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Research Article
Biovar 2 of *Ralstonia solanacearum* Species Complex Causes Tomato Bacterial Wilt Disease in Tanzania

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Abstract

Background and Objective: Bacterial wilt disease (BWD) caused by complex species of *Ralstonia solanacearum* (RSSC) has been categorized as one of the most significant plant diseases in the world. It is a serious problem of tomato and causes significant economic losses of tomato in Tanzania. The purpose of this study was to determine biovars of RSSC causing tomato BWD in Tanzania. **Materials and Methods:** Tomato stems showing typical symptoms of BWD were collected from main agro-ecological regions and were characterized by pathological and carbohydrate oxidation tests. The least significance difference (LSD) procedure was used for mean separation ($p = 0.05$) of disease incidence and severity. **Results:** A total of 29 out 40 RSSC isolates from infected tomato stems produced typical colonies of RSSC on triphenyl tetrazolium chloride medium out of which 19 (52%) were pathogenic on tomato variety Tanya. Carbohydrate oxidation test showed that most (90%) predominating isolates in main agro-ecological regions belong to biovar 3 while the rest (10%) belong to biovar 2 and prevail in the southern zone of Tanzania. This is the first report of prevalence of biovar 2 of Ralstonia in Tanzania and suggests a recent introduction of biovar 2 in tomato fields in Tanzania. **Conclusion:** Biovar 2 of RSSC is reported for the first time to cause tomato bacterial wilt disease in Tanzania. This alerts plant health regulators to embark on necessary phytosanitary measures to prevent further spread and/or introduction of the disease considering its quarantine status in different countries.

Key words: Carbohydrate oxidation, pathogenicity, isolates, TTC medium, virulence

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most important vegetable crop for improving nutrition and income globally[1]. Nutritionally, tomato contains a substantial amount of protein and essential vitamins, minerals and trace elements to enhance human diet[2,3]. The bacterial wilt disease (BWD) caused by *Ralstonia solanacearum* species complex (RSSC)[4] significantly affect production of crops such as tomato worldwide[1,5]. RSSC is a soil-borne gram-negative bacterium with the ability to infect about 450 plant species from 54 different botanical families such as tomato, potato, eggplant and many native plant species causing huge yield losses[6]. Losses caused by BWD are known to be enormous but cannot be accurately estimated as it varies with cultivar, soil, environment and bacterial isolates. Approximately more than 80 countries are affected by BWD which causes economic loss of more than $1billion annually[7,8]. The bacterium normally invades plant roots from the soil through wounds or natural openings, colonizes the intercellular space of the root cortex, vascular parenchyma and eventually enters the xylem vessel and spreads up into the stem and leaves. Infected plants stunt, wilt and die rapidly while young leaves remain green.

Various management strategies are used to manage BWD[1]but the diversity of RSSC challenges effectiveness of the management strategies[9]. Use of resistant cultivars for instance is considered the main management strategy but the stability of resistance is highly affected by the biology of RSSC[10]. Being a species complex plant pathogen, Ralstonia has a broad pathological, genetic and physiological diversity. For example by using host range, pathological, biochemical and molecular descriptors, RSSC was classified into races[11-13], virulent/avirulent and pathogenic/non-pathogenic, biovarsor phylotypes[14-20].

New strains of RSSC may be introduced in uninfected environments from various sources such as planting materials, water, soil, insects and nematodes[21]. In 2016 and 2017, tomato growing seasons, two outbreaks of bacterial wilt disease occurred in Tanzania. Tomato plants in different fields were reportedly wilting without yellowing at flowering growth stage[22]. Severe symptoms with complete plant death were commonly observed. Recently, there has been a regular outbreak of bacterial wilt disease in tomato fields in Tanzania. However, there is little information on the characteristics of the causative pathogens. The causative agent of tomato bacterial wilt disease in the coastal zone of Tanzania belong[23] to RSSC biovar 3. However, this information is limited in terms of its coverage and management, therefore this study was undertaken to determine Biovars of RSSC causing tomato BWD in Tanzania to alert the plant quarantine officers to embark on necessary phytosanitary measures.

