Incidence, severity and characteristics of *Ralstonia solanacearum* species complex in greenhouses in Southern Ghana

N.Y. Sarfo[1], E.W. Cornelius[1] and S.K. Torkpo[1,2*]

[1] *Department of Crop Science, School of Agriculture, College of Basic and Applied Sciences, University of Ghana, Legon*

[2] *Forest and Horticultural Crops Research Centre, Okumaning-Kade, School of Agriculture, College of Basic and Applied Sciences, University of Ghana, Legon*

*Corresponding Author: *sktorkpo@ug.edu.gh; stephentorkpo@gmail.com*

Abstract

Tomatoes are important component in Ghanaian diets, but bacterial wilt disease has become a major threat to the production of this high value vegetable. Consequently, relevant stakeholders over the past decade have sited greenhouses in the South of the country to boost tomato production, with some operators reporting wilt symptoms of tomato in their greenhouses. This study was therefore conducted with the aim of determining incidence and severity of the bacteria wilt disease of tomato; isolate causal organism from diseased tissues, establish sources of the bacteria inoculum in greenhouses and characterize isolates using morphological, biochemical and molecular techniques. Koch's postulate was also carried out on the isolates. Bacteria wilt disease was recorded in 12 out of 54 greenhouses surveyed. Incidence and severity of the disease within greenhouses ranged from 5.9%-18.5% and 0.5 – 1.2, respectively. *Ralstonia solanacearum* species complex was confirmed through streaming, morphological and biochemical tests. Strains of Phylotypes I and III (now R. *pseudosolanacearum*) were determined by multiplex polymerase chain reaction. Sources of bacteria inoculum in the greenhouses were found to be irrigation water and the growth substrate used. Koch's postulate was fulfilled. Accurate diagnosis of the pathogen is a major step towards developing effective disease management tools in the country and beyond.

Keywords: greenhouse, tomato, Ralstonia, wilt, phylotypes

Introduction

Tomato (*Solanum lycopersicum* L) is the most important vegetable in terms of value (Hanson et al., 2001). Global tomato production in 2012 was 161,793,834 tons, and that for Ghana stood at 321,000 tons (FAO, 2013), increased to 420,000 tons in the country in 2019 (MoFA, 2020) but declined to 374,554.49 in 2023 (Tridge, 2025). Tomato is cultivated mainly by smallholder farmers in all the agro-ecological zones in Ghana (Diao, 2010). According to Osei et al. (2010) the monetary expenditure on tomatoes exceeds any other vegetable in the country. Nutritionally, tomato fruits and products are described as "protective foods", owing to the presence of lycopene, and other anticarcinogenic and antioxidant compounds (Alam et al., 2007).

Despite the importance of tomato, average yield in Ghana of 7.5 tons/ha is far below potential tomato yield of 20 tons/ha (MoFA, 2017), and lower than the 10 tons/ha recorded in Burkina Faso (FAO, 2019). Low tomato yield in Ghana could be attributed to factors including plant protection challenges (Oduro 2000; Offei et al., 2008). This leads to shortfalls in meeting local demands for tomato in the country, resulting in high yearly importation of fresh tomato from neighbouring countries, especially Burkina Faso (Robinson & Kolavalli, 2010). It has been estimated that the country informally imports about 100,000 tons of tomato annually from neighbouring countries, particularly, Burkina Faso (MoFA-IFPRI, 2020).

Ghana's tomatoes are mostly produced in open fields under rainfed or irrigation production systems (MoFA, 2017), with its attendant challenges such as diseases and pests' problems. To enhance productivity and ensure secure and sustained supply of tomatoes throughout the year, and reducing imports of vegetables especially tomato, successive

Governments of Ghana in collaboration with relevant stakeholders have sited greenhouses in the South of the country to boost production. Hochmuth (2018) has stated that tomatoes are very popular for cultivation under greenhouse production systems.

Unfortunately, operators of some of these greenhouses in the country have reported incidences of devastating wilt disease of tomato, which could result in total yield loss. Similar symptoms have been reported in open field tomato in the north of country, and the suspected pathogen, *Ralstonia solanacearum* species complex reported Subedi et al. (2014). In neighbouring Togo, Kunwar et al. (2020) also reported occurrence of R. *solanacearum* species complex on some solanaceous crops. Safni et al. (2014) has proposed an amendment to the taxonomy of R. *solanacearum* species complex to allow for proper diagnosis.

