 hosts and long survival period in the soil (Taylor *et al.*, 2011; Wicker *et al.*, 2007). Bacterial wilt was reported to cause up to 64% losses in open fields and 100% in greenhouses (Ochieng' *et al.*, 2016; Mbaka *et al.*, 2013). Spread of the pathogen has been effective through planting tomato in infected soil, using infected tomato seedlings, use of contaminated irrigation water, recycling of irrigation water and use of uncertified seeds in the farm as is often practiced by farmers to reduce production costs (Kanyua, 2018).

Management approaches against bacterial wilt such as cultural, chemical (Aslam *et al.*, 2017) and biological have been associated with challenges of inefficiency, health complications and increasing the production cost

(McManus *et al*., 2002; Perry and Wright, 2013; Latifah *et al.*, 2018). According to Yuliar and Toyota (2015) introduction of the use of tolerant varieties in plant disease management is the most effective eco-friendly method to control bacterial wilt disease. *Ralstonia solanacearum* resistant variety Hawii 7996 was developed by Wang *et al*. (2000) at the World Vegetable Centre. Kim *et al*. (2016) screened 285 tomato cultivars obtained from different parts of the world for resistance against bacterial wilt and found out that four genotypes were resistant. In Pakistan, evaluation of 30 varieties for resistance to the disease was also done by Aslam *et al*. (2017) and found out that only two varieties Lerica and Early king were resistant to bacterial wilt disease. In Kenya, tomato hybrid varieties are majorly imported by private seed merchants and less attention is given to tomato improvement in the country (Kathimba *et al*, 2018). Manani *et al*. (2020) evaluated six tomato varieties in the western region of Kenya and identified Heirloom Tall vine and Goliath pear varieties were tolerant and the rest were susceptible. Adoption of tolerant tomato varieties would potentially address the challenge of bacterial wilt with minimal use of synthetic fungicides (Scott *et al.*, 2004). This study aims to identify tolerant tomato varieties through screening of hybrid and non-hybrid varieties commonly grown in Kajiado County in order to mitigate the spread, damages and yield losses caused by bacterial wilt disease.

MATERIALS AND METHODS

Experimental site

This study was conducted in a greenhouse at Upper Kabete Campus, University of Nairobi in Kenya. The area is located at an Agro-ecological zone of upper midland zone three (UM3), on latitude 1º 15'South and longitude 36º 44' East at an altitude of about 1800 m above sea level (Jaetzold, 2006). The mean daily temperature in the greenhouse was 18 ºC between the period of April and July 2021.

Isolation of *Ralstonia solanacaerum*

The bacterium, *Ralstonia solanacearum* was isolated from diseased plants that showed typical symptoms of bacterial wilt in the laboratory using Kelman's Agar medium (2,3,5 Triphenyl Tetrazolium Chloride) consisting of 10g bacto-peptone (Difco), 1g casamino acids, 5ml glycerol, 15g bacto agar (Difco), 1000ml distilled water sterilized at 121 ºC for 15 minutes (Kelman, 1954). After isolation, a single colony was purified and pathogenicity was conducted on susceptible tomato variety to confirm its virulence.

Tomato varieties collection

Eighteen tomato cultivars comprising 15 hybrids and 3 non hybrids were obtained from seed companies and registered seed distributers. They included Stallion F1, Rambo F1, Kilele F1, Commando F1, Star F1, Danny F1, Bravo F1, Big Rock F1, Assila F1, Terminator F1, Gem F1, Sifa F1, Shanty F1, Onyx F1, Ranger F1, Isisementi, Rionex and Riogrande varieties. These varieties were selected because they were popularly grown by the farmers in Kajiado County based on a focused group discussion that was conducted in sixteen farms prior to this experiment.

Screening tomato varieties for resistance to *Ralstonia Solanacearum*

Tomato seedlings were raised in a greenhouse in germination trays containing hygro-mix obtained from the local agrovets shops. The seedlings were well watered three times a week. The screening of the eighteen tomato cultivars was carried out in the greenhouse. Polythene pots measuring 20.3 cm by 35.6 cm-by-35.6 cm were filled with pasteurized media composed of sand and soil in the ratio of 1:3 and each bag contained 6 kg of the media. The experiment was laid out in a randomized complete block design with three replications Aslam *et al*. (2017). Thirty-day old seedlings of each variety were transplanted and each variety was allocated four pots per block and three seedlings were transplanted in each pot. A concentration of 1×10^8 cfu/ml was adjusted using pour plate method (Singh *et al*., 2018). One week after transplanting, seedlings of each variety were inoculated with 30 ml of 1×10^8 cfu/ml bacterial suspension through soil drenching. Pricking was done with a sharp scalpel on the roots before drenching to increase chances of penetration of the pathogen.