MATERIALS AND METHODS

Survey and sampling: A field survey was conducted from September, 2017 to December, 2018 to determine biovars of RSSC causing BWD in Tanzania. Purposive sampling was adopted by selecting ten districts namely Arumeru, Babati, Manyoni, Nyamagana, Kilolo, Temeke, Chake Chake, Mbeya Urban, Kibondo and Kongwa to cover the major agro-ecological zones of Tanzania (Fig. 1). A multistage random sampling procedure was used in selecting the wards, villages and the farms. Four wards were selected at random in a district and five farmers' fields were sampled from each village. Within the farm, five plots of 50 m^2 were sampled by critically observing symptoms of BWD. For a quick field diagnosis, the streaming of milky white masses of bacterial cells distinguished BWD caused by RSSC from vascular wilts caused by fungal pathogens and nematodes.

Assessment of disease incidence and severity: Data on wilt incidence were recorded in five plots within a field by counting number of plants with BWD symptoms in a plot[15,22]. Then the percent wilt incidence was calculated by the following equation:

$$\text{Wilt incidence } (\%) = \frac{\text{Mean of wilted plants in a field}}{\sum \text{plants assesed in a field}} \times 100$$

Disease severity was recorded based on a 1-5 scale[15,22]. Briefly, 1: No symptom, 2: Top young leaves wilted, 3: Two leaves wilted, 4: Four or more leaves wilted and 5: Plant dies.

Average disease severity per district was calculated using a equation:

$$\text{Severity } (\%) = \frac{5A + 4B + 3C + 2D + E}{5N} \times 100$$

where, A is the number of plants on scale 5, B is the number of plants on scale 4, C is the number of plants on scale 3, D is the number of plants on scale 2, E is the number of plants on scale 1 and N is the total number of plants evaluated. Twenty samples of tomato stems with typical bacterial wilt symptoms were collected from each district (200 samples in total) and sent to the laboratory for the isolation and characterization of RSSC.