According to EPPO (2004), R. *solanacearum* had been listed as A2 quarantine pest thereby endangering export of Ghanaian tomatoes in international trade. Also, Mansfield et al. (2012) ranked the bacterium as the second most scientifically and economically important phytopathogenic bacterium in 2012, after *Pseudomonas syringae*. Similarly, R. *solanacearum* has been described as a major constraint to tomato production, in sub-Saharan Africa and beyond (Aloyce et al., 2017; Shutt et al., 2018; Balamurugan et al., 2020; Paudel et al 2020; Mekonnen et al., 2022; Traoré et al., 2023) These are worrying developments for the recently introduced greenhouse tomato production system in the country, and its success will greatly depend on proper mitigation of challenges posed by R. *solanacearum* species complex. Accurate identification of R. *solanacearum* species complex is very critical for proper management of the disease. Therefore, the objectives of the study were to determine the incidence and severity of bacterial wilt disease in greenhouse tomatoes in southern Ghana; identify and characterize the causal organism of the bacterial wilt disease in southern Ghana and investigate the sources of inoculums for bacterial wilt disease in greenhouses.

Materials and Methods

Selection of study area

Greenhouses in each region were purposefully selected using a database acquired from the West African Agricultural Productivity Programme (WAAPP), which had records of all greenhouse operators in respective regions unevenly clustered into groups, and with the help of Agricultural Extension Agents (AEA's) and relevant scientists. A total of 54 greenhouses were sampled and surveyed in communities (Fig. 1) spread across Greater Accra, Eastern, Central and Volta Regions of Ghana. The study areas in the Greater Accra

Fig. 1 Location of greenhouses in communities in the study area
FOHCREC: Forest and Horticultural Crops Research Centre

Region, and Shia Osudoku in the Eastern Region are characterized by temperature of 24.9 °C to 28.6 °C with annual precipitation of 691 mm; Eastern Region had 24.2 °C to 27.4 °C with annual precipitation of 1413 mm; Central Region had 24.°C to 27.2 °C with annual precipitation of 979 mm; Volta Region had 24.9 °C to 28.6 °C with annual precipitation of 1412 mm. With the exception of Kade, which had February and July as the warmest and coolest months, respectively, the rest had March and August as the warmest and coolest months, respectively (https://compare.climate-data.org/rates/search/index.php? Accessed: April 19, 2021).

Determination of incidence and severity of bacterial wilt disease of tomato in greenhouse in the study area

In each community, disease incidence and severity were assessed between August and December 2017. A quick diagnosis of diseased tomato plants was carried out using wilting of leaves without foliar yellowing, streaming and an examination of vascular tissue of plant for discolouration to distinguish between bacterial wilt and other wilts. Stream of milky exudates from bacteria stream test was diagnostic for Ralstonia wilt (Goszczynska et al., 2000).

Disease incidence and severity assessment were carried out within each greenhouse visited.

$$\textbf{Greenhouse incidence} = \frac{Total\ number\ of\ greenhouses\ affected}{Total\ number\ of\ greenhouses\ assessed} \times 100$$

$$\textbf{Disease incidence in greenhouse} = \frac{Number\ of\ diseased\ plant\ in\ greenhouse}{Total\ number\ of\ plants\ in\ greenhouse} \times 100$$

Similarly, disease severity was scored on a 0-4 disease assessment scale modified after Horita & Tsuchiya (2001) as described by Shenge et al. (2007).

$$Disease\ severity\ in\ greenhouse = \frac{Sum\ of\ all\ ratings}{Total\ rating} \times Maximum\ disease\ grade$$

Isolation and identification of causal organism of the bacterial wilt disease of tomato

Tomato plants showing symptoms of bacterial wilt were obtained from various greenhouses during the survey and transported to the Plant Pathology Laboratory of the Department of Crop Science, University of Ghana, Legon. Stem segments (about 10 cm in length) from the collar region of wilted plants were washed thoroughly under running tap water to remove soil and debris. The cut tissues were surfaced sterilized with 70% ethanol for 60 seconds and rinsed with sterile distilled water under a laminar flow chamber. Disinfected stems were cut longitudinally and suspended in a beaker containing sterile distilled water (SDW) for five minutes. A loop full of the turbid stream suspension was then streaked on a 9 cm Petri dish containing nutrient agar (NA) using disposable inoculation loops and incubated for 2-3 days at 20-30°C. Daily observations were then made for colony growth.

The bacteria isolates were sub-cultured on nutrient agar and 2, 3, 5-triphenyl tetrazolium chloride medium (Casamino acid - casein hydrolysate), 1g; peptone, 10 g; Glucose, 5 g; Bacto- Agar, 17 g per litre of distilled water, autoclaved at 121˚C for 20 min and 5 ml of 2, 3, 5-triphenyl tetrazolium chloride added after allowing to cool) to obtain pure colony forming units (CFU) of the bacterium. Pure isolates were kept in slants on nutrient agar and stored at 4˚C in a refrigerator until needed.

Biochemical and morphological tests

Gram staining and Catalase oxidase tests were conducted on isolates according to Schaad (1988). Similarly, Potassium hydroxide (KOH) solubility test was carried out according to Suslow et al. (1982). Colony growth characteristics on 2, 3, 5-triphenyl tetrazolium chloride (TZC medium) was studied based on Kelman (1954).

