Characterization of *Ralstonia* spp. in Tanzania and Potential Integrated Pest Management Strategies for Managing Bacterial Wilt in Tomatoes

Dissertation

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Abstract

Tomato is widely cultivated in Tanzania as a cash crop for local consumption and export. The crop comes in different varieties adapted differently into the diversified climatic habitats of Tanzania. The varieties respond differently to diseases and pests thus a great variation to susceptibility to diseases and pests. However, pests and diseases constrain tomato production especially in the key tomato producing regions. Bacterial wilt disease caused by the soilborne bacterium *Ralstonia* spp. has emerged as one the most devastating diseases in these tomato producing regions of Tanzania. The bacterium is very versatile inhabiting a wide range of plant species, thus complicating its management in the tomato farming systems, therefore calling for understanding its ecology.

To understand the diversity and virulence of *Rastonia* spp. in Tanzania, symptomatic Solanaceous plants were collected from Arusha, Tanga, Morogoro, Iringa, and Mbeya. Sixty-one strains of *R. pseudosolanacearum* were recovered from infected plant tissues, including 45 strains from tomato, 14 strains from eggplant, and two strains from sweet pepper. All 61 strains were confirmed as *Ralstonia.* spp. using PCR with species-specific primers. The strains were further classified into phylotype using multiplex PCR with phylotype-specific primers, whereby 93.2% (N=57) were confirmed as phylotype I, and 6.8% (N=4) as phylotype III. The partial endoglucanase gene (*egl)* was sequenced for twelve representative strains, which were assigned sequevars 18 (N=4), 20 (N=1), 22 (N=1), and 31 (N=6). All 61 strains grew anaerobically in Van den Moote

medium, with 3.7% (N=2) producing nitrogen (N_2) gas. The virulence of 61 strains was assessed on different hosts; 82% were virulent to tomato 'RioGrande', 77.7% were virulent on sweet pepper 'Yolo wonder', and 39.3% were virulent on tomato 'Hawaii 7996', with a mean disease incidence of 45.7%, 26.6%, and 7.3%, respectively. Latent infection was significantly (P<0.0001) higher in 'Yolo wonder' sweet pepper than in tomato 'RioGrande' and 'Hawaii 7996'. Among the three cultivars, 'RioGrande' tomato was the most susceptible (AUDPC= 201.3), followed by 'Yolo wonder' pepper (AUDPC=97.1), and 'Hawaii 7996' tomato with (AUDPC=26.5) (P<0.0001).

To identify potential rootstocks for grafting, we screened thirteen tomato and one eggplant *(Solanum melongena)* lines for resistance to a collection of Tanzanian and Asian *R, pseudosolanacearum* strains. From the preliminary screening, we selected tomato lines 'MT56' and 'WG120' and eggplant line 'EG190' for further evaluation for resistance against *R. pseudosolanacearum* strains collected from key tomato growing regions of Tanzania. All three selected lines varied in their resistance phenotype (P<0.0001) and supported latent infections with varying degrees of infection levels (P=0.0024). Lines 'MT56' and 'EG190' were selected as rootstocks for grafting with the susceptible tomato variety 'Moneymaker'. Grafting success rate ranged from 60%-75% regardless of rootstock-scion combination. Bacterial wilt incidence was significantly reduced in seedlings grafted with'MT56' or 'EG190' compared to self-grafted 'Moneymaker' (P=0.0024) and disease progression also varied significantly (P=0.0190).

Anaerobic soil disinfestation (ASD) with four carbon sources (wheat bran, rice bran, molasses, and cow manure) were evaluated as management strategy to bacterial wilt in naturally infested soils, in three villages (Misufini, Mlali, and Image) in Tanzania. Invitro bioassays using soils from the same field were also conducted. Significant differences in pH (P>0.0001), paint removal (P>0.0001) and temperature (P =0.01) between the carbon sources were detected in both the field (at all three locations) and greenhouse assays. The treatments reduced bacterial wilt incidence in tomatoes grown in ASD-treated soils compared to non-treated control soils in field trials at Misufini 1 (P=0.0205), Misufini 2 (0.0061), and Mlali 2 (P =0.019). This trend was also observed in the bioassays in which bacterial wilt incidence and area under disease progress curves in tomato seedlings grown in field-treated soils from Image, Mlali, and Misufini were significantly lower than in non-treated controls (P <0.0001). A significant difference (P <0.05) was observed in latent infection scores from DAS- ELISA among the asymptomatic seedlings sampled from ASD treated soils. However, ASD treatments varied significantly (P<0.0001) in reducing bacterial wilt incidence among carbon sources for eight of nine soils, but results were inconsistent between fields for all carbon sources. Yield was assessed on three farms with low (<10%) wilt incidence and was not significantly (P>0.05) affected by ASD treatment compared to controls.

Multivariate analysis of variance revealed non-significant (P>0.05) relationship in most of ASD independent factors with disease incidence and yield except for a few significant positive or negative relationships observed for pH, reducing conditions, and

temperature (P>0.05) in on-farm trials. Similarly, there were very few significant (P<0.005) correlations between disease incidence, area under the disease progress curve (AUDPC), and latent infection to pH and reducing conditions in the bioassay.

The development and adoption of effective and sustainable management practices require a better understanding of bacterial wilt as well as farmers' knowledge of the disease and their specific management practices. Five key tomato producing regions of the Tanzania mainland, Morogoro, Iringa, Mbeya, Tanga, and Arusha were surveyed to evaluate the prevalence of bacterial wilt disease as well as farmers' awareness of the disease and practices to manage it. On-farm surveys were conducted in July 2017 and November 2019 in which a total of 229 farms producing Solanaceous crops were surveyed, and 103 tomato farmers were interviewed using a questionnaire. During the farmers' survey, 16 tomato varieties emerged as preferred varieties based on fruit shape, size, and yield. Farmers' awareness of bacterial wilt disease and symptoms was high. Some of the farmers surveyed practiced bacterial wilt management using fungicides and/or insecticides (32.9%) or roguing diseased plants (26.2%).

Bacterial wilt disease was present in (61.2%) of all Solanaceous crop farms in 2017 and (67.3%) in 2019. The prevalence varied significantly between regions (P =0.0026) with Mbeya region having the highest prevalence and Iringa the lowest among five key tomato producing regions. Bacterial wilt prevalence in tomato farms was (38.5%) in 2017 and (41%) in 2019 with no significant differences between regions (P= 0.9270) and years (P=0.5894). Bacterial wilt incidence and severity were assessed in all tomato fields in

which the disease was present. Bacterial wilt incidence did not vary significantly between regions in 2017 (P =0.8657) and 2019 (P =0.1040). Significant variation (P<0.05) was observed within Mbeya region in 2017 and Iringa and Morogoro in 2019. Bacterial wilt severity varied significantly between regions in 2017 (P =0.0106) and non-significantly in 2019 (P = 0.1177). Arusha region had the average highest severity (70%) and Iringa the lowest (13.1%). Bacterial wilt incidence was significantly negatively regressed (β=-0.146, P=0.006) with farmers' tomato variety preference and positively with seed source (β=1.05, P=0.014) and farmers' awareness of the disease (β=2.202, P=0.024) among all farmers agronomical practices and descriptive characteristics tested in this survey.

Dedication

This dissertation is dedicated to my mother; Theodora Vasos for prayers, my husband Nsajigwa Mbije, my children Victoria, Emmanuel, Innocent and Jacqueline for encouragement, support, and prayers.

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Fields of Study

Major Field: **Plant Pathology**

Table of Contents

List of Tables

List of Figures

Chapter 1. Introduction

Tomato production

Tomato is widely cultivated in Tanzania, with an estimated annual production of 314,986 tonnes, contributing 64% of the total vegetable and fruit crop production in the country (De Putter et al., 2011; Luzi-Kihupi et al., 2015, Mutayoba and Ngaruko, 2017). Cultivated tomato is partly grown for local consumption to meet nutritional needs as well as income generation through sales in Tanzania and neighboring countries including Kenya, Rwanda, and Zambia (Minja et al., 2011; Maerere et al. 2006; Mutayoba and Nguruko, 2017). Recently the number of farmers involved in tomato production has increased exponentially due to increased demand and favorable conditions for tomato production (De Putter et al., 2011). In Tanzania tomato is mainly cultivated in the southern highlands (Mbeya, Njombe, and Iringa), northern highlands (Kilimanjaro, Arusha, and Manyara), and coastal and central regions (Morogoro and Tanga (Lushoto)). Smallholder farmers dominate Tanzanian tomato farming systems with an average of 1 Ha/household devoted to tomato production (Maerere et al., 2010).

In its production chain, tomato is constrained by many factors such as deteriorating soil fertility, the use of vulnerable and low-yielding varieties, unreliable rainfall, diseases, insect pests, and poor farming practices (Opena et al., 1990; Minja et al., 2011; De Putter et al., 2011; Testen et al., 2016). Widespread diseases, especially bacterial wilt have

extensively affected tomato production in parts of subtropical and tropical nations (Hayward 1991; Elphinstone, 2005).