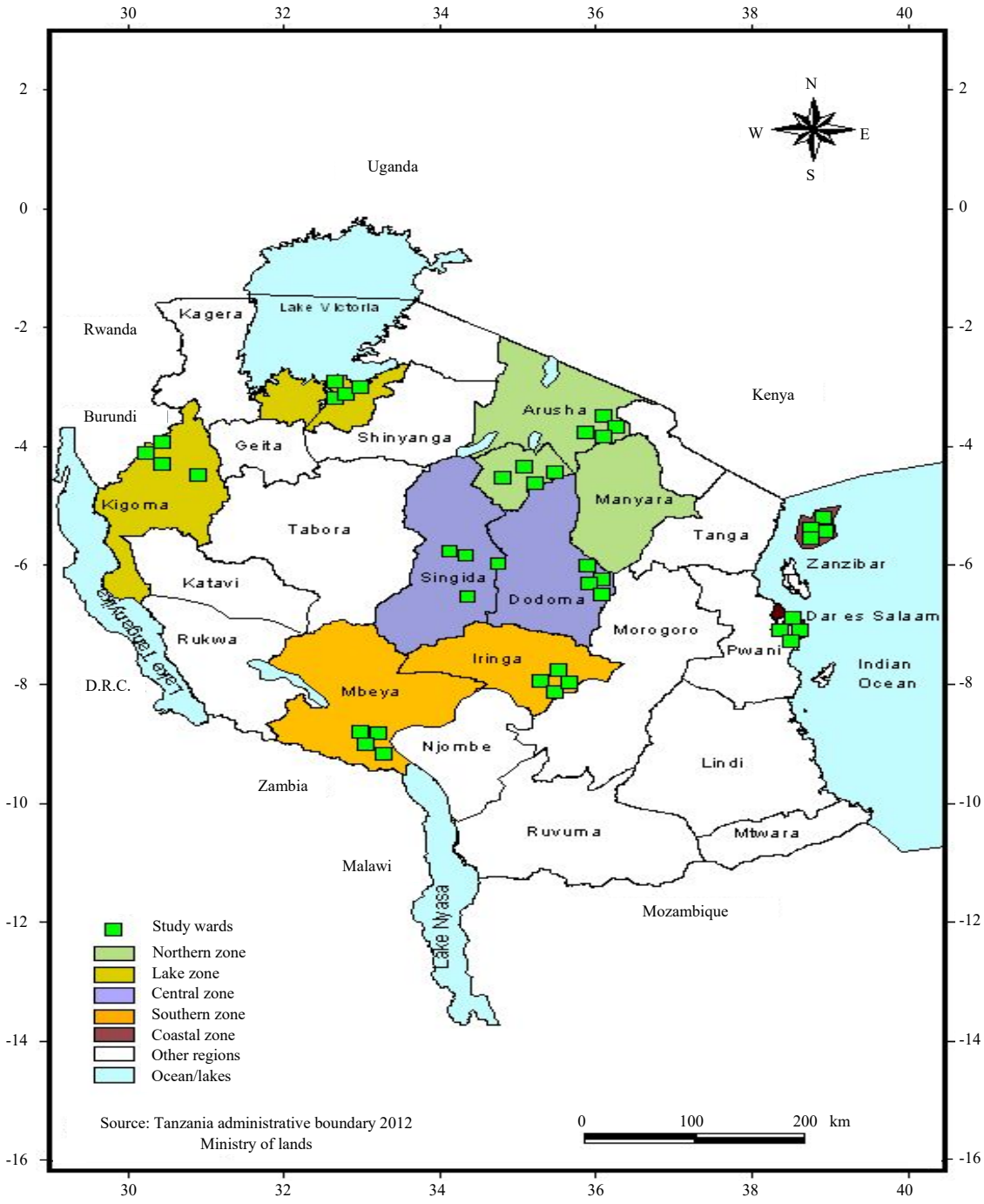

Fig. 1: Study areas visited during the bacterial wilt disease survey in Tanzania

Preparation of bacterial suspension: From the 200 samples of wilted tomato stems originating from the agro ecological zones, eight from each zone were randomly picked to represent a ward in the study area and coded accordingly. A total of forty samples were thus selected for the biovar (s) determination. Selected samples were washed with running water from the tap to remove soils and then immersed in 70% ethanol for 2-3 min to remove any saprophytic or epiphytic bacteria from stem surfaces. Surface-sterilized stems of each isolate were macerated in sterile water to obtain a bacterial suspension.

Table 1: Protocol adopted for selected pathological and biochemical descriptors

				Biovar						
					2					
Approach	Method	Component	Descriptor	1	2A	2T	3	4	5	References
Pathological	Cultural	TTC medium	Colony appearance	Virulent: Pink center, white margins Avirulent: Red colony with off white margins						Kelman[14]
	Pathogenicity test	Seedlings bioassay	Wilt symptoms	+	+	+	+	+	+	He *et al.*[13], Horita and Tsuchiya[24], Janse and Ruissen[15]
Carbohydrate oxidation test	Disaccharides	Dextrose	Color change	Y	Y	Y	Y	Y	Y	He *et al.*[13], Hayward[16], McLaughlin and Sequeira[25], Kumar *et al.*[27]
		Salicin	Color change	G	G	G	G	G	G	
		Lactose	Color change	G	Y	Y	Y	G	Y	
		Maltose	Color change	G	Y	Y	Y	G	Y	
		Cellobiose	Color change	G	Y	Y	Y	G	Y	
		Trehalose	Color change	Y	G	G	Y	Y	Y	
		D-ribose	Color change	Y	G	Y	Y	Y	Y	
		D-trehalose	Color change	Y	G	Y	Y	Y	Y	
		L-tryptophan	Color change	Y	G	Y	Y	Y	Y	
	Sugar alcohols	Mannitol	Color change	G	G	G	Y	Y	Y	
		Sorbitol	Color change	G	G	G	Y	Y	G	
		Dulcitol	Color change	G	G	G	Y	Y	G	

+: Pathogenic, G: Greenish, Y: Yellow

Protocol development: The virulence, pathogenesis and biovars of RSSC isolates were tested by using the pathological and biochemical approaches as shown in Table 1. Based on virulence, isolates of RSSC can be characterized as virulent which are those isolates which produced typical pink center colonies with white margins while avirulent isolates produce red off white margins colonies. Through the pathogenicity test, the pathogenic isolates of RSSC cause wilting of tomato plants as opposed to the non-pathogenic isolates. In addition, oxidation of different types of carbohydrates is used to characterize isolates of RSSC into biovar(s). This is indicated by a color change of medium from green to yellow after inoculating with the isolate of RSSC.

Preparing media, disaccharides and sugar alcohols solution: Solution of TTC and mineral media, disaccharides and sugar alcohols were prepared as detailed in Table 2. D-Trehalose, L-Tryptophan and D-Ribose were included in the test to differentiate between the sub-phenotypes 2A and 2T of biovar 2. Negative controls were set up without any carbohydrate, where salicin and sterile water were used. Dextrose, the most commonly utilized carbohydrate by all biovars was included as a positive control.