Hypersensitivity reaction (HR) test on tobacco (Nicotiana tabacum)

A 5 ml sterile hypodermal syringe without a needle was used to inject the bacterial suspension into the underside of four tobacco leaves of the test plant. Sterile distilled water was injected in the underside of the control plant. The plants were kept in a screen house at 25-30^{o}C and observed for leaf yellow chlorosis or necrosis (Lelliott & Stead 1987; Schaad et al., 2001; Wick, 2010).

Pathogenicity tests

Five WACCI tomato varieties (WACC 1, WACC 2, WACC 3, WACC 4, and WACC 5) and Eva F1 (a preferred variety of greenhouse farmers in Ghana but reported as susceptible to the BWD) were used in pathogenicity tests

at WAAPP greenhouse cluster at Borteyman in November 2017.

Seedlings were sown in 72 seed-cell trays with a soil starter (Fertiplus® potting mix) and conventional nursery management practices were observed. Seedlings were transplanted after 2 weeks into 12 L pots containing naturally infested coco peat and incubated in a screen house. The pots were arranged in a completely randomized design (CRD) with six treatments (variety as stated in paragraph one above) replicated 10 times. Adequate quantities of fertilizers were applied as follows: NPK 19:19:19 soluble fertilizer (Poly-feed, Haifa chemicals®) was applied daily at a rate of 200g /15L through fertigation using a drip irrigation system. A nutrient supplement (Agriful, Haifa chemicals®) was also applied at the rate of 20 ml/15L every 5 days. Additionally, foliar application of Mix and Max at a rate of 20 ml/15L was sprayed on the plants every 5 days as supplement (using 15L knapsack sprayer). An application of Ca-B (Calcium oxide + Boron) at 65 ml/15L gallon was applied once every week for prevention of calcium deficiencies. Multi K at 200 ml/15L was also applied after the third week.

The plants were sprayed with Agrithane® 80 WP (Mancozeb) at a rate of 2.7 g/L to control fungal diseases, and 2 ml/L lambda cyhalothrin insecticide to control insects using a 15L manual knapsack sprayer.

Bacteria cultures were obtained from Ralstonia affected tomato plants in greenhouses at Dawhenya. These were suspended in sterile distilled water to make a suspension containing about (1x108 CFU/mL) and used to inoculate transplants using a stem puncture technique (Winsted & Kelman, 1952). This involved the puncturing of stems of plants with a sharp needle through which a 5 mL of bacterial suspension was injected into the axil of the second or third expanded leaf below the stem apex using a sterile syringe. The plants were monitored for wilt symptoms and disease incidence and severity determined. Re-isolation was done from symptomatic plants to confirm the presence of the pathogen during the trail.

Disease severity was scored on a 0-4 disease assessment scale modified after Horita & Tsuchiya (2001) as described by Shenge et al. (2007). Plants were evaluated at 0, 7, 14, 21, 28, 35, and 42 days post inoculation.

Deoxyribonucleic acid (DNA) extraction

Genomic DNA was extracted from bacteria isolates using a modified CTAB (cetyl trimethyl ammonium bromide) protocol described by William et al. (2012). A pure 24 h bacterial colony cultured on nutrient agar was used to inoculate 5 ml of CPG broth (Bacto casamino acids, 1.0 g; Bacto peptone, 10 g; and glucose, 10 g in 1 liter of water) in falcon tubes. The cultures were then grown at 28 °C for 48 h in a shaking incubator at 200 resolutions per min (rpm) and harvested by centrifugation at 10,000 rpm for 5 minutes and the supernatant discarded. The resulting cell pellets were suspended and lysed in 740 µl of TE buffer (10 mm Tris-HCL, 1 mm EDTA, pH 8.0) by vigorous pipetting. A 20 µl lysozyme (conc. 100 mg/ml) was added to the mixture and incubated for 30 min at 37°C. Sodium dodecyl sulfate (10%) and 18 µl of proteinase K (20 mg/ml) were added to the mixture and incubated for one hour. A 100 µl of 5 M NaCl solution and 100 µl of 10% CTAB solution in 0.7 M NaCl were added and thoroughly mixed to remove most protein and cell debris. The ensuing suspension was incubated for 10 min at 65°C and then kept on ice for 15 min. The mixture was then centrifuged at 10000 rpm for 10 min after the addition of 0.5 ml chloroform: isoamly alcohol (24:1). Isopropanol of 0.6 ml was added and incubated at -20 °C for 2 hours and centrifuged at 10000 rpm for 15 min at 4 °C. The DNA pellets were washed with cold 500 µl of 70% ethanol, air dried at room temperature for about three hours and re-dissolved in 50-µl TE buffer. DNA quality was assessed by running 5 µl of extracted DNA on 1% agarose gel, and then stored at -20 °C until needed.