Data Collection

Assessment of bacterial wilt severity

Severity was monitored for four weeks post inoculation and data was collected based on a modified key described by (Uwamahoro *et al.,* 2018; Aslam *et al.*, 2017). Severity rating score were: 0-No symptoms, 1-25% of all the leaves wilted, 2-50% of all the leaves wilted, 3- >75% all the leaves wilted, 4-Dead plant. Area under disease progress curve was calculated from the formula as described by

(Simko and Piepho 2012)

$$AUDPC = \sum_{i=1}^{k} [(SCBW_i + SCWB_{i+1})(t_{i+1} - t_i)]/2$$

Where SCBW is severity score of bacterial wilts, t is time and *i* is the *i*th observation

Assessment of bacterial wilt incidence

Disease incidence was done by counting the number of wilted plants per pot after every week for four weeks. Percentage of bacterial wilt incidence was determined in each treatment by the formulae:

WI= NPSWS/TNPPT *100

Where WI-wilt incidence, NPSWS-number of plants showing wilt symptoms and TNPPT-total number of plants per treatment (Ayana *et al*., 2011). The categorization of individual varieties to disease index reaction (Table I) was based on a scale as described by Dossoumou *et al*., (2021).

TABLE I- DISEASE INDEX REACTION

%Incidence of wilted plant	Disease Reaction
0%	Highly resistant (HR)
1-10%	Resistant (R)
>10-20%	Moderately resistant (MR)
>20-30%	Moderately susceptible (MS)
>30-70%	Susceptible (S)
>70%	Highly susceptible (HS)

Data analysis

Data was subjected to analysis of variance using R statistical software version 4.1.2 and means were separated by Tukey's Honest Significant test (HSD).

RESULTS

Response of tomato cultivars to Ralstonia solanacearum incidence and severity of bacterial wilt

Disease incidence and severity were assessed for four weeks post inoculation. The response of tomato varieties after inoculation with *Ralstonia solanacearum* showed significant differences in both incidence and severity rating scores. Five varieties namely Bravo F1, Kilele F1, Terminator F1, Big Rock F1 and Ranger F1 were tolerant to *Ralstonia solanacearum* with a disease index reaction (DI) range of 1-10% (Table I). 50% of the plant population in Isisementi, Rionex, Riogrande, Danny F1 and Sifa F1 varieties showed bacterial wilt symptoms by week 3 whereas Bravo F1, Big Rock F1, Kilele F1, Terminator F1 and Ranger F1 had less than 10% wilted plant population (Table I). Six varieties that include three non hybrid varieties Riogrande, Isisementi, Rionex and three hybrid varieties Sifa F1, Danny F1 and Onyx F1were susceptible to bacterial wilt with disease index reaction (DI) range of >30-70% while Assila F1, Commando F1 and Gem F1 varieties were observed to be moderately susceptible with (DI) of >20-30%. Stallion F1, Star F1, Shanty F1 and Rambo F1 were grouped as moderately tolerant with a D1 range of >20-30%. (Table I).

Out of the 18 varieties screened, non hybrid varieties Riogrande, Isisementi, Rionex and Sifa F1 began wilting as early as week 2 (Table I). These varieties were also noted to have significantly high area under disease progress curve (AUDPC) with a disease index reaction ranging greater than 30% to 70% and were grouped as susceptible to *Ralsonia solanacearum* (Table III). The mean severity scores of Bravo F1, KileleF1, Terminator F1 and Big rock F1 were observed to be less than 0.5 throughout the four weeks and these varieties had significantly low area under disease progress curve AUDPC of less than 50 (Table III and Figure 1).