Identification of RSSC isolates by using pathological descriptors

TTC medium: The bacterial suspension was streaked into the TTC medium agar plates and incubated at 28°C for 48 h. Single growing colonies were picked and sub-cultured onto a fresh medium to obtain pure cultures. Identification of presumptive colonies were made when typical colonies of virulent showed a characteristics light red colored center and whitish margin while those of avirulent isolates were smaller, off-white and non-fluidal[14]. The virulent isolates were selected for the subsequent experiment.

Pathogenicity test: Seeds of tomato variety called Tanya, a commonly cultivated but susceptible variety to BWD in Tanzania were used in this experiment. The seeds were sown in a 1 L pot filled with forest soil and sand at 3:1. The pots were placed in green-house at the average temperature of 25°C and watering were conducted after every other day. After two weeks, seedlings were inoculated with the inoculum of the isolates which had presumptive RSSC colony appearance on TTC medium[15]. Three seedlings in replicate were inoculated with suspension of isolates at the rate of 10^9 CFU mL^{-1} with punctures made with a sterile needle in stem between two cotyledons. Three replicates per bacterial suspension were used so that a total of nine seedlings were inoculated with each bacterial isolate. Seedlings inoculated with sterile water were included as negative control. Prior to inoculation, seedlings were not irrigated for 24 h.

The experiment was designed in a completely randomized design with three replications and held at 25°C in screen-house. Development of wilting symptoms was observed and severity was recorded weekly[13,24] on a 0-5 scale where, 0: No symptoms, 1: Leaf above inoculation wilted, 2: Two leaves wilted, 3: Three leaves wilted, 4: Four or more

Table 2: Preparation of media, disaccharides and sugar alcohols solution

Medium	Ingredients	Amount	Procedure
TTC	Casein	1.0 g	Ingredients 1-3 were dissolved in DW
	Bacteriological agar	15.0 g	Autoclaving at 121°C for 15 min
	MR-VP medium	17.0 g	Filter-sterilized TTC salt were added to the
	Distilled water (DW)	1000 mL	medium after cooling to 45-50°C
	TTC salts	50 mg L^{-1}	Medium was poured into sterile plates
Mineral medium	Ammonium dihydrogen phosphate ($NH_4H_2PO_4$)	1.0 g	Ingredients 1-6 were dissolved in DW Medium was boiled with constant stirring
	Potassium chloride (KCl)	0.2 g	pH of the medium was raised to 7.0 by drop wise
	Magnesium sulphate ($MgSO_4.7H_2O$)	0.2 g	addition of 1.0 N sodium hydroxide
	Peptone	1.0 g	Medium was divided into container with 90 mL of
	Bromothymol blue	0.03 g	medium
	Agar	3.0 g	Autoclaved at 121°C, 15 psi for 20 min
	DW	1.0 L	
	Sodium hydroxide (NaOH)	1.0 N	
Disaccharides solution	Cellobiose	1.0 g	Each disaccharide or sugar alcohol was dissolved
	Lactose	1.0 g	in 10 mL DW separately
	Maltose	1.0 g	Filter-sterilize by 0.22 µm filters
	Salicin	1.0 g	
	Dextrose	1.0 g	
	D-trehalose	1.0 g	
	L-tryptophan	1.0 g	
	D-ribose	1.0 g	
Sugar alcohols solution	Dulcitol	1.0 g	
	Sorbitol	1.0 g	
	Mannitol	1.0 g	

leaves wilted and 5: Plant died. Score of BWD severity were related with the BWD severity recorded in the field by computing a correlation coefficient by using CoStat data analysis software.

When typical symptoms were observed, re-isolation of the bacteria was made on TTC medium. After 48 h incubation at 28°C, presence of RSSC looking colony was examined and recorded. Isolates with RSSC colony characteristics were subjected to biovar(s) determination.

Identification of biovars of RSSC by carbohydrates oxidation test

Preparing inoculum: Ten isolates identified as the most virulent according to Koch's rule (culturing and pathogenicity test) were used. Single colony of each isolate was streaked on TTC medium and incubated for 48 h at 28°C[14]. A loop-full of each isolate was taken and mixed into 1 mL sterile water in a 2.0 mL centrifuge tube (Eppendorf) to make suspension containing about 10^9 CFU mL^{-1}.