Multiplex polymerase chain reaction (PCR) and gel electrophoresis

Phylotype identification described by Fegan and Prior (2005) was used for strain identification of isolates. Phylotype specific multiplex PCR (Pmx-PCR) was carried out in a 25 µl final volume of reaction mixture, containing 1xTaq Master mix (PCR buffer,1.5mM $MgCl_2$, 200 mM of each dNTP, 50 mM KCL, 10 mM Tris-HCL and 1.25U of Taq DNA polymerase) (England Biolabs INC., U.K), 6 pmoles of primers Nmult:21:

1F, Nmult: 21: 2F, Nmult: 22: InF,18 pmoles of the primer Nmult: 23: AF, Nmult: 22: RR and 4 pmoles of the primers 759 and 760 and 3.0 µl DNA (Sagar et al., 2014). The negative control had sterile water instead of DNA.

The following cycling conditions were used in a thermocycler (BIO-RAD, cycler, USA): 96˚C for 5 min; then 30 cycles of 94˚C for 30 s, 59˚C for 30 s and 72˚C for 30 s, followed by a final extension at 72˚C for 10 min (Kumar et al., 2004; Fegan and Prior 2005). Amplicons were separated by electrophoresis on 1.5% agarose gel in 1XTBE buffer at 100V for 2 hours. Gels were viewed on a UV trans-illuminator (BioDoc Imaging System, U.K).

Establishing the source of inoculums of bacterial wilt disease of greenhouse tomato in southern Ghana

In establishing the source of origin of the causal bacterium of the wilt disease, samples of plant growth media, tomato seeds and irrigation water were collected from each greenhouse during the disease survey, labelled and sealed in sterile transparent polythene bags, kept in an ice box and transported to the Plant Pathology Laboratory, Department of Crop Science, University of Ghana.

Isolation of bacteria from tomato seeds affected by bacterial wilt disease

The method used by Umesha & Avinash (2015) was adapted for the isolation of the bacterium from the seed samples. Tomato seed samples collected from greenhouses during the survey were surface sterilized with 70% ethanol for 60 seconds and rinsed with sterile distilled water under a laminar flow chamber. Liquid assays of the collected seeds were prepared by macerating 5 g of the seeds using sterile mortar and pestle in 10 ml of sterile distilled water (SDW). One ml of the resultant supernatant was mixed into 9 ml of SDW to obtain a dilution of 10^{-1} which serially diluted to 10^{-5}. A loop full of each dilution was streaked on nutrient agar and incubated for bacteria isolation and purification.

Isolation of bacteria from plant growth media

A magnetic shaker was used to thoroughly mix 20 g of the nutrient agar in 100 ml SDW in 250 ml Erlenmeyer flasks for 10 minutes, and five-fold serial dilutions made from the resultant supernatant. A loop full of each dilution was aseptically streaked on NA media and the plates were incubated for colony observation and purification (Nguyen & Ranamukhaarachchi, 2010; Umesha & Avinash, 2015).

Isolation of bacteria from irrigation water

The collected water samples were transferred to sterile falcon tubes (50 ml) and serial dilutions of up to 10-5 of each water sample were made. Each dilution was then streaked on nutrient agar and observed for colony growth and further characterization.

DNA was isolated from 2-3 days old cultures collected from the various sources (as previously described) and numerically coded for further confirmation using multiplex polymerase chain reaction and data was presented as positive or negative for the presence of the bacterium. Universal primers 759 and 760 were used for amplification.

Results

Incidence and severity of bacterial wilt disease of tomato in the study area

Fifty-four (54) greenhouses were assessed for incidence of BWD of tomato (Table 1a), out of which 12 greenhouses experienced TBWD across the study area. Disease incidence within greenhouses ranged from 5.9% to 18.5%, and severity ranging from 0.5-1.2. Out of the four regions, Central region and Volta regions did not record BWD in any of the domes. Greater Accra and Eastern regions recorded 22% greenhouse incidence (12 out of 54 total domes). Dawhenya in the Greater Accra region had disease incidence and severity of 18.5% and 1.2, respectively. Borteyman also recorded disease incidence and severity of 7.7% and 0.6 respectively. Kade in the Eastern region recorded an incidence of 8.2% with severity of 0.8 whilst Natriku had 5.9% incidence with a severity of 0.5 (Table 2a). Over 10 tomato varieties were grown by greenhouse tomato farmers in the study area with Eva been the dominating variety and closely followed by

TABLE 1
Number of greenhouses in communities in the study area

Region	District	Communities	No. of Greenhouse	GPS Coordinates greenhouse
Greater Accra	Ningo Prampram	Dawhenya	5	5.791870° N, 0.069999° E
	Adenta Municipal	Amrahia (Dairy farms)	1	5.76536° N, 0.15139° W
	Tema-West	Borteyman	5	5.7348137° N, 0.0302469° W
	Ashiaman Municipal	Adjei Kojo	13	5.696074° N, 0.058505° W
Central	KEEA	Nsadwir	4	5.075194° N, 1.468694° W
Eastern	New Juabeng	Akwadum	10	6.112164° N, 0.338263° W
	Nsawam Adoagyiri	Praso	1	6.19946° N, 1.16987° W
	Denkyembour	FOHCREC*	1	6.142516° N, 0.902476° W
	Shai Osudoku	Natriku	5	6.108386° N, 0.108424° E
Volta	South Dayi	Adidome	2	6.6863946° N, 0.3292465° E
		Dzemeni	2	6.596766° N, 0.1607779° E
	North Dayi	Vakpo	4	6.837465° N, 0.310207° E
Total			54	

*Forest and Horticultural Crops Research Centre, Kade

Nimo Necta (Table 2b).