TABLE II- PERCENTAGE INCIDENCE OF BACTERIAL WILT IN EIGHTEEN TOMATO VARIETIES

Variety	Week 1	Week 2	Week 3	Week 4	Cumulative mean	Disease Reaction
Assila F1	3.70a	23.81abcd	27.98abcd	37.43bcdef	24.07cde	MS
Big Rock F1	3.70a	4.17cd	8.33bcd	12.50ef	7.87fgh	R
Bravo F1	0.00a	0.00d	0.00d	12.50ef	3.24h	R
Commando F1	7.40a	20.83abcd	25.00abcd	45.83abcde	24.54cde	MS
Danny F1	3.70a	45.83ab	64.29a	62.50abcd	45.37a	S
Gem F1	4.17a	16.67abcd	29.17abcd	41.67bcde	26.39cd	MS
Isisementi	26.85a	52.98a	61.90a	79.17a	56.48a	S
Kilele F1	0.00a	4.17cd	4.17cd	4.17f	4.17h	R
Onyx F1	11.11a	19.91abcd	43.98abc	54.17abcd	31.48bc	S
Rambo F1	0.00a	4.7cd	13.10bcd	26.79def	11.57efgh	MR
Ranger F1	3.70a	4.76cd	9.52bcd	14.29ef	7.41gh	R
Riogrande	18.98a	50.00a	50.00ab	73.21ab	52.31a	S
Rionex	22.22a	43.98abc	50.00ab	72.69abc	45.37a	S
Shanty F1	3.70a	12.50abcd	24.07abcd	37.50bcdef	18.06defg	MR
Sifa F1	16.20a	51.43a	51.43ab	56.19abcd	43.52ab	S
Stallion F1	0.00a	8.33bcd	16.67bcd	30.36def	13.43defgh	MR
Star F1	7.40a	13.10abcd	21.43abcd	35.71cdef	20.83cdef	MR
Terminator F1	0.00a	4.77cd	4.76cd	8.93ef	4.17h	R
Mean	7.38	21.22	28.1	39.2	24.5	
CV (%)	135.05	62.1	50.7	31	118.1	
LSD	30.65	40.6	43.8	37.4	13.37	
Pvalue<0.05	0.05	<0.001	<0.001	<0.001	<0.001	

Means in the same column followed by the same letter are not significant. Means were separated using Tukey Honest significant Test (P<0.05).

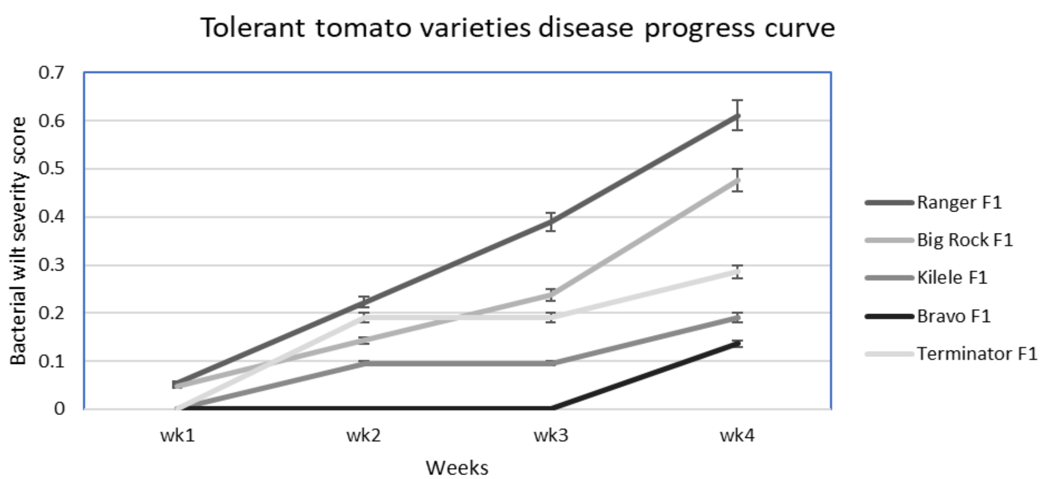

Figure 1: Response of bacterial wilt disease over time

TABLE III -SEVERITY RATING SCORES OF BACTERIAL WILT AND AREA UNDER DISEASE PROGRESS CURVE OF EIGHTEEN TOMATO VARIETIES EVALUATED IN THE GREENHOUSE EXPERIMENT

	Week					
Variety	**1**	**2**	**3**	**4**	**Cumulative mean Severity**	**AUDPC**
Assila F1	0.15a	0.65abc	0.79abcd	1.20bcdefg	0.65cde	107.30defg
Big rock F1	0.04a	0.13bc	0.21cd	0.44efg	0.19gh	31.50gh
Bravo F1	0.00a	0.00c	0.00d	0.12g	0.04h	3.50h
Commando F1	0.11a	0.55abc	0.96abcd	1.54abcdefg	0.77cd	135.30cdef
Danny F1	0.04a	0.85abc	1.80ab	2.33abc	1.24b	191.30bcd
Gem F1	0.08a	0.25bc	0.72abcd	1.29bcdefg	0.57cdef	94.50defgh
Isisementi	0.42a	1.83a	2.25a	2.88a	1.82a	305.50a
Kilele F1	0.00a	0.07bc	0.08cd	0.17g	0.08h	14.00gh
Onyx F1	0.11a	0.48abc	1.17abcd	1.76abcdef	0.86c	155.20cde
Rambo F1	0.00a	0.23bc	0.32bcd	0.68defg	0.30efgh	51.30fgh
Ranger F1	0.04a	0.17bc	0.32bcd	0.50efg	0.25fgh	39.70fgh
Riogrande	0.31a	1.20abc	1.39abcd	1.93abcde	1.22b	215.80abc
Rionex	0.56a	1.42ab	1.63abc	2.54ab	1.51ab	262.50ab
Shanty F1	0.11a	0.35bc	0.64bcd	0.96cdefg	0.51defg	88.70defgh
Sifa F1	0.38a	1.34ab	1.80ab	2.07abcd	1.32b	212.30abc
Stallion F1	0.00a	0.25bc	0.42bcd	0.79defg	0.36efgh	59.50efgh
Star F1	0.04a	0.51abc	0.55bcd	1.08bcdefg	0.54cdefg	88.70defgh
Terminator F1	0.00a	0.19bc	0.19cd	0.27fg	0.14h	25.70gh
Mean	0.13	0.58	0.84	1.25	0.689	115.6
C.V (%)	139.5	75.6	59.8	38.9	183	53.9
LSD	0.58	1.36	1.55	1.5	0.3583	103.49
P value(p<0.05)	0.0103	<.001	<.001	<.001	<.001	<.001