Allocation of disaccharides and sugar alcohol solution into mineral medium, inoculation and data collection: After autoclaving, the media was cooled to 65°C. Ten milliliter of carbon source was each mixed with 90 mL of the mineral medium to make sugar/alcohol amended medium. Three hundred microliter of sugar/alcohol-amended medium were dispensed in each 2.0 mL centrifuge tube and inoculated with 10 µL of suspension of each isolate then incubated at 28°C. Observations were recorded on changing pH as indicated by color change for 7 days incubation[25].

Statistical analysis: Data of disease incidence and severity were pooled together by calculating the average of incidence and severity of each ward in a district, this resulted into forty samples which were thereafter subjected to the analysis of variance (ANOVA). The mean separation were carried out by using the least significance difference (LSD) procedure at $p = 0.05$. The Costat data analysis software program facilitated analyses.

RESULTS

BWD incidence and severity in main agro-ecological zones of Tanzania: The results indicated that on average bacterial wilt disease was present in 55% of the visited farmers' tomato fields. The mean of disease incidence and severity in the study area ranged from 12-37 and 22.47-89.43%, respectively (Table 3).

Virulence and pathogenesis of isolates of RSSC: Results showed that out of forty isolates of RSSC evaluated for virulence from different zones29 produced typical colonies of

Table 3: Bacterial wilt disease incidence and severity in the main agro-ecological zones of Tanzania

Zones	Code of the ward	Incidence (%)	Severity (%)
Northern	NAA1	23.89^{b}	70.97^{fg}
Northern	NAA2	21.02^{b}	48.19^{c}
Northern	NAA3	18.89^{a}	44.27^{bc}
Northern	NAA4	24.11^{b}	63.19^{ef}
Northern	NMB1	25.80^{b}	68.99^{f}
Northern	NMB2	20.67^{a}	51.33^{de}
Northern	NMB3	21.55^{b}	40.57^{bc}
Northern	NMB4	23.52^{b}	66.67^{f}
Southern	SIK1	29.73^{c}	82.40^{a}
Southern	SIK2	32.47^{c}	77.38^{g}
Southern	SIK3	30.11^{c}	82.04^{h}
Southern	SIK4	37.00^{d}	89.43^{i}
Southern	SMM1	26.03^{bc}	74.00^{fg}
Southern	SMM2	23.67^{b}	64.43^{ef}
Southern	SMM3	24.02^{b}	60.57^{ef}
Southern	SMM4	23.79^{b}	59.33^{e}
Central	CSM1	24.11^{b}	54.00^{de}
Central	CSM2	21.50^{b}	52.31^{de}
Central	CSM3	23.44^{b}	69.00^{f}
Central	CSM4	20.80^{b}	53.09^{de}
Central	CDK1	23.06^{b}	59.19^{e}
Central	CDK2	17.02^{ab}	39.31^{b}
Central	CDK3	22.78^{b}	60.33^{ef}
Central	CDK4	22.50^{a}	69.65^{f}
Lake	LMN1	22.96^{b}	57.49^{e}
Lake	LMN2	21.47^{b}	60.05^{ef}
Lake	LMN3	20.67^{b}	54.10^{de}
Lake	LMN4	12.96^{a}	22.47^{a}
Lake	LKK1	20.42^{ab}	50.33^{de}
Lake	LKK2	18.99^{ab}	30.63^{b}
Lake	LKK3	12.78^{a}	22.65^{a}
Lake	LKK4	23.49^{b}	57.91^{e}
Coastal	CZC1	12.00^{a}	42.16^{b}
Coastal	CZC2	13.63^{a}	26.89^{ab}
Coastal	CZC3	24.09^{b}	73.07^{fg}
Coastal	CZC4	21.20^{b}	45.80^{cd}
Coastal	CDT1	23.07^{b}	23.16^{a}
Coastal	CDT2	22.00^{b}	61.63^{ef}
Coastal	CDT3	12.63^{a}	37.97^{b}
Coastal	CDT4	21.99^{b}	57.02^{e}
Mean		21.45	51.47
F-test		*	**
LSD		4.07	3.19

*p≤0.01, **p≤0.05, means with the same letter(s) within the column are not significantly different based on LSD (p = 0.05)

Table 4: Virulence and pathogenesis reaction of isolates of *Ralstonia solanacearum* species complex (RSSC)