Tomato diseases

Bacterial spot, bacterial wilt, bacterial canker, and bacterial speck limit tomato production in the key production regions of mainland Tanzania (Black et al., 1999; Shenge and Mabagala, 2007; Shenge et al., 2010; Mbega et al., 2012; Testen et al., 2018). Among the diseases caused by bacterial pathogens, bacterial wilt is the leading disease affecting tomato in Tanzania (Black et al., 1999; Baitani, 2017; Aloyce,2020). Bacterial wilt is caused by members of the *R. solanacearum* species complex (RSSC), the second most recognized bacterial pathogen after *Pseudomonas syringae* pathovars in causing devastating crop and yield loss globally, especially in Solanaceous crops (Champoseu and Momol, 2009; Mansfield et al., 2012; Meng, 2013). Members of the RSSC are pathogenic soil proteobacteria, widespread in subtropical, tropical, and temperate areas (Hayward, 1991; Yabuuchi et al., 1995; Elphistone, 2005). Previously, only tropical lowland areas characterized by warm climate were reported to support the perpetuation of RSSC bacteria (Hayward, 1991). However, Elphinstone (2005) identified members of the RSSC that belong to race 3 biovar 2 (R3B2) that survive in cooler and high-altitude areas and cause southern wilt, bacterial wilt, and brown rot in geranium, tomato, and potato respectively (Champoseau and Mommol, 2009).

Bacterial wilt disease is present in 26 African countries (Elphinstone, 2005; Muthoni et al., 2012) and emerged recently as one of the most damaging tomato diseases in key tomato producing regions of Tanzania (Black et al., 1999; Mwankemwa, 2015; Baitani, 2017; Testen et al., 2018; Aloyce,2020). It was first reported on tomato and eggplant in Zanzibar, the northern and southern highlands of Tanzania Mainland in 1998 (Black et al., 1999). Since that time, the disease is spread in other important production regions of Tanzania such as Mbeya, Arusha, Morogoro, Iringa, central, lake and coastal regions of mainland Tanzania and Unguja (Zanzibar). Bacterial wilt disease severity especially in Solanaceous crops (tomato, eggplant, peppers, and potato) is increasing at alarming paces in these regions (Mwankemwa, 2015; Baitani, 2017; Aloyce,2020).

Ralstonia spp. characterization

Historically, *Ralstonia* spp. strains were characterized based on their ability to break down a series of sugars/alcohols and attack a series of crops, hence falling into five biovars and races (Martin and French, 1985; French et al., 1998; EPPO, 2004; Oslon, 2005; Denny, 2006). In the biovar classification scheme, biovar 1 strains cannot utilize carbohydrates and alcohols, strains of biovar 2 can utilize carbohydrates only, those of biovar 3 break down alcohols and carbohydrates while members of biovar 4 can metabolize alcohols only, and biovar 5 strains can utilize carbohydrates and mannitol (Hayward, 1991). In the race classification race 1 strains are native to tropical countries and known to attack mostly Solanaceous crops, such as tomato, tobacco, potatoes, and eggplant, and Solanaceous weeds. Race 2 is native to the Caribbean islands, the Philippines, and tropical Americas, and strains in this group are known to attack triploid bananas, plantain, and

Heliconia causing moko and bugtok diseases (French et al., 1998; EPPO 2004). Race 3 strains are distributed in all continents and are known to attack Solanaceous crops including potato and tomato, Solanaceous weeds, and geraniums; these members fall in biovar 2 (Deny, 2006; Pradhanang et al., 2005; Genin, 2010). Isolates of potato that fall in biovar 2 were further divided based on habitat into Andean (2A) and tropical (2T) biovars (French, 1994). Race 4 strains inhabit Hawaii and Asia and are known to attack members of the Zingiberaceae family (ginger). Race 5 strains inhabit China and cause mulberry wilt (He et al., 1983). However, it has been difficult to define the race and biovar system classification. For example, in classical race classification, bacteria belonging to the same race are differentiated based on their ability to infect single host species, which is not the case with the RSSC (Alvarez, 2005). The biovar system may lead to false-positive results due to the richness of the growth medium that favors rapid-growing saprophytes (Singh et al., 2010). Biovar characterization does not also provide information on genetic relationships between members of the same group, such as biovar 2, which contains strains of Asian, African, and South American origin (Denny, 2006). Furthermore, it is difficult to correlate the relationship between biovars and races in the RSSC as one race may accommodate several biovars and vice versa except for R3B2 strains in which biovar and races can be correlated (Champoseau and Momol, 2009; Ahmed et al., 2013).

The RSSC encompasses the following genetically related wilt pathogens: *R. solanacearum* (bacterial wilt), *R. syzgii* (clove Sumatra disease), and BDB (blood banana disease) (Fegan and Prior 2005; Allen et al., 2005; Denny, 2006). Grouping within the RSSC was facilitated by DNA-DNA hybridization, partial sequencing of 16s DNA, and

phenotypic studies (Denny, 2006). Variation of genetic groups within the RSSC raised the need for more studies and the application of phylogenetic analyses. Thus, multiple gene sequencing approaches that involved sequencing of whole genomes, the internal transcribed spacer region (ITS) of 16s - 23 rRNA, or megaplasmid virulence genes such as the hypersensitive response gene (*hrpB*), endoglucanase (*egl*), and housekeeping genes such as DNA mismatch repair (*mutS)* gene were explored (Poussier et al., 2000; Fegan and Prior, 2005; Lewis Ivey et al., 2007; Remenant et al., 2011; Wicker et al., 2011; Sagar et al., 2014; Wang et al., 2017; Patil et al., 2017; Kurm et al., 2021). Cook and colleagues (1994) used restriction fragment length polymorphism (RFLP) analysis to study 62 strains from Oceania, the Americas, and Asia. Their analysis led to two divisions of classification in which division I consisted of Asia strains and included members of biovar 5, 4, and 3, and division II included members from the Americas that were from biovars 2 and 1. Taghavi et al. (1996) and Poussieur et al. (2000) did further work through sequencing and analysis of 16S DNA that further divided division II into three subgroups. Subgroup IIA included American strains, subgroup IIB was comprised of BDB and *R. syzgii,* and subgroup IIC included African strains.

Closely related strains from the RSSC were further divided into four phylotype clusters that generally correlated with geographic origin by Fegan and Prior (2005). Phylotyping was conducted using a multiplex PCR assay with primers that targeted the ITS region of the chromosome. Fegan and Prior (2005) identified four phylotype groups: phylotype I that included Asian strains, phylotype II comprised of strains originating from the Americas, phylotype III included strains from Africa, and Indonesia populations were

in phylotype IV. With partial sequence analysis of *egl* analysis, additional sequence variants (sequevars) were identified from among the phylotypes (Poussier et al., 2000). Partial egl gene revealed that African biovar 1 strain was found to be closely related and grouped into the Asiaticum rather than Americanum division (Poussier et al., 2000). Clonal populations within sequevars have been identified using DNA fingerprinting methods such as AFLP analysis and repetitive element sequence-based (rep-PCR) (Lewis Ivey et al., 2007; Poussier et al., 2000). Wicker and colleagues (Wicker et al., 2011) explored distinct evolutionary patterns within the four phylotypes. They used chromosomal maintenance genes including *mutS* and pathogenicity-related genes *egl* and *fliC* to further divide the four phylotypes into eight clades based on multi-locus sequence analysis (MLSA). Using recombinant gene analyses, they concluded that all phylotypes originating from Indonesia as phylotype 4 and other phylotypes arose from adaptation to new environments and host plants because of migration (Wicker et al., 2011).

Polyphasic approaches that include sequencing 16S-23S rRNA, ITS, and *egl*, and DNA-DNA hybridization have been used to further classify RSSC phylotypes into three main genospecies by analysis of average nucleotide identity earlier by Remenant et al. (2011) and later reclassification by Safni et al. (2014). In the proposed reclassification (Safni et al., 2014), *R. pseudosolanacearum* is composed of members of phylotypes I and III, *R. solanaceraum* includes members of phylotype II and *R. haywardii* includes phylotype IV strains. Phylotype IV is further divided into the three subspecies *solanacearum*, s*yzigii,* and *celebensis* that correspond to *R. solanacearum, R. syzigii,* and blood BDB based on phenotype and life cycle. Prior et al. (2016) strongly supported the

separation of the RSSC into three genomic species with genomic comparison, proteomic, metabolic, and DNA-DNA hybridization analyses.

Strains of *Ralstonia* spp. can grow anaerobically and make use of nitrate as final electron acceptor (Dalsing et al., 2015; Prior et al., 2016). Nitrogen metabolism ensures *Ralstonia* spp. survival and protection as well as successful colonization and pathogenicity (Prior et al., 2016). Bacterial wilt-causing strains convert nitrate through a series of chemical reactions to nitrogen gas as the final product (Dalsing et al 2015). These are important pathways used by the bacterium to adapt to fluctuations in oxygen levels in the xylem and protect against host defenses, hence contributing to the aggressiveness and successful establishment of the bacterium (Dalsing et al., 2015). Strains of *R. pseudosolanacearum* (phylotype I and III) can complete the process of denitrification by possessing genes that code for N_20 reductase (NosZ) that convert nitrous oxide (N_2O) to nitrogen gas (N2 gas) while strains of phylotype II and IV lack NosZ and thus cannot complete the process of denitrification to nitrogen gas (Dalsing et al., 2015; Prior et al., 2016). Denitrification helps *Ralstonia* spp. to conquer and detoxify the functioning of nitrate species nitrite and convert nitric oxide (NO_2–and NO) and then to nitrous oxide which is not lethal to the pathogen thus enabling successful pathogen invasion (Dalsing et al., 2015).