Gram stain, Potassium hydroxide (KOH) solubility and Catalase oxidase tests

Bacterial isolates from samples showing symptoms of the BWD revealed red short rod-shaped bacteria when subjected to Gram stain reaction. Mucoid and slimy threads was observed when bacterial colonies from diseased samples were mixed with 3% KOH, stirred and picked with a disposable inoculation loop. Production of gas bubbles was observed by the naked eye and under a stereomicroscope 25X for Catalase oxidase test.

TABLE 2a
Incidence and severity within greenhouses that had BWD of tomato in the study

Region	District	Community	Number of affected greenhouse	Incidence (%)	Severity (1-4)
Greater Accra	Ningo prampram	Dawhenya	1	18.5	1.2
	Tema West	Borteyman	5	7.7	0.6
Eastern	Denkyembour	FOHCREC*	1	8.2	0.8
	Shai Osudoku	Natriku	5	5.9	0.5
Mean				10.0	0.7

*Forest and Horticultural Crops Research Centre, Kade

TABLE 2b
Tomato varieties grown by greenhouse farmers in the study areas

Varieties grown in Greenhouse	Frequency	Percentage (%)
Eva	44	88
Nimo Necta	30	60
Tatiana	27	54
Pectomech	23	46
Anna	17	34
Padma	11	22
Napoli	6	12
Roma	5	10
8014	3	6
2013-4	3	6

TABLE 2b *cont*
Tomato varieties grown by greenhouse farmers in the study areas

Varieties grown in Greenhouse	Frequency	Percentage (%)
ABM 152	3	6
Jingping	2	4
Bigguy	2	4
Martima	1	2
Jaguar	1	2
Money maker	1	2
Platinum	1	2
NKansah HT	1	2
Inlay	1	2

Farmers had multiple responses

Bacteria colony morphology on TZC medium on isolates

Bacterial cultures from diseased samples grown on TZC medium at 25-30^{o}C for 2-5days showed characteristic red center and whitish margin which are fluidal after 24 hours of incubation. This morphology characterizes them as virulent isolates (Fig 2).

Hypersensitivity test of bacterial isolates

Tobacco plants inoculated with Bacteria isolates from symptomatic plant tissue collected from the study areas resulted in positive hypersensitive reaction 4 days after inoculation. The site of inoculation exhibited yellow chlorotic or necrotic symptoms which turned brown after 7 days (data no shown).

Pathogenicity tests

All six varieties (WACC 1, WACC 2, WACC 3, WACC 4, WACC 5, and Eva F1) inoculated with bacteria suspension were susceptible to the bacterial wilt disease. Symptoms of the disease were observed at different times (7, 14, 21, 28, 35 and 42 days after inoculation), and the most susceptible variety was the Eva variety with WACC 5 being the least susceptible. The area under disease progressive curve (AUDPC) of the disease for all varieties increased steadily with time. Significant differences ($P < 0.05$) in disease severity (AUDPC) were observed amongst the varieties with WACC 2, WACC 4, WACC 5 having similar disease severity, but significantly higher than WACC 1 and lower than WACC 3 and Eva varieties ($P < 0.05$) (Table 3a & 3b).

Molecular identification of Ralstonia solanacearum isolates using Multiplex PCR amplification

Result from phylotype specific multiplex PCR (Pmx- PCR) showed that R. *solanacearum*

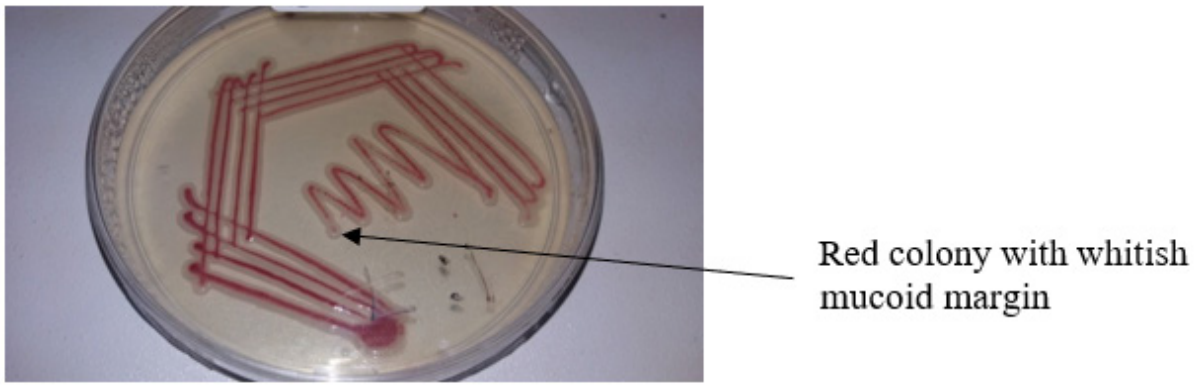

Fig. 2 Colony morphology of suspected R. *solanacearum* from irrigation water on TZC medium