Zones	Isolate code	Virulence	Pathogenesis	Koch's rule
Northern	NAA1	+	+	+
Northern	NAA2	+	-	-
Northern	NAA3	-	-	-
Northern	NAA4	+	+	+
Northern	NMB1	+	+	-
Northern	NMB2	+	+	-
Northern	NMB3	+	-	-
Northern	NMB4	-	-	-
Southern	SIK1	+	+	+
Southern	SIK2	+	-	-
Southern	SIK3	+	+	-
Southern	SIK4	+	+	+
Southern	SMM1	-	-	-
Southern	SMM2	+	+	-
Southern	SMM3	+	-	-
Southern	SMM4	-	-	-
Central	CSM1	+	-	-
Central	CSM2	+	+	-
Central	CSM3	-	-	-
Central	CSM4	+	+	+
Central	CDK1	+	+	-
Central	CDK2	+	-	-
Central	CDK3	-	-	-
Central	CDK4	+	+	-
Lake	LMN1	+	+	+
Lake	LMN2	-	-	-
Lake	LMN3	+	+	-
Lake	LMN4	-	-	-
Lake	LKK1	+	+	+
Lake	LKK2	+	-	-
Lake	LKK3	-	-	-
Lake	LKK4	+	+	-
Coastal	CZC1	+	+	+
Coastal	CZC2	+	-	-
Coastal	CZC3	+	+	+
Coastal	CZC4	+	-	-
Coastal	CDT1	+	-	-
Coastal	CDT2	-	-	-
Coastal	CDT3	-	-	-
Coastal	CDT4	+	+	+

+: Virulence/pathogenic, -: Avirulence/non-pathogenic

virulent isolates on TTC medium (Table 4). Irregular and fluidal colony appearance with pink center and white margins color were consistently observed (Fig. 2).

Figure 2 represents colony appearance of RSSC on TTC medium: Virulent (Left) and avirulent (right). In pathogenicity test, 19 isolates showed typical wilting (Fig. 3), whereas the remaining 11 were non-pathogenic and could be rated as saprophytic although they had similar colony appearance with RSSC. Subsequently, when the isolates were re-isolated on TTC medium, they produced virulent colonies of RSSC. Furthermore, wilting severity in tomato seedlings relatively differed among isolates. RSSC isolates from the Southern zone were significantly (p≤0.05) more pathogenic than the isolates from other agro ecological zones (Table 5). The scatter graph indicated positive correlation between disease severity and pathogenesis of RSSC (Fig. 4).

Biovars of RSSC: Results showed that all the tested bacterial isolates were able to oxidize the four basic carbon sources (Dextrose, sucrose, mannitol and lactose) in 3 days (Table 6). Furthermore, the result of the biovar test showed that nine isolates oxidized disaccharides (sucrose, lactose and maltose) and sugar alcohols (mannitol, sorbitol and dulcitol) while an

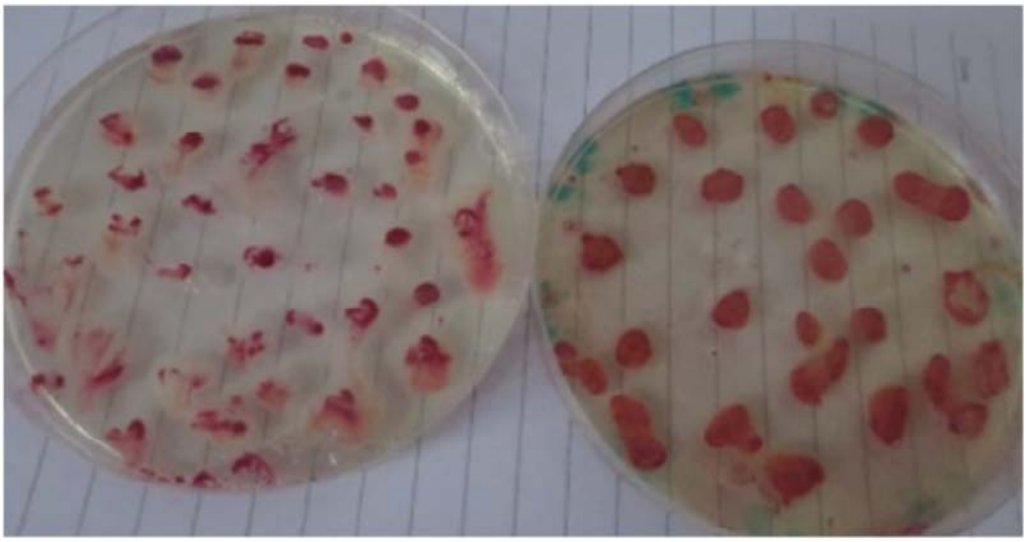

Fig. 2: *Ralstonia solanacearum* colony appearance on TTC medium: Virulent (Left) and avirulent (right)