Means in the same column followed by the same letter are not significant. Means were separated using Tukey Honest significant Test (P<0.05).

DISCUSSION

Using resistant varieties is a simple, safe and economical strategy of managing bacterial wilt. This study identified hybrid varieties Kilele F1, Terminator F1, Bravo F1, Big rock F1 and Ranger F1 as tolerant and non hybrid varieties Riogrande, Rionex and Isisementi as susceptible to *Ralstonia solanacearum*. A similar study in Kenya by Manani *et al.* (2020) screened six varieties against *Ralstonia solanacearum* and found that Heirloom Tall vine and Goliath pear hybrid were resistant to bacterial wilt. Riogrande variety was noted to be susceptible to *R. solanacearum* in studies conducted by (Aslam *et al.,* 2017 and Kathimba *et al.,* 2018). In addition, Dossoumou *et al.* (2021) evaluated 21 tomato genotypes against *R. solanacearum* and found out that only one genotype Cobra 26 was resistant to bacterial wilt disease. In the current study, expression of bacterial wilt incidence and severity was lower during the entire period of the experiment. Similar observations were made by Abebe *et al.* (2020) who evaluated 27 varieties and found out low means of severity and incidence and concluded that the varying levels of severity and incidence of bacterial wilt could be ascribed to different genetic make-up of the varieties. Manani *et al.,* (2020) and Vanitha *et al.* (2009) concluded that low mean severity and incidence in the resistant tomato genotypes could be due to the production and activity of phenylalamine ammonia liase (PAL) and Polyphenol oxidase (PPO). PAL and PPO are found to be responsible for production and oxidation of phenolic compounds that enhance plant defense system against *R. solanacearumas* as reported by (Vanitha *et al*, 2009). In this study, five cultivars showed tolerant to bacterial wilt disease based on the scale described by (Dossoumou *et al.,* 2021). Grimault *et al.* (1995) and Singh (1961) reported that resistance was due to certain single dominant genes and recessive genes that are present in the plant host genome. Based on Oliveria *et al*. (1999) additive effects of the genes can be attributed to resistance against bacterial wilt disease. The susceptible varieties expressed wilting early and succumbed easily due to the pathogen colonization in the vascular tissues that block water passage. According to Kim *et al*. (2016) and Nakoha *et al.* (2000), the resistance exhibited to tomato varieties against bacterial wilt could be due to thickened pit membranes that stops the movement of *R. solanacearum* in the xylem vessels which was not the case in susceptible varieties which they observed to have high concentration of *Ralstonia solanacearum* in the primary and secondary xylem. Similarly, Grimault and Prior (1993) described that the resistance feature was linked with thickened pits of the plant that halted the establishment of *R. solanacearum* in the vascular system.

In this current study, susceptibility and tolerance of tomato varieties to *R. solanacearum* can be linked with wilt development. In tolerant varieties, wilts developed at week four while susceptible varieties at week 2 after inoculation. Similar findings were reported by Dossoumou *et al.*, 2021. The stet experiment was conducted during the cold season of May to June, general bacterial wilt symptoms expression was slower and this is in line with the results by Hanson *et al.* (1996) who reported that bacterial wilt resistance is influenced by environmental conditions. Bittner *et al*. (2016) reported that low temperature of between 15 °C to 20 °C delays bacterial wilt disease expression symptoms.

CONCLUSION

Out of the 18 varieties popularly grown in Kajiado county, majority are susceptible to *Ralstonia salanacearum* without five found to be tolerant. Non hybrid varieties Riogrande, Rionex and Isisementi performed poorly and succumbed to bacterial wilt disease. Therefore, the five tolerant tomato varieties namely Kilele F1, Terminator F1, Bravo F1, Big rock F1 and Ranger F1 can be recommended with the integrated diseases management strategies to farmers in areas that are prone to bacterial wilt disease in Kajiado County for up-scaling tomato production.

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