strains from Southern Ghana were Phylotype I (280 and 144 bp amplicon), Phylotype III (280 and 91 bp amplicon) and Phylotype IV (280 and 213 bp amplicon) (Fig. 3). Phylotype I are from Asia, and were characterized by 280 and 144bp amplicon, Phylotype III are from Africa characterized by 280 and 91 bp amplicon, Phylotype IV are from Tropics (Indonesia, Japan and Australia) characterized by 280 and 213 bp amplicon. All samples were negative for Phylotype II, which is from America and characterized by 280 and 372 bp amplicons.

The bacterium enters the greenhouses through various sources (Table 4). Twenty-nine (29)

TABLE 3a

Severity of the tomato bacteria wilt disease in six tomato varieties artificially inoculated with *Ralstonia solanacearum*

Tomato variety	**AUDPC***
WACC 1	24.50
WACC 2	29.40
WACC 3	31.85
WACC 4	29.05
WACC 5	28.35
EVA	51.10
LSD($p<0.05$)	1.50

*AUDPC (area under the disease progress curve) for tomato was calculated using the formula of Shaner and Finey (1977)

TABLE 3b

Analysis of Variance table for area under disease of six tomato varieties screened for resistance to bacterial wilt

Variate: AUDPC					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accessions	5	3.522	0.704	0.67	0.65
Residual	18	18.798	1.044		
Total	23	22.32			

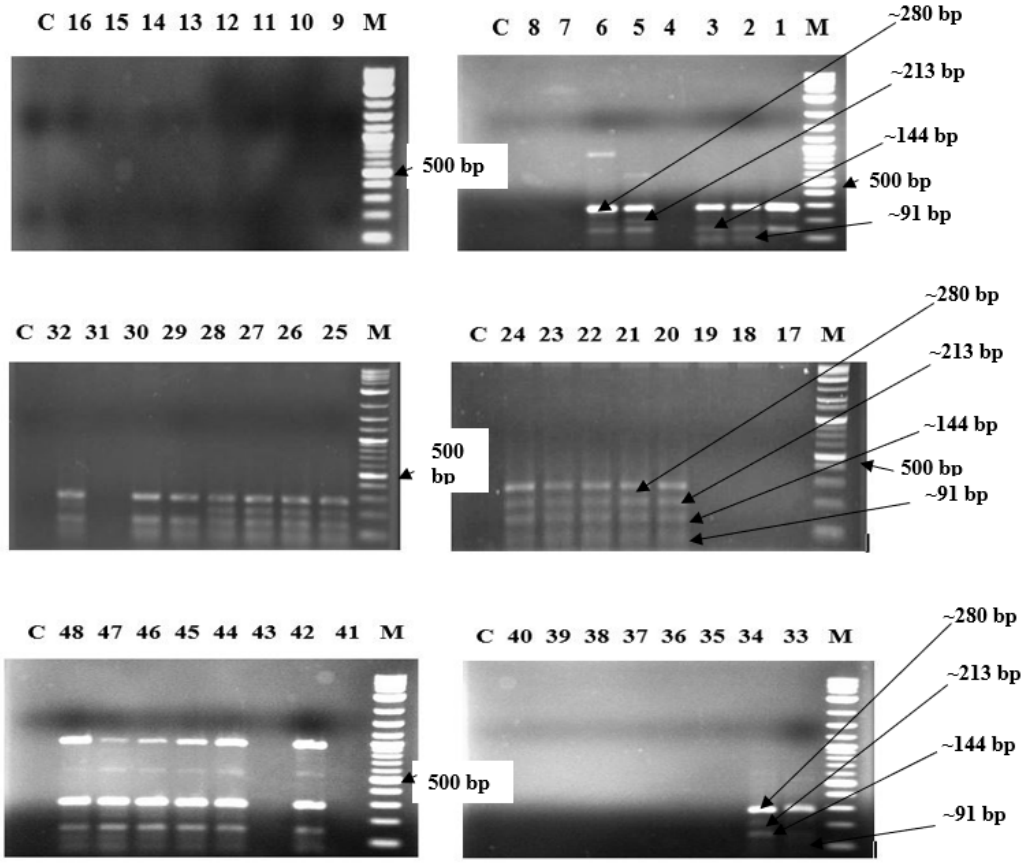

Fig. 3 Phylotype specific multiplex PCR detected isolates from collected samples. Lane M represents 100 bp DNA ladder, lane 1-48 represents individual suspected strains of R. *spp*; Lane C represents negative control

out of 53 samples collected were positive for Phylotype I while fewer samples tested positive for phyloypes III and IV (Table 4) Twelve samples each of plant and wet samples were positive for Phylotype I. Phylotype I was detected in two dry substrate and two irrigation water samples, and a dust sample (Table 4). Samples collected from Dawhenya community