Fig. 3: Pathogenicity test of bacterial wilt disease on tomato seedlings

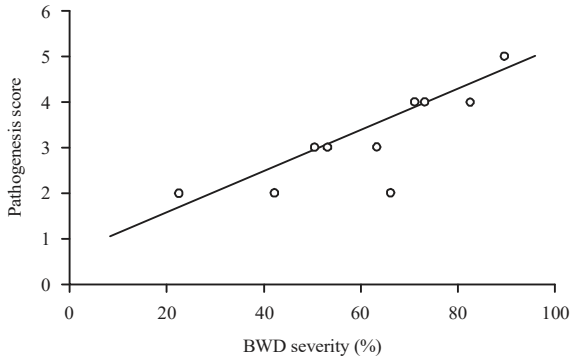

Fig. 4: A scatter graph for severity in the field and pathogenesis of BWD isolates

Table 5: Differences in wilting severity among the pathogenic isolates of *Ralstonia solanacearum* species complex

Zones	Isolate code	Mean of wilt severity
Northern	NAA1	3.33^{c}
Northern	NAA4	2.00^{b}
Northern	NMB1	3.33^{c}
Northern	NMB2	2.00^{b}
Southern	SIK1	4.00^{d}
Southern	SIK3	3.33^{c}
Southern	SIK4	4.33^{d}
Southern	SMM2	2.67^{bc}
Central	CSM2	1.67^{ab}
Central	CSM4	1.67^{ab}
Central	CDK1	2.67^{bc}
Central	CDK4	2.33^{b}
Lake	LMN1	2.00^{b}
Lake	LMN3	1.67^{ab}
Lake	LKK1	1.33^{a}
Lake	LKK4	1.67^{ab}
Coastal	CZC1	1.33^{a}
Coastal	CZC3	2.33^{b}
Coastal	CDT4	2.00^{b}
Mean		2.84
F test		*
LSD		1.07

*Significant at $p \leq 0.05$, mean with the same letter(s) within the column are not significantly different based on LSD test (p = 0.05)

Table 6: Utilization reaction of the basic carbon sources by isolates of *Ralstonia solanacearum* species complex

Isolates	Sucrose	Lactose	Mannitol	Dextrose	Salicin	DW	Inference
NAA1	+	+	+	+	-	-	RSSC
NAA4	+	+	+	+	-	-	RSSC
SIK1	+	+	+	+	-	-	RSSC
SIK4	+	+	+	+	-	-	RSSC
CSM4	+	+	+	+	-	-	RSSC
LMN1	+	+	+	+	-	-	RSSC
LKK1	+	+	+	+	-	-	RSSC
CZC1	+	+	+	+	-	-	RSSC
CZC3	+	+	+	+	-	-	RSSC
CDT4	+	+	+	+	-	-	RSSC

+: Positive reaction, -: Negative reaction, RSSC: *Ralstonia solanacearum* species complex, DW: Distilled water

Table 7: Differentiation of isolates of *Ralstonia solanacearum* species complex into biovar(s)

Isolates	Maltose	Lactose	Cellobiose	Mannitol	Dulcitol	Sorbitol	Ribose	Trehalose	Tryptophan	Dextrose	Salicin	DW	Biovar
NAA1	+	+	+	+	+	+	+	+	+	+	-	-	3
NAA4	+	+	+	+	+	+	+	+	+	+	-	-	3
SIK1	+	+	+	+	+	+	+	+	+	+	-	-	3
SIK4	+	+	+	-	-	-	+	+	+	+	-	-	2-2T
CSM4	+	+	+	+	+	+	+	+	+	+	-	-	3
LMN1	+	+	+	+	+	+	+	+	+	+	-	-	3
LKK1	+	+	+	+	+	+	+	+	+	+	-	-	3
CZC1	+	+	+	+	+	+	+	+	+	+	-	-	3
CZC3	+	+	+	+	+	+	+	+	+	+	-	-	3
CDT4	+	+	+	+	+	+	+	+	+	+	-	-	3
XYZ	+	+	+	-	-	-	+	+	+	+	-	-	2-2T

+: Positive reaction, -: Negative reaction, 2T: Sub-group of biovar 2, DW: Distilled water

isolate SIK4 was not able to utilize the sugar alcohols (Table 7). The isolate coded as XYZ was isolated from the wilted round potato stems and was include as an out group, it behaved similarly as isolate SIK4. On the other hand, all the control plates of carbon sources (dextrose and salicin) and DW remain unchanged (Table 7 and Fig. 5).