Multi-host range and virulence in *Ralstonia spp.* have been attributed to multiple virulence factors. The bacteria possess n-acetylated extracellular polysaccharide (EPS I) that facilitate the wilting process by blocking movement of water in xylem tissue (Champoseu and Momol,2009). EPS also protects the pathogen by limiting recognition of

pathogen-associated molecular patterns (PAMPs) by host plant immune system (Razou et al., 1998). The Type III Secretion System (T3SS) also plays important role in virulence. It is codded as a group of mega plasmid genes (hrp) that induce hypersensitive response (HR) (Van Gijsegem et al., 1995; Meng .2013). Possession of flagella and type IV pili facilitate invasion and attachment to host plant issue (Tans-Kersten et al., 2001; Tans-Kersten et al.,2004) through swimming and twitching motility. Cell Wall Degrading Enzymes (CWDE's also play important role in virulence by aiding host cell invasion. Possession of Egl (endoglucanase) polygalacturonase A, B and C (PehA, B and C), cellobiohydrolase A (CbhA). And pectinmethylesterase (Pme) facilitates wilting process (Huang and Allen; 1997; Zhang et al., 2005).

Management of bacterial wilt disease

The fact that *Ralstonia spp.* is highly diverse with very wide host range complicated control and management strategies of bacterial wilt disease in tomato and other important crops. As with many soilborne diseases, bacterial wilt management is complex and difficult to accomplish using popularly known disease management strategies such as phytosanitary measures, cultural practices, biological control, and chemical treatments to host resistance (Saddler, 2005; Elphinstone, 2005; Champoseau and Momol, 2009, Shutt et al., 2018). This is attributed in part to the ability of the pathogen to survive long term in the soil with favorable high humidity and warm temperature conditions (French et al., 1998; Arwiyanto et al., 2015). Among these strategies, none of the single strategies has proven to be fully effective in controlling bacterial wilt. However, a combination of disease management strategies may reduce bacterial load in pathogen-infested soils. Integrated pest management

strategies are known to reduce or eliminate diseases and therefore sometimes are practiced by farmers. However, limited, and inadequate information on plant diseases and their management, especially in African countries due to deficiency of extension services, is the main reason behind the non-utilization of IPM in plant disease management (Uwamahoro et al., 2018; Testen et al., 2018). Accurate disease diagnosis is key to successful and sustainable disease management programs. Farmers must learn how to properly diagnose diseases, decide on management options, and assess the risks associated with the adoption of the strategy (Savary et al., 2011). In addressing this issue scientists are encouraged to involve farmers in various programs that are aimed at mitigating disease effects (Meijer et al., 2015). Current agriculture extension programs emphasize the involvement of farmers in all extension programs (Kiptot et al., 2007). Involvement of farmers or incorporation of farmers' skills has led to the successful adoption of technology. Thus, introducing and improving farmers' practices is of paramount importance as farmers play a key role in disseminating and integrating the findings into their farming systems (Testen et al., 2016). Farmer participation in scientific research includes demonstration plots as well as participatory research such as mother and baby trials (Testen et al., 2016). Studies show that farmers adapt to new or improved technologies by learning and weighing benefits, for instance, costs, risks, and the contribution of technology to enhanced crop quality and yield compared to local practices (Meijer et al., 2015; Oo et al., 2012). Therefore, knowledge of farmers' farming practices management is important for incorporating into scientific packages for improving or introducing appropriate and effective disease management practices. Thus, our first objective was aimed at learning farmers' practices, their

knowledge and strategies used in disease management for possible incorporation into a broader band effective management scheme.

Host resistance is the preferred means of bacterial wilt management; however, most popular farmers' preferred tomato varieties are susceptible to bacterial wilt (Opena et al., 1990; Wang et al., 1998; Huang et al., 2015). Host resistance has been explored in Solanaceous crops that include pepper (*Capsicum* spp.), eggplant, and tomato (Wang et al., 1998; Oda, 1995; Lin et al., 2008; Du et al., 2016; Salgon et al., 2017). Currently available tomato varieties resistant to bacterial wilt have been bred using wild Solanaceous species, namely *Solanum lycopersicum var. cerasiforme* (*Lycopersicon esculentum* var. *cerasiforme)* and *Solanum pimpinellifolium (L. pimpinellifolium*) (Scott et al., 2005). However, several factors limit breeders in the development of resistant tomato varieties (Rivald et al., 2012). Tomato bacterial wilt resistance can be polygenic or monogenic and in some cases strain- or location-specific (Grimault and Prior, 1994; Hanson et al., 1998; Wang et al., 1998). In tomato line 'Hawaii 7996', bacterial wilt resistance is controlled by two quantitative trait loci (QTL) and resistance is durable in multiple environments (Wang et al., 2013). Strain-specific resistance is non-durable (Scott et al., 1996) and variety performance may be variable across multiple locations (Wang et al., 2013). Favorable conditions for disease occurrence that include soil type, soil microbiota, temperature, and moisture may also play a part in resistance failure (Hanson et al., 1998). Some resistant plants that do not show the wilting symptom exhibit latent infection, restricting the pathogen to the lower stem (Prior et al., 1998; Nakaho et al., 2004; Lebeau et al., 2011). Latently infected plants facilitate bacterial wilt pathogen dissemination.

Grafting has the potential to be a crucial tool in controlling bacterial wilt in tomatoes and other crops in the Solanaceae family. Grafting in vegetable crops makes use of host resistance to plant pathogenic microbes and environmental stresses, hence improving the yield and quality of grafted plants (Rivard et al., 2012). In grafting for disease resistance, the rootstock choice is based on its ability to resist or tolerate soilborne disease, whereas scion choice is based on fruit quality, yield potential, horticultural characteristics, and farmer or market preferences (Black et al., 2003; Rivard et al., 2012). The technique has been in practice since 1920 in Korea and Japan where initially it was used to control soil-dwelling pathogens and nematodes in cucurbits (Rivero et al., 2003). Likewise, in recent times grafting has been established as an appropriate integrated pest management (IPM) technique for Solanaceous crops that not only improves plant vigor, quality, and yield by facilitating plant nutrient and water uptake but also decreases plant susceptibility to pests and root and foliar diseases (Black et al., 2003; Louws et al., 2010; Guan et al., 2012).

Many Solanaceous rootstocks have been identified and explored for their performance in reducing bacterial wilt on susceptible horticulturally preferable varieties. Researchers in China deployed wild tomato species lines (CH-2-21, 25 and 26) as rootstocks for grafting fresh market tomato with 80-100% success in controlling bacterial wilt disease (Lu et al., 1992). Tomato breeding lines 'Hawaii 7996', Hawaii 7997, and Hawaii 7998 are known for reduced bacterial wilt incidence and severity in worldwide locations (Grimault et al. 1995, Hanson et al., 1998, Scott et al., 2005, Lebeau et al 2011; Wang et al 2013). Rahmawat and Arwiyanto (2020) reported about 40% bacterial wilt

incidence when ‘Hawaii 7996’ was grafted to susceptible tomato scions in Indonesia. Rivard and Louws (2008) reported a 100% survival rate in heirloom tomato plants grafted onto ‘Hawaii 7996’ and CRA-66 rootstocks. Similar results were observed in Brazil using ‘Hawaii 7996’ as rootstock for commercial tomato varieties (Cardoso et al., 2012) and Louisiana, USA against phylotype I and II isolates (Lewis Ivey et al., 2021). In the same context Scott et al. (1995) crossed a susceptible tomato variety with Hawaii 7997 to obtain the variety Neptune with bacterial wilt resistance. However, a limited spectrum of resistance was observed and in 2009 they released lines Fla8109 and Fla8109b that had similar pedigrees as Hawaii 7997 (Scott et al., 2009). Other commercial tomato rootstocks Cheong Gang (Seminis), Shield (Rijk Zwaan), and RST-04-106-T (DP Seeds) were reported to fully control bacterial wilt disease when grafted to susceptible tomato scions (Suchoff et al., 2019).

Eggplant is preferred to tomato as a rootstock because of eggplant’s durable resistance to *Ralstonia* spp. and ability to survive in flooded soils (Lee et al., 2013). In eggplant, bacterial wilt resistance genes segregate as single genes (*ERs1* and *RE-bw*) (Salgon et al., 2017). The eggplant rootstock EG203 (AVRDC The World Vegetable Center) was reported to survive at a rate of >95% in bacterial wilt-infested soils. Fresh market wilt-susceptible tomato varieties TStarE and Victoria grafted onto five eggplant rootstock accessions VI041979A, VI041809A, VI041984, VI041945, and VI041943 from AVRDC exhibited 0-20% bacterial wilt incidence (Manickam et al., 2021). Other eggplant lines or varieties including SM164, SM6, Surya, and AF9125 exhibited promising resistance to phylotype I and II *Ralstonia* spp. strains (Lewis Lewis Ivey et al., 2021).

Tanzanian tomato farmers consider qualities such as fruit shape, size, yield, flavor, and market preferences in choosing varieties (Testen et al., 2016). The lack of availability of varieties resistant to bacterial wilt and/or soilborne diseases and having preferred horticultural traits curtails tomato production, especially on smallholder farms in Tanzania and other East African countries (Luzi Kihupi et al., 2015; Akemo et al., 2002). The inadequate supply of quality seeds and breeding programs for developing locally adapted varieties (Minja et al., 2011; Testen et al., 2016) limits the availability of disease resistant cultivars in Tanzania. Grafting farmer-preferred tomato varieties onto locally adapted, bacterial wilt-resistant rootstocks would provide an effective means of disease management while preserving farmer preferences. However, commercial rootstock varieties tested and deployed in other countries are not available or prohibitively expensive for smallholder farmers in Tanzania. Affordable open-pollinated (non-hybrid) rootstock varieties or lines that enable farmers to save and share seeds are needed. Therefore, our second objective was aimed at characterizing Tanzanian *Ralstonia* spp. strains from key tomato producing regions of Tanzania. And the third objective was aimed at screening selected rootstocks with strains collected from key tomato producing regions of Tanzania. The approach would save in limiting phylotype specific host resistance thus leading to targeted bacterial wilt management approach.

In practice studies conducted on fumigation of bacterial wilt infected fields have proved that pesticide/fumigation application can be efficient method for managing bacterial wilt Buddenhagen, 1986; Pradhanang et al., 2005, Muthoni et al., 2012) despite being costly and difficult to practice in large scale farming (Messiha et al., 2007;

Muthoni, 2012). Though farmers in third world countries are not commonly practicing soil fumigation, they are using a lot of pesticides in trying to manage soilborne diseases including bacterial wilt. Farmers especially tomato growers in Tanzania have been reported to depend on pesticides as their main pest management strategy (Ngowi et al. 2007; Maerere et al. 2010). These researchers have also reported inappropriate and overuse of these pesticides among vegetable growers in Arusha, Manyara and Morogoro region. The use of tank mixes and pesticide concoctions is a common practice among many vegetable farmers in these regions to the extent of having reported cases of pesticide poisoning during/after spray (Ngowi et al 2007). This is not only hazardous to humans and animals but also very deleterious to the environment.

Recently, increasing concerns of their hazardous health effects and environmental contamination, and probably pesticides resistance builds up raised the need for safe and effective alternative disease management strategies. Among the potential strategies, the use of soil rehabilitative treatments such as ASD is promising for the management of bacterial wilt (Rivard and Louws, 2008; Momma, 2008; Mazzola and Hewavitharana, 2014). Among soil treatments to suppress bacterial wilt, solarization has been effective only for *Ralstonia* biovar 2 strains that inhabit cool areas, as strains from other biovar groups easily adapt to higher temperatures (French, 1994). Anaerobic soil disinfestation (ASD) has been shown in numerous studies to reduce *R. solanacearum* populations to undetectable levels or symptoms have been delayed or reduced in susceptible plants (Blok et al., 2000; Momma et al., 2006; Messiha et al., 2007; Van Overbeek et al., 2013). No ASD studies have been reported in Eastern Africa or Tanzania in particular.

Anaerobic soil disinfestation is a soil rejuvenation process that involves the creation of an anaerobic environment in water-saturated soil amended with high carbon-based organic materials. Anaerobic conditions are generated by flooding amended soil and covering it with plastic sheeting to limit exchanges of gases for 2-15 weeks (Blok et al., 2000; Momma et al., 2006; Messiha et al., 2007). The addition of organic matter promotes the multiplication of soil microbial flora that produce compounds with antimicrobial activity and create an anaerobic environment harmful to aerobic microorganisms, including most plant pathogens (Momma, 2008; Runia et al., 2014; Huang et al., 2016; Testen and Miller, 2018). Anaerobic organic matter consumption by soil microbes leads to the release of abundant acetic and n-butyric acids among other organic acids (Momma et al., 2006; Sanabria et al., 2020) into the soil (Momma, 2008; Butler et al., 2012). Other organic acids include isovaleric, isobutyric, and propionic acids that are released in small quantities (Sanabria et al., 2020).

The efficacy of carbon sources used in ASD is a function of the quantity and types of metabolic products that are produced as they are broken down (Shrestha et al., 2016). The quantity and type of carbon source selected determine the type of organic acids and other toxic products released during the ASD (Hewavitharana et al., 2014). In some cases, availability may also guide the choice of carbon source used in ASD. Anaerobic soil disinfestation with cover crops such as mustard greens, plant by-products including rice husk, grape pomace, and wheat bran, molasses, and animal manure as carbon sources has been reported to effectively reduce diseases caused by soilborne pathogens (Blok et al., 2000; Momma, 2008; Testen and Miller 2019; Testen et al., 2021; Khadka and Miller

2021). Acetic and n-butyric acids reduced *R. solanacearum* populations in ASD-treated soils amended with wheat bran and other organic carbon sources with subsequent decreases in bacterial wilt incidence to below measurable rates in tomato (Momma et al., 2006; Momma, 2008). Fresh grass, commercial media containing plants with high protein content, and potato haulms have been used as ASD carbon sources to treat soils contaminated by *R. solanacearum* with great success (Messiha et al., 2007; Van Overbeek et al., 2013). Messiha et al. (2007) reported *R. solanacearum* population reductions of over 90% after ASD treatment.

Ralstonia spp. strains can grow anaerobically as they invade and colonize plants by using nitrate respiration to generate energy, transforming inorganic nitrate into gaseous form (Chapter 2; Dalsing et al., 2015; Prior et al., 2016). However, a combination of factors and mechanisms in addition to soil anaerobicity contributes to the efficacy of ASD in suppressing soilborne pathogens (Momma et al., 2006; Runia et al., 2014). Microbial metabolic activities responsible for the accumulation of organic acids in ASD-treated soil also result in the release of toxic gases such as methane, nitrous oxide, ammonia, and hydrogen sulfide that have antimicrobial effects and help to reduce soil pathogen populations (Runia et al., 2014). Also, the incorporation of high-carbon amendments and limited oxygen promotes shifts towards anaerobic microbial populations that include *Clostridia*, *Klebsiella*, *Enterobacter* species (Huang et al., 2016; Testen and Miller, 2018). Members of these genera and others act as natural biocontrol agents through the production of soil anaerobic conditions, reduced soil pH, and formation of toxic compounds (Hewavitharana et al., 2014; Choi et al., 2018). They also out-compete and suppress the

growth of soilborne pathogens by their rapid multiplication and adaptation to changes in soil conditions.

Previous ASD research concentrated on optimization of the process and effectiveness of the treatment. Recently ASD treatments became operational and used in Japan (Momma et al., 2006), Netherlands (Blok et al 2000). In the United States, ASD has been effectively used in Tennessee, Ohio, Florida, California, Washington (Testen et al., 2018; Sanabria et al., 2020; Mazolla and Hewavitharana, 2014; Butler et al., 2012; McCarty et al., 2014; Achmon et al., 2016; Browne et al., 2018). Likewise, ASD has been effectively used in the management of bacterial wilt pathogen in Japan (Momma, 2008) and Netherlands (Van Overbeek et al., 2013). In all these experiments, populations of *R. solanacearum* have been reduced to the extent of being undetectable or delayed/reduced symptoms in susceptible plants. NO ASD studies have been recorded in Eastern Africa and Tanzania in particular. Therefore, the fourth objective of our research was aimed at investigating the efficacy of ASD treatment in reducing populations of *R solanacearum* and reducing disease symptoms in farmers' preferred tomato varieties that are highly susceptible to bacterial wilt. In the study, different locally available carbon sources were tested for their efficacy in both field and greenhouse conditions. Obtained results will be used in suggesting recommendations that will be added in IPM packages and extended to farmers for the management of soilborne diseases in tomatoes.

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Chapter 2. Identification and Characterization of *Ralstonia pseudosolanacearum* from Solanaceous Crops in Mainland Tanzania.

Abstract

Tomato is one of the most important horticultural crops in Tanzania in terms of productivity and economic importance. However, cases of bacterial wilt caused by *Ralstonia* spp. are increasing at an alarming pace, especially in prime tomato producing regions. To understand the diversity and virulence of *R. pseudosolanacearum* in Tanzania, symptomatic Solanaceous plants were collected from the major tomato-producing regions Arusha, Tanga, Morogoro, Iringa, and Mbeya. Sixty-one strains of *R. pseudosolanacearum* were recovered from infected plant tissues, including 45 strains from tomato, 14 strains from eggplant, and two strains from sweet pepper. All 61 strains were confirmed as *R. pseudosolanacearum* using PCR with species-specific primers. The strains were further classified into phylotype using multiplex PCR with phylotype-specific primers, whereby 93.2% (N=57) were confirmed as phylotype I, and 6.8% (N=4) as phylotype III. The partial endoglucanase gene (*egl)* was sequenced for twelve representative strains, which were assigned sequevars 18 (N=4), 20 (N=1), 22 (N=1), and 31 (N=6). All strains grew anaerobically and two of the strains also produced nitrogen. The virulence of 61 strains was assessed on different hosts; 82% were virulent to tomato 'RioGrande', 77.7% were

virulent on sweet pepper ‘Yolo wonder’, and 39.3% were virulent on tomato ‘Hawaii 7996’, with a significant variation (P<0.0001) in mean disease incidence of (45.7%,) (26.6%), and (7.3%), respectively. Latent infection was significantly (P<0.0001) higher in ‘Yolo wonder’ sweet pepper than in tomato ‘RioGrande’ and ‘Hawaii 7996’. ‘RioGrande’ progressed the fasted with mean area under disease progress curve (AUDPC) value of 201.3 followed by ‘Yolo wonder’ pepper (AUDPC=97.1), and ‘Hawaii 7996’ tomato (AUDPC=26.5). The study revealed the high level of genetic and phenotypic diversity among the *R. solanacearum* strains from Tanzania, calling for a targeted evaluation of potential management practices to suit the pathogen population diversity.

Introduction

Tomato contributes about 64% of the total vegetable crop production in Tanzania (De Putter et al., 2011; Luzi-Kihupi et al., 2015; Mutayoba and Ngaruko, 2017). Tanzania has an average production of 2.2 -16.5 t/Ha, which is far lower than the mean global tomato production of 27.5 t/Ha (FAO, 2005; Maerere et al., 2006). Deteriorating soil fertility, low-yielding varieties, unreliable rainfall, disease, pests, and poor farming practices are the main factors that contribute to low tomato production (Minja et al., 2011).

Insect pests and diseases are the leading constraints accounting for 56% and 88% of tomato yield losses, respectively (Minja et al., 2011). Up to 100% yield losses have been recorded in areas with high disease and pest pressure (Opena et al., 1990; UMADEP, 2003; CABI, 2017); Minja et al., 2011; De Putter et al., 2011; Testen et al., 2016). Fungal, bacterial, and viral diseases extensively affect tomato production in parts of subtropical and tropical nations (Hayward, 1991; Elphinstone, 2005). Bacterial diseases in tomato include those caused by *Xanthomonas* spp. (bacterial spot), *Ralstonia* spp. (bacterial wilt), *Clavibacter michiganensis* subsp. *michiganensis* (bacterial canker), and *Pseudomonas syringae* pv. *tomato* (bacterial speck) (Black et al., 1999; Shenge and Mabagala, 2007; Shenge et al., 2010; Mbega et al., 2012; Testen et al., 2018).

Among the diseases caused by bacterial pathogens, bacterial wilt is the leading disease affecting tomato in Tanzania (Black et al., 1999; Baitani, 2017; Aloyce, 2020). Bacterial wilt is caused by members of the *R. solanacearum* species complex (RSSC), the second most recognized bacterial pathogen after *Pseudomonas syringae* pathovars causing

devastating crop and yield loss globally, especially in Solanaceous crops (Champoseu and Momol, 2009; Mansfield et al., 2012; Meng, 2013). Members of the RSSC are pathogenic soil proteobacteria, widespread in subtropical, tropical, and temperate areas (Hayward, 1991; Yabuuchi et al., 1995; Elphistone, 2005). Previously, only tropical lowland areas characterized by warm climate were reported to support the perpetuation of RSSC bacteria (Hayward 1991). However, Elphinstone (2005) identified members of the RSSC that belong to race 3 biovar 2 (R3B2) that survive in cooler and high-altitude areas and cause southern wilt, bacterial wilt, and brown rot in geranium, tomato, and potato respectively (Champoseau and Mommol, 2009). Depending on host and location, strains of *Ralstonia* spp. may differ in virulence (Lopez et al., 1996). Variability of pathogen populations, soil and environmental conditions may also influence strain virulence as shown in several studies over the past decade (Hanson et al., 1998)

Historically, *Ralstonia spp.* strains were characterized based on their capability to break down a series of sugars/alcohols and attack a series of crops, hence falling into five biovars and races (Martin and French, 1985; French et al, 1998; EPPO, 2004; Oslon, 2005; Denny, 2006). In the biovar classification scheme, biovar 1 strains cannot utilize carbohydrates and alcohols, strains of biovar 2 can utilize carbohydrates only, those of biovar 3 break down alcohols and carbohydrates while members of biovar 4 can metabolize alcohols only, and biovar 5 strains can utilize carbohydrates and mannitol (Hayward, 1991). In the race classification race 1 strains are native to tropical countries and known to attack mostly Solanaceous crops, such as tomato, tobacco, potatoes, and eggplant, and Solanaceous weeds. Race 2 is native to the Caribbean islands, the Philippines, and tropical

Americas, and strains in this group are known to attack triploid bananas, plantain, and heliconia causing moko and bugtok diseases (French et al., 1998; EPPO 2004). Race 3 strains are distributed in all continents and are known to attack Solanaceous crops including potato and tomato, Solanaceous weeds, and geraniums; these members fall in biovar 2 (Deny, 2006; Pradhanang et al., 2005, Genin, 2010). Isolates of potato that fall in biovar 2 were further divided based on habitat into Andean (2A) and tropical (2T) biovars (French, 1994). Race 4 strains inhabit Hawaii and Asia and are known to attack members of the Zingiberaceae family (ginger). Race 5 strains inhabit China and cause mulberry wilt (He et al., 1983). However, it has been difficult to define the race and biovar system classification. For example, in classical race classification, bacteria belonging to the same race are differentiated based on their ability to infect single host species, which is not the case with the RSSC (Alvarez, 2005). The biovar system can lead to false-positive results due to the richness of the growth medium that favors rapid-growing saprophytes (Singh et al., 2010). Besides, biovar characterization does not provide information on genetic relationships between members of the same group, such as biovar 2, which contains strains of Asian, African, and South American origin (Denny, 2006). Furthermore, it is difficult to correlate the relationship between biovars and races in the RSSC as one race may accommodate several biovars and vice versa except for R3B2 strains (Champoseau and Momol, 2009; Ahmed et al., 2013).

The RSSC encompasses the following genetically related wilt pathogens: *R. solanacearum* (bacterial wilt), *R. syzgii* (clove Sumatra disease), and BDB (blood banana disease) (Fegan and Prior 2005; Allen et al., 2005; Denny, 2006). Grouping within the

RSSC was facilitated by DNA-DNA hybridization, partial sequencing of 16s DNA, and phenotypic studies (Yabuuchi et al., 1995; Poussier et al., 2000; Fegan and Prior, 2005. Safni et al., 2014). Variation of genetic groups within the RSSC raised the need for more studies and the application of phylogenetic analyses. Thus, gene sequencing approaches that involved sequencing of whole genomes, the Internal Transcribed Spacer region (ITS) of 16s - 23 rRNA, or megaplasmid virulence genes such as the hypersensitive response gene (*hrpB*), endoglucanase (*egl*), and housekeeping genes such as DNA mismatch repair (*mutS)* gene were explored (Poussier et al., 2000; Fegan and Prior, 2005; Lewis Ivey et al., 2007; Remenant et al., 2011; Wicker et al., 2011; Sagar et al., 2014; Wang et al., 2017; Patil et al., 2017; Kurm et al., 2021). Cook and colleagues (1994) used restriction fragment length polymorphism (RFLP) analysis to study 62 strains from Oceania, the Americas, and Asia. Their analysis led to two divisions of classification in which division I consisted of Asia strains and included members of biovar 5, 4, and 3, and division II included members from the Americas that were from biovars 2 and 1. Taghavi et al. (1996) and Poussieur et al. (2000) did further work through sequencing and analysis of 16S DNA that further divided division II into three subgroups. Subgroup IIA included American strains, subgroup IIB was comprised of BDB and *R. syzgii,* and subgroup IIC included African strains.

Closely related strains from the RSSC were further divided into four phylotype clusters that generally correlated with geographic origin by Fegan and Prior (2005). Phylotyping was conducted using a multiplex PCR assay with four forward primers and one reverse primer that targeted the ITS region of the chromosome. They identified four

phylotype groups: phylotype I that included Asian strains, phylotype II comprised of strains originating from the Americas, phylotype III included strains from Africa, and Indonesia populations were in phylotype IV. With partial sequence analysis of *egl* and amplified fragment length polymorphism (AFLP) analysis, additional sequence variants (sequevars) were identified from among the phylotypes (Poussier et al., 2000). These analyses grouped African biovar 1 strain into the Asiaticum rather than Americanum division, apart from other biovar 1 strains (Poussier et al., 2000). Clonal populations within sequevars have been identified using DNA fingerprinting methods such as AFLP analysis and repetitive element sequence-based (rep-PCR) (Lewis Ivey et al., 2007; Poussier et al., 2000). Wicker and colleagues (Wicker et al., 2011) explored distinct evolutionary patterns within the four phylotypes. They used chromosomal maintenance genes including *mutS* and pathogenicity-related genes *egl* and *fliC* to further divide the four phylotypes into eight clades based on multi-locus sequence analysis (MLSA). Using recombinant gene analyses, they concluded that all phylotypes originating from Indonesia as phylotype 4 and other phylotypes arose from adaptation to new environments and host plants because of migration (Wicker et al., 2011).

Polyphasic approaches that include sequencing 16S-23S rRNA, ITS, and *egl*, and DNA-DNA hybridization have been used to further classify RSSC phylotypes into three main genospecies by analysis of average nucleotide identity earlier by Remenant et al. (2011) and later reclassification by Safni et al. (2014). In the proposed reclassification (Safni et al., 2014), *R. pseudosolanacearum* is composed of members of phylotypes I and III, *R. solanaceraum* includes members of phylotype II and *R. haywardii* includes

phylotype IV strains. Phylotype IV is further divided into the three subspecies *solanacearum*, s*yzigii,* and *celebensis* that correspond to *R. solanacearum, R. syzigii,* and blood BDB based on phenotype and life cycle. Prior et al. (2016) strongly supported the separation of the RSSC into three genomic species with genomic comparison, proteomic, metabolic, and DNA-DNA hybridization analyses.

Strains of *Ralstonia* spp. were also characterized on their ability to grow anaerobically and make use of nitrate as a terminal electron acceptor (Dalsing et al., 2015; Prior et al., 2016). These are important pathways used by the bacterium to adapt to fluctuations in oxygen levels in the xylem and protect against host defenses, hence contributing to the aggressiveness and successful establishment of the bacterium (Dalsing et al., 2015). Strains of *R. pseudosolanacearum* (phylotype I and III) can complete the process of denitrification by possessing genes that code for N_20 reductase (NosZ) that convert nitrous oxide (N_2O) to nitrogen gas (N_2 gas) while strains of phylotype II and IV lack NosZ and thus cannot complete the process of denitrification to N_2 gas (Dalsing et al., 2015; Prior et al., 2016). Denitrification helps *Ralstonia* spp. to detoxify the nitrate (NO_3) and nitrite (NO_2) compounds to nitrous oxide (N_2O) which is not lethal to the pathogen thus enabling successful pathogen invasion (Dalsing et al., 2015). Depending on host and location, strains of *Ralstonia* spp. may differ in virulence (Lopez et al., 1996). Variability of pathogen populations, soil and environmental conditions may also influence strain virulence (Hanson et al., 1998)

The aim of this study was to understand the genotypic and phenotypic (virulence) variation in RSSC populations in Tanzania. Characterization work will pave the way

towards improving bacterial wilt management strategies for Tanzania. The following are the specific objectives of this study:

a. Isolate and identify *Ralstonia* spp. strains affecting tomato/Solanaceous crops in fields surveyed for bacterial wilt disease, and group them taxonomically using molecular methods.
b. Assess variations in virulence of the isolated, identified strains on tomato and peppers.
c. Determine the ability of the isolated strains to grow anaerobically and convert nitrate to nitrogen gas.

Materials and Methods

Sample collection. Symptomatic plants from Solanaceous crops (tomato, sweet pepper, potato, and eggplants) were collected from five key tomato producing regions in mainland Tanzania: Iringa, Mbeya, Morogoro, Arusha, and Tanga. Bacterial wilt was putatively identified based on symptoms described by Champoseu and Mommol (2009) and confirmed using Agdia *Ralstonia*-specific immunostrips (lateral flow device) (Agdia Inc., Elkhart, IN, USA) as per the manufacturer's instructions. The samples were considered positive for *Ralstonia* when immunostrips revealed two red lines. Approximately 100 g o f diseasedstem and upper root of symptomatic tomato, pepper, or eggplant plants were packed in plastic bags, put inside cooler boxes, and transported to Sokoine University of

Agriculture (SUA). Field information associated with each sample was recorded including the host plant, field and plant, location geocodes, and collection date.

Pathogen isolation. Stems (5cm long) were cut just above the root (crown), submerged in 70% ethanol for one minute then rinsed once with sterile distilled water. The stems pieces were blotted with sterile paper towels before being immersed in tubes containing 5ml sterile distilled water and left to stream for 1-2hrs. The resulting bacterial suspension was streaked onto tetrazolium chloride (TZC) medium and incubated at 29°C for 48hrs. Colonies with a morphology typical of virulent *Ralstonia* spp (Yabuuchi et al 1995) were selected and sub-cultured into fresh TZC medium until pure cultures were obtained. In total sixty-one strains were isolated and stored for genotypic and phenotypic (Table A. 2). Phylogenetic analysis was conducted with a smaller subset (N=12) of these strains (Table 2.1).

Preparation of bacterial suspensions. Isolated colonies of *R. solanacearum* (Fig. 2.1A) were obtained from TZC medium and sub-cultured onto fresh plates containing casamino acid-peptone-glucose (CPG) medium. The plates were incubated for 24 hrs at 28°C and single colonies were picked using sterile plastic loops to make suspensions in sterilized distilled water. A Helios Epsilon spectrophotometer (Thermo Electron Corp, USA) was used to check the concentration of the bacterial suspensions at an optical density (OD) of 600 nm, and the concentrations were adjusted to $5x10^8$ CFU/ml (OD_{600} = approx. 0.5).

Serology. The Agdia double antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA) kit for *Ralstonia* spp*.,* 96-well plate format, was used to confirm the identity of isolated, purified strains to the genus level. For RSSC the threshold for detection in

DAS-ELISA is 20-200 CFU/g (Priou et al., 2005). The assay was conducted according to the manufacturer's instructions. Approximately of 1x 10^8 CFU/ml bacterial cell suspension (100µl) from each of the 61 strains was pipetted into wells with three replicate wells per sample. Positive and negative controls were provided with the kit. Following the process of sample incubation, conjugate enzyme, and substrate addition a bright blue color change was expected for positive samples and no color change for negative samples. Negative and positive controls were used as visual guides for results scoring. The test was conducted twice per sample group.

DNA extraction and purification. Thoroughly mixed bacterial suspensions (0.5ml, approx. 5x 10^8 CFU/ml) from each of the 61 strains were either pipetted into new tubes for freezing and thawing or pipetted onto Whatman Flinders Technology Associates (FTA) cards (GE Health Care UK Limited, Buckinghamshire, UK) as instructed on the label. The cards were labeled with the strain identity and left to dry in a desiccator at room temperature (25-26°C) for 3-7 days. Each card contained four samples and each strain was placed on two different FTA cards. An FTA punching tool was cleaned by wiping with a paper towel presoaked in 70% alcohol before each use was used to punch 10mm FTA discs from well-dried FTA cards. The FTA discs were transferred into separate Eppendorf tubes (10 discs/tube), then 200µl of FTA purification reagent solution was added into each tube and incubated at room temperature for 15 minutes before removing the solution with a sterile pipette. The process was repeated twice. Then 200 µl of 1X Tris-EDTA (TE) buffer pH 8.0 was added to the cleaned FTA discs, incubated for 15 minutes, and removed using a sterile pipette. This was repeated twice. Tubes with cleaned FTA discs were left to dry at room

temperature and stored for use as DNA templates at room temperature. Frozen bacterial cultures were thawed at room temperature and used directly as DNA templates for endoglucanase gene PCR amplification. Another set of DNA templates was also prepared from freeze and thawing the pure colonies of *Ralstonia* spp. colonies. The template was prepared from single colonies of 24hrs old cultures from CPG medium. The colonies were suspended in 100µl sterile distilled water frozen in -16°C for 2hrs and thawed for 1 hr. Freeze and thaw process was repeated twice.

Genotypic characterization of bacterial strains. Genotypic characterization included confirmation of species identity of 61 strains (Table 2.1) by PCR assays with species- and phylotype-specific primers, and sequencing of the partial endoglucanase gene (*egl*) as described below.

Species-specific PCR. PCR reaction mixture of 25 µl was prepared using Go Taq Green master mix (Promega Inc, Madison, Wisconsin, USA), 4µm each of forward and reverse primers (Table A. 3), and FTA discs (1 disc per reaction) as DNA template. PCR amplification conditions were 3 minutes of DNA denaturing at 94°C, 1-minute annealing at 53°C, and 1.5 minutes of extension at 72°C; 30 cycles at 94°C, 60°C and 72°C for 30 seconds and five minutes of final extension at 72°C. Amplified products were separated in a 1.5% agarose gel by electrophoresis in Tris-acetate EDTA buffer (1× TAE) at 115V for 1hr, and post stained by adding 100µl of 1% ethidium bromide solution to the molten precooled agarose gel. The PCR products were visualized and photographed under a UV gel documentation system (Uvitec, Cambridge, UK). All samples with expected amplicons (280bp) were identified as species-members of the RSSC**.**

Phylotyping. Five phylotype-specific primers (Fegan and Prior, 2005) and two species complex-specific primers (Opina et al. 1997) (Table A. 4) 4µm each were mixed with Master mix and FTA discs (one/reaction) as DNA templates. DNA extracts (50ng) of *R. solanacearum* GMI 1000, K60, UW386, and UW443 obtained from Dr. Caitilyn Allen, University of Wisconsin, Madison, WI, USA, were used as controls for phylotypes I, II, III, and IV, respectively. The PCR amplification regime was 3 minutes of DNA denaturing at 94°C, 1-minute annealing at 53°C, and 1.5 minutes of extension at 72°C; 30 cycles at 94°C, 60°C and 72°C for 30 seconds and five minutes of final extension at 72°C. Amplified products were separated in a 1.5% agarose as described visualized and photographed under UV gel documentation system. Phylotypes I, II, III, and IV were identified by the presence of 372, 144, 213, and 91 bp amplicons, respectively (Fegan and Prior, 2005) in addition to the 280 bp product (Opina et al., 1997).

Partial endoglucanase gene (*egl*) sequencing. PCR to *egl* (750 bp) was conducted using primers Endo-F and Endo-R (Fegan and Prior, 2005; Poussier et al., 2000) (Table A. 4). A PCR reaction mixture of 25 µl was prepared using Go taq green master mix (Promega Inc), 4µm each of forward and reverse primers, and the DNA template was 2µl of frozen and thawed colonies. PCR amplification conditions were 9 minutes for DNA denaturing at 96°C, 30 cycles of 95°C, 70°C for 1 minute and 72°C for 2 minutes, final extension 10 minutes at 72°C. Amplicons were separated by electrophoresis in a 1.2% agarose gel as described in the previous section. Amplified samples for which a clear single band (750bp) was observed in the gel were selected and aliquoted into new PCR tubes (at least 20 µl/sample). The tubes with aliquoted PCR products were incubated for 5 minutes at 95°C,

sealed with laboratory film and packed in zip lock bags for shipping. Sanger sequencing was performed at Macrogen Europe (Amsterdam, the Netherlands).

Phylogenetic analysis. Sequences were edited and aligned using MacVector version 18.0.1 software (Apex, NC). About 31 reference strains (Table A. 4) including African (28), American (one), and outgroup (two) strains were selected from the NCBI database. BLASTn and similarity matrix analysis was performed using MacVector Software (Vers. 18). The aligned multiple FASTA files were uploaded into MEGA 7 (Tamura et al. 2011). software and the phylogenetic trees were constructed using the Neighbor-joining method using Jukes and Cantor's models (Juke and Cantor 1969). with 1000 bootstrap resampling.

Pathogenicity testing. Sixty-one *Ralstonia* sp. strains isolated from symptomatic tomato, sweet pepper, and eggplant collected from Morogoro, Iringa, Mbeya, and Tanga were tested for pathogenicity (Table 2.1, Table A. 2). The protocol used was adapted from Wang et al. (2017) with modification on inoculation method in which soil drenching method was used. Five plants per cultivar in three replications were used to lay a split-plot in a randomized complete block design. The replications were blocked over time. Varieties were the main plots and strains were subplots. Seedlings for these experiments were raised from tomato 'RioGrande' selected from the list of most preferred tomato varieties and 'Hawaii 7996' background of 'WG120' Ohio line and sweet pepper 'Yolo wonder' selected as one of the susceptible pepper variety from previous experiments. Seeds of selected varieties were sown in polythene bags (0.5kg) with pre-autoclaved forest soil amended with NPK fertilizer (YaraMila Winner 15-9-20) for 4 weeks. The plants were maintained in a screenhouse located at the Sokoine University of Agriculture. The

screenhouse had a daily average temperature of 30°C and nighttime of 22°C and plants were watered once daily. Four-week-old seedlings were inoculated with individual *Ralstonia* sp. strains by drenching the soil at the base of each plant with 50 ml of a 48-hr *R. pseudosolanacearum* bacterial suspension grown on Casamino acid-Peptone-Glucose (CPG) medium. The inoculum was prepared by flooding pure cultures with sterile distilled water, scraping the cells with a sterile spreader, and pouring the resulting suspension into a flask containing a known amount of sterile distilled water. The concentration of inoculum was checked with a Helios Epsilon spectrophotometer (Thermo Electron Corp, USA) at OD_{600} and adjusted to 10^8 CFU/ml concentration (OD_{600}= 0.1) Control plants from each of the varieties were mock inoculated with sterile distilled water. Plants were watered once daily and fertilized with YaraMila NPK once every two weeks. Pots with inoculated plants were placed on 15cm Petri plate bottoms and maintained in double plastic trays to trap overflowing water and prevent cross-contamination between inoculated plants. At the end of the experiment, all plant tissues and soil were autoclaved while pots, tables, and floor were washed and disinfected with 2% sodium hypochlorite diluted from concentrated industrial bleach (15%).

Disease assessment: Plants were assessed weekly for the incidence and severity of wilting symptoms (Fig 2.1B). Disease incidence was assessed by counting the total number of plants (N) and the number of plants with bacterial wilt symptoms (n) for each line and isolate inoculated. The incidence of bacterial wilt was calculated using the equation

$$Incidence\ of\ bacterial\ wilt = \frac{n}{N} * 100$$

Disease severity values were obtained by rating diseased plants for each line x strain combination using a 1-5 scale (Horita and Tsuchiya, 2001) in which 1=asymptomatic, 2=two leaves withered, 3=three leaves wilted, 4=at least four leaves wilted, and 5=dead plant. Each severity score was converted to a leaf damage scale (0-100 %), where 0 represented asymptomatic leaves and 1, 2, 3, 4, and 5 represented 20, 40, 60, 80, and 100% wilted leaves, respectively. Mean disease severity was expressed as the mean of all leaf damage percentages for each line x isolate combination. The area under the disease progress curve (AUDPC) was calculated according to the formula (Madden et al., 2007).

$$\text{AUDPC} = \sum_{i=1}^{n} \left(\left(\frac{y_i + y_{i+1}}{2} \right) + (t_i + 1 - t_i) \right)$$

Where y_i = measures of disease level at ith observation and t_i= time of disease measure at ith observation.

To assess latent infection, plants remaining asymptomatic for 8 weeks after inoculation were sampled by cutting a 2 cm stem section from the base of the plant and placing it in a tube containing 2.5 ml of sterile distilled water to allow for bacterial streaming for one hour. Aliquots (100µl) of the suspensions were pipetted into wells of a 96-well microtiter plate and an enzyme-linked immunosorbent assay (*Ralstonia* ELISA kit, Agdia Inc.) was conducted according to manufacturer instructions. Negative and positive controls were provided with the kit and these were used as color change guides to score positive or negative results. Wells with blue color visibly darker than the negative control were scored as positive. Samples that showed no color or lighter blue than the negative control were scored as negative for latent infection. The percentage of plants with latent infection was

calculated by dividing the number of plants that tested positive *for R. solanacearum* by the total number of plants sampled for latent infection multiplied by 100.

Anaerobic growth determination. The method of Prior et al. (2016) was used for assessment of anaerobic growth of 61 *Ralstonia* strains (Table A. 2). Fifteen ml test tubes containing 9ml of Modified Van den Moote medium (VDM) with and without potassium nitrate were inoculated with the strains at an initial concentration of 0.1 (OD_{600}) with three replications. Tubes inoculated with sterile distilled water were used as control in this experiment. The inoculum was prepared from 48-hr *R. pseudosolanacearum* cultures grown on CPG medium. The inoculum was prepared by flooding pure cultures with sterile distilled water, scraping the cells with a sterile spreader, and pouring the resulting suspension into a flask containing a known amount of sterile distilled water. The concentration of inoculum was checked with a spectrophotometer at OD_{600} and adjusted to 5x 10^8 CFU/ml. From this suspension, an aliquot of 1ml was pipetted into 3 replicate tubes containing 9ml of VDM medium with or without potassium nitrate for each bacterial culture. Both inoculation and incubation were carried out under anaerobic conditions in an anaerobic incubator (Memmert Inc. 153, East Frisian, Germany). The tubes were incubated at 28-30°C without shaking for 72hrs. OD_{600} measurements were taken by pipetting 1ml of culture into cuvettes for spectrophotometric analysis. The ratio of OD_{600} values between growth on VDM medium with and without nitrate was calculated; ratios > 1.0 were scored as positive for anaerobic growth and < 1.0 as negative for anaerobic growth. The remaining 9ml of culture was left to grow to 96 hrs and visually assessed for bubble formation.

Cultures were scored as positive for N_2 gas if bubbles were produced and negative if there was no production of bubbles after 96 hrs. of growth.

Data analysis

Statistical analyses were carried out in SAS statistical software (SAS 9.4.4 2017) using Proc GLM (SAS Institute, location). All data were tested for equality of variance with Levene's test for treatments and experiment as fixed effects. When no significant difference was observed between treatment and replications and their interaction the two experiments were combined for analysis. Analysis of variance (ANOVA) was used to compare the responses of varieties (tomato and sweet pepper) and strains using F and T-tests where applicable. Means were separated using the Least Significant Difference (LSD). Area under disease progress curve (AUDPC) values were compared in SAS using Proc GLM. A phylogenetic tree was created using the neighbor-joining Jukes and Cantor model (Juke and Cantor 1969). at 1000 bootstrap values using MEGA 7 (Tamura et al. 2011). software. Cluster analysis was done for grouping strains based on the region of origin, host plant.

Results

Identification to genus using DAS ELISA: All 61 strains tested positive in the Agdia *Ralstonia* ELISA, producing a deep blue coloration in the test wells similar to the positive control, the negative control remained colorless.