TABLE 4

Phylotype categorization of *Ralstonia spp* from samples collected from communities in the study area

Communities	Phyloptype I (Asia- 144bp)	Phyloptype II (America- 372bp)	Phyloptype III (Africa- 91bp)	Phyloptype IV (Tropical- 213bp)	Universal RS (280bp)
Dawhenya					
Plant samples	+	-	-	-	+
Wet substrate	+	-	+	+	+
Dry Substrate	+	-	+	+	+
Irrigation(Mains)	-	-	-	-	-
Dust	+	-	-	+	+
Irrigation (drip)	+	-	-	+	+
Seed	-	-	-	-	-
Seed	-	-	-	-	-
Seed	-	-	-	-	-
Amrahia (Diary farm)					
Plant tissue	-	-	-	-	-
Wet Substrate	-	-	-	-	-
Dry substrate	-	-	-	-	-
Irrigation	-	-	-	-	-
Seed	-	-	-	-	-
Seed	-	-	-	-	-
Nungua (Agric. farms)					
seed	-	-	-	-	-
seed	-	-	-	-	-
seed	-	-	-	-	-
Irrigation	-	-	-	-	-
Dry substrate	+	-	+	+	+
Plant sample GH1	+	-	+	+	+
Plant sample GH2	+	-	+	+	+
Plant sample GH3	+	-	+	+	+
Plant sample GH4	+	-	+	+	+
Plant sample GH5	+	-	+	+	+
Wet substrate GH1	+	-	+	+	+
Wet substrate GH2	+	-	+	+	+
Wet substrate GH3	+	-	+	+	+
Wet substrate GH4	+	-	+	-	+
Wet substrate GH5	+	-	+	-	+
Akwadum					
Wet substrate	-	-	-	-	-
Kade					
Plant tissue	+	-	+	+	+
Irrigation	-	-	-	+	+
Wet substrate	+	-	-	+	+
Seed	-	-	-	-	-

+ = present

- = absent

TABLE 4 *cont*

Phylotype categorization of *Ralstonia spp* from samples collected from communities in the study area

Communities	Phyloptype I (Asia- 144bp)	Phyloptype II (America- 372bp)	Phyloptype III (Africa- 91bp)	Phyloptype IV (Tropical- 213bp)	Universal RS (280bp)
Nsadwir					
Dry Substrate	-	-	-	-	-
Irrigation	-	-	-	-	-
Praso					
Plant samples	-	-	-	-	-
Irrigation water	-	-	-	-	-
Substrate (in-use)	-	-	-	-	-
Vakpo					
Dry Substrate	-	-	-	-	-
Natriku					
Irrigation water	+	-	-	+	+
Dry Substrate	-	-	-	-	-
Plant Sample GH1	+	-	-	+	+
Plant Sample GH2	+	-	-	+	+
Plant Sample GH3	+	-	-	+	+
Plant Sample GH4	+	-	-	+	+
Plant Sample GH5	+	-	-	+	+
Wet Substrate GH1	+	-	-	+	+
Wet Substrate GH2	+	-	-	+	+
Wet Substrate GH3	+	-	-	+	+
Wet Substrate GH4	+	-	-	+	+
Wet Substrate GH5	+	-	-	+	+

+ = present

- = absent

which includes plant samples, wet substrate, dry substrate, dust sample and irrigation water were positive for RS but seed samples collected from the community tested negative for the bacterium. In Borteyman community, samples collected which included plant tissues, wet substrate and dry substrate in all domes or greenhouses were positive whereas irrigation water and seed samples were negative for the bacterium. At Kade, plant tissue, wet substrate and irrigation water samples were RS-positive but seed samples were RS-negative. All samples collected from Natriku were positive for the bacterium (Table 4). Samples from communities such as Nsadwir, Vakpo, Praso, Akwadum and Amrahia were all negative for the bacterium.

Discussion

There were varying levels of disease incidence and severity amongst greenhouses in the study area. Out of the 54 greenhouses (domes) surveyed, 12 had an incidence of bacterial wilt disease. The highest incidence was recorded in Dawhenya, followed by Borteyman, Kade and Natriku. Incidence and severity were low at the time of survey but progressed with time, with greenhouses in Dawhenya experiencing total crop loss two weeks after assessment was made. Higher crop losses with an average of 45% were also recorded in counties in Kenya resulting from high plant death (Onduso 2014). The sudden spread and prevalence of the disease could be attributed to the high temperature and moisture in the greenhouses, which creates thriving conditions for the growth of the pathogen (Tsitsigiannis et al., 2008).