Fig. 5: Biochemical reaction of *R. solanacearum* isolates from different agro ecological zones of Tanzania

DISCUSSION

BWD is widely spread in Tanzania affecting more than 55% of farmers' field at significantly ($p \leq 0.05$) different levels of incidence and severity. The level of virulence and pathogenesis among isolates significantly varied within and across the agro ecological zones. Variations in the incidence and severity of BWD in the agro ecological zones may be attributed to factors such as diversity of RSSC isolates soil types and the production environment[26]. In terms of production environment, hot weather and with high (80-90%) relative humidity are suitably favor survival of RSSC increasing the incidence and severity of bacterial wilt disease. Such environmental conditions are common in most screen-houses especially in developing countries and therefore prevention measures are critically important to avoid introduction of RSSC to such environments. RSSC survives well in moist soils with a pH values from 6-7. Since soils in the agro ecological zones of Tanzania are not homogenous, variations of bacterial wilt disease incidence and severity may be caused by differences in soil moisture and pH levels. Higher (>85%) relative humidity and changes in soil pH increase severity and incidences of bacterial wilt disease[22].

There was a strong association (coefficient of correlation $r = 0.84$) of BWD severity in the field and pathogenesis of the causing isolates which supported Koch's postulates. The pathological descriptors adopted were able to distinguish virulent from avirulent isolates and further pathogenic from non-pathogenic isolates. Moreover, the use of biochemical descriptors successfully differentiated isolates of RSSC into biovars as previously reported by He *et al.*[13], Hayward[16] and Kumar *et al.*[27] that biovar 3 oxidizes both disaccharides and sugar alcohols, biovar 2 oxidizes only disaccharides whereas biovar1utilizes hexose alcohols and biovar 4 oxidizes only alcohols. Isolates of RSSC utilizes various carbon sources for maintenance and growth[28].

Out of ten isolates of RSSC used in this study, 90% were identified as biovar 3, while the remaining (10%) were recognized as biovar 2 (2-T). Biovar 3 was recorded from all the zones while biovar 2 was present in the Southern zone of Tanzania. To the best of our knowledge, this is the first report of the prevalence of biovar 2 of RSSC in Tanzania and hence is an alarm to design management strategies to prevent spread to other geographical locations.

Biovar 2 of RSSC infects both tomato and potatoes and thus considered to be of more economic importance. The prevalence and survival of biovar 2 of RSSC in the Southern agro ecological zones of Tanzania could be associated with the continuous cultivation of host plants such as round potato, tomato, pepper and eggplants[21]. This could indicates that the farmers are not aware of the demerits of continuous cultivation of the same host plant in the same piece of land every season. It therefore call upon the extension service providers to help farmers to adopt plant protection practices such as crop rotation for improved disease management. RSSC biovar 2 is distributed worldwide, occurring in temperate regions, subtropical areas and at higher altitudes in the tropics, because of its lower optimum temperature. Results suggest that biovar 2 of RSSC was recently introduced in Tanzania, although its origin is unknown. Generally, biovar 2 strains around the world appear to be spreading rapidly into previously uninfected areas and implementation of stronger standard phytosanitary measures are recommended to prevent further spread.

CONCLUSION

The study has investigated and discovered that two major biovars of *Ralstonia solanacearum* species complex are associated with tomato BWD in Tanzania. Biovar 3 was previously known as the only RSSC biovar responsible for tomato BWD in Tanzania. However, findings from this study have discovered that RSSC biovar 2 also causes tomato BWD in Tanzania. This alerts plant health regulators to implement necessary phytosanitary measures to prevent further spread and/or introduction of the disease considering its quarantine status in different countries.

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SIGNIFICANCE STATEMENT

This study discover the existence of biovar 2 of RSSC as one of the pathogens of bacterial wilt disease in Tanzania that can be beneficial for plant health regulators to take stronger phytosanitary measures to prevent further spread and /or introduction to uninfected areas. This study will help the researcher to uncover management strategy of tomato BWD based on the characteristics of prevailing pathogen strain in a given agro-ecological region that many researchers were not able to explore. Thus, an effective and sustainable management strategy of BWD that is pathogen targeted may be developed.

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