Species identification and phylotyping. All bacterial strains were identified as *Ralstonia* sp. using conventional end-point PCR with primers 759/760, with the expected 280 bp product. Using phylotype multiplex PCR, a 142 bp product was amplified for most of the strains (93.4%; N=57), classified as phylotype I. A 91 bp product was amplified for the remaining four strains (6.6%), classified as phylotype III (Table 2.1).

Sequence analyses and sequevar grouping. Of the 61 strains that produce amplicons of the expected 750 bp size, only 12 produced a quality sequence, thus phylogenetic analysis was conducted on these 12 strains only (Table 2.1). The sequences of the remaining 49 strains produced poor quality base calls or no base calls. Tanzanian strains fell into two clusters with bootstrap values >90 (Fig. 2.2). Both phylotype III strains from Tanzania (TZ32 and TZ54, Iringa) clustered with phylotype III reference strains from Zimbabwe (AF295278 and AF295277) that are sequevar 20 and 22 respectively. The second cluster contained ten Tanzanian phylotype I strains of which four strains clustered with Guyana (DQ657595) reference strain (Sequevar 18) and six strains clustered with African strains that belong to sequevar 31 (Fig 2.2) Cluster three contained reference strains used as outgroups (*R. syzygii subsp. indonesiensis* and *R. syzygii subsp. celebesensis*). The similarity index of strains to reference strains ranged from 91.1% to 100% (Figure A. 1)

Pathogenicity and aggressiveness of Tanzanian *R. pseudosolanacearum* strains. The virulence of 61 *R. pseudosolanacearum* strains as indicated by bacterial wilt incidence varied significantly among the strains and host plants ($P<0.0001$) and their interaction was also significant ($P=0.0016$) (Table A.1). Average bacterial wilt incidence across all three varieties ranged from 0-58.8% (Table 2.1). The distribution of virulent strains varied significantly ($P=0.0005$) among the tested tomato and pepper varieties. Of the 61 strains, 50 were virulent on tomato 'RioGrande', 45 on sweet pepper 'Yolo wonder', and 24 on tomato 'Hawaii 7996' (Table 2.2). Nineteen strains were virulent on all three varieties and average bacterial wilt incidence across the varieties was significantly different ($P>0.0001$) among the strains. Eight strains were avirulent on all three varieties (Table 2.1). Bacterial wilt incidence was marginally significantly ($P = 0.0532$) higher across all three varieties for strains isolated in Tanga (32.8%) and Mbeya (31.4%) than Iringa (14.7%) (Table 2.2). Average bacterial wilt incidence in 'RioGrande' (45.8%) was significantly higher than in 'Yolo wonder' (26.3%) and 'Hawaii 7996' (6.4%) (Table 2.1).

The presence or absence of latent infection in plants that survived until the termination of the experiment (8 weeks) differed significantly ($P>0.0001$) among strains and varieties, and there was also a significant ($P<0.0001$) strain x variety interaction (Table A.1). On average across the three varieties, latent infection ranged from 0% to 77.7% in plants inoculated with the 61 strains (Table 2.1). The susceptibility of the tomato and pepper varieties to the 61 strains tested varied significantly ($P<0.0001$) (Table 2.3). Tomato 'RioGrande' with a mean bacterial wilt incidence of 45.8% was significantly more susceptible than 'Yolo wonder' (26.3% incidence) and 'Hawaii 7996' (6.4% incidence).

The area under the disease progress curve also varied significantly (P>0.0001) between varieties but not between strains and the variety x strain interaction was not significant (Table A.1). The AUPDC was highest for 'RioGrande' (201.3) and lowest for 'Hawaii 7996' (26.3). Mean latent infection was significantly ($P < 0.0001$) higher in 'Yolo wonder' (43.2%) than in 'Hawaii 7996' (29.5%) or 'RioGrande' (29.5%). The distribution of virulent strains varied significantly (P=0.0004) among the two phylotypes but not among the four sequevars (P=0.3888) (Table 2.4).

Anaerobic growth of Tanzanian *R. pseudosolanacearum* strains. Anaerobic growth ratios for all 61 strains were >1.0, ranging from 1.1 to 7.7 with no significant differences (P=0.1612) detected among strains. All the strains grew anaerobically but only TZ 25 and TZ 31 produced nitrogen gas (N_2) (Data not shown).

Discussion

Bacterial wilt disease management is complicated by the soilborne nature of *R. solanacearum* species complex (RSSC) the complexity, and variability of strains, and their ability to grow anaerobically. Targeted management strategies that require an understanding of local populations of the pathogen are crucial. Within the key tomato-producing regions of Tanzania strains of RSSC were identified as belonging to phylotype I and III (*R. pseudosolanacearum)* although phylotype I strains were more commonly isolated from these regions.

Phylotype I and III strains are originally considered to be predominate in Asia and Africa, respectively (Fegan and Prior), However, recent reports from different parts of

Africa have shown phylotype I strains to dominate the sampled population (Somo Toukam et al., 2009; Subedi et al., 2014; Chesneau et al., 2018; Shutt et al., 2018). In addition, Aloyce (2020) and Mwankemwa (2015) found a preponderance of phylotype III and II strains on tomato and potato respectively, in their Tanzania RSSC populations. This study did not demonstrate high phylotype diversity, in agreement with Mwankemwa's (2015) findings on potatoes in the Southern Highlands of Tanzania. However, our results differed somewhat from those of Aloyce (2020), who isolated phylotype II and III strains among only four strains from tomatoes in the Southern ecological zone of Tanzania, which includes Mbeya and Iringa. We found only phylotype I strains in Mbeya and phylotype III in Iringa. Members of phylotype I are prevalent on Solanaceous crops and have a wide distribution especially in tropical lowlands (Cellier and Prior 2010). In our study phylotype I strains were isolated from tomato, sweet pepper, potato, and eggplants indicating wide host preference of this population. Little is known about the presence of members of phylotype III in Tanzania except for the few strains from Aloyce (2020) study but reports from other African countries like Cameroon and Ivory Coast confirmed the presence of phylotype III (Mahbou Somo et al 2009, N'guessan et al., 2012).

Phylotype III strains are known to inhabit cool highland environments (temperate climate) and have a limited host range (tomato and potato) (Somo Toukam et al., 2009). We isolated phylotype III strains from Iringa region which is characterized by cooler temperature throughout the year (averages below 25^0C). The difference between our results and those of Aloyce (2020) could be explained by the possibility of highly diverse phylotype distribution in the Southern Highlands of Tanzania. However, intensive

sampling should be carried out in the area to obtain enough representative strains from a wide area to explain the diversity. Subedi (2015) indicated the presence of only phylotype I in Asia. The presence of phylotype I strains in Africa but not phylotype III strains in Asia could be the result of the movement of latently infected planting materials from Asia to Africa and none or minimal from Africa to Asia. It also indicates that phytosanitary measures and systems such as quarantines and border inspections are successfully implemented and operating in these countries. In the current time trade between Asian countries such as India, Taiwan, and China with Africa has grown, and goods include seeds and other planting materials. In a similar context, Massawe (2020) observed the occurrence of maize lethal necrosis viruses that are closely associated with viruses of Asian origin in different seasons and locations suggesting trade as a contributor to pathogen introductions and diversity.

Phylotype specificity of bacterial wilt resistance has been reported in different Solanaceous rootstock cultivars (Wang et al., 1998). Phylotype specificity may complicate the suggestion of host-resistant candidates for wide use in several locations including Tanzania. For example, tomato 'Hawaii 7996' gas shown high level of resistance to bacterial wilt caused by phylotype I and phylotype II strains of RSSC (Wang et al.,1998; Wang et al. 2013). However, in other studies including this study the level of resistance to 'Hawaiii 7996' based on incidence and severity has varied considerably between strains of phylotype I and between phylotypes I, II or III (Lebeau et al., 2011; Lewis Ivey et al., 2021). In this study, bacterial wilt incidence was low (mean 6.4%, range 0 – 67%) in tomato line 'Hawaii 7996'. The results differ from those of Subedi (2015) and Lewis Ivey

et al. (2021) in which phylotype I strains did not cause bacterial wilt symptoms in 'Hawaii 7996'. This suggests that the *R. solanacearum* virulence factors are controlled by complex and specialized cellular signaling mechanisms as well as surrounding environmental conditions and soil characteristics (Genin, 2010; Khokhani et al., 2017). Cardwel et al (2017) explained delayed colonization and restriction of pathogens as the major mechanisms that are used by Hawaii 7996 in resistance against *Ralstonia* spp. Hawaii 7996 has been tested on multiple locations and conferred resistance in multiple locations (Wang et al., 1998, Lebeau et al., 2011) However resistance breakage on this variety have been reported in some locations suggesting that host resistance can also be host specific and environmental specific (Hanson et al., 1998). We did not have representative strains of phylotype II and IV in our study, therefore, intensive evaluation is needed to make recommendations based on sufficient representation of phylotypes including phylotype II and III for recommending 'Hawaii 7996' as a potential candidate for host resistance in Tanzania. We recovered few phylotype III strains from Tanzania. The suggestion is based on the findings by N'guessan et al. (2012) that highlighted the possibility of resistance breakage in 'Hawaii 7996' by strains of phylotype I and III in Ivory Coast.

Within phylotype I strains that were sequenced in this