Presence of red short rod-shaped bacteria from the Gram stain reaction indicates that bacteria isolated from samples in this study is Gram-negative - a characteristic of *Ralstonia pseudosolanacearum*. Further confirmation that the bacteria isolates are plant pathogenic was the mucoid and slimy threads observed from the KOH test, which is also a characteristic

of *Ralstonia pseudosolanacearum*. Suslow et al. (1982) used the KOH test as a faster way to differentiate between Gram-negative and Gram-positive bacteria. The 3% KOH breaks down the outer wall membrane of Gram-negative bacteria leading to the formation of slimy viscous thread whilst the Gram-positive bacteria tend to resist the breakdown due to its thick cell wall.

Leaf yellow chlorosis or necrosis observed for the hypersensitivity test further confirms the causative organism of the wilt observed on tomato to be caused by bacteria (Lelliott & Stead 1987; Schaad et al., 2001; Wick, 2010). Similarly, the observed growth, colony morphology and colour characteristic on TZC medium confirmed the status of the isolated bacteria during the present study as R. *pseudosolanacearum* sp. nov. (Kelman, 1954). Additionally, positive results from the pathogenicity tests further confirm the wilt causing bacteria in the greenhouses is species of *Ralstonia solanacearum* species complex.

Strains from southern Ghana were identified as Phylotype I from Asia, Phylotype III from Africa and Phylotype IV from Tropics. These results were in line with Fegan & Prior (2005) who described the Phylotypic classification system consisting of four phylotypes. Our findings on detection of Phylotpes I and III from greenhouses is consistent with Subedi et al. (2014), who identified Phylotype I and Phylotype III on tomatoes grown in open fields in Ghana. Also, Mahbou Somo Toukam et al. (2009) detected these two phylotypes on tomatoes in Cameroon. Detection in the present study of Phylotype I (now R. pseudosolanacearum sp. nov.) (Safni et al., 2014) concurs with Kunwar et al. (2020), who reported Phylotype I from all tomatoes, gboma and pepper samples collected from farms in Togo. Similarly, strong presence of Phylotype I have been reported from tomatoes in Burkina Faso (Traoré et al., 2023) and South Africa (Shutt et al., 2018). Further, the detection of Phylotpe III in our study was consistent with the finding of Mekonnen et al. (2022) in Ethiopia, where the authors also identified Phylotpe II but not Phylotpe IV.

This is the first report of Phylotype IV (now R. *syzygii* by Safni et al., 2014) in Ghana. Phylotype IV was reclassified as *Ralstonia syzygii* subsp. *syzygii* subsp. nov (reported on Clove in Indonesia), *Ralstonia syzygii* subsp. *indonesiensis* subsp.nov (reported on Tomato and Chilli pepper in Indonesia, and potato in Japan and Indonesia) and *Ralstonia syzygii* subsp. *celebesensis* subsp. nov. (reported on banana, Indonesia). One could assume that the Phylotype IV reported in this study might be strains of *Ralstonia syzygii* subsp. *indonesiensis* subsp.nov, occurring in mixtures with Phyloypes I and III. It is imperative to note that non-detection of Phylotype II strain from America (now R. *solanacearum*) in the study area could be attributed to its absence in the country. Phylo-typing is considered important for the strategic management and control of diseases as a variety said to be resistant to a phylotype can be susceptible to another phylotype (Sagar et al., 2014).

Bacteria inoculums were found in irrigation water in communities such as Kade and Natriku. Waiganjo et al. (2006) have reported the spread of the disease through contaminated irrigation water. Similarly, Traoré et al. (2023) have suggested favourable environment for development of disease to the proximity of their test site to water course. Additionally, monocultures of solanaceous crops such as tomatoes, peppers, and eggplants in fields contribute to high rate of R. *solanacearum* inoculum (Traoré et al., 2023).

In Dawhenya, the source of inoculums was contaminated substrate and Borteyman community had bacteria inoculums in substrate and plant tissues. Seed samples collected from all the greenhouses were not contaminated with the bacterium. However, in other studies, seeds have been noted to be a potential source of the disease (Abdurahman et al. 2017). The use of infected seedlings was among the most important means by which the disease spread (Ajanga, 1993). The adoption of good sanitation practices including the use of disinfectants in footbath at entrance of greenhouses, sterilization of substrates and the cleaning of equipment before and after use

has been reported to reduce spread of diseases (Dudek, 2008; Meng, 2013).

There is the need for a concerted effort by stakeholders in farmer training, movement of materials, among others to safeguard greenhouse tomato production in the country in particular, Africa as a whole and the world as Large. More samples of the causal organism should be isolated from other parts of the country for strain identification and sequencing for phylogenic analysis, and use of the isolates in screening of resistant tomato varieties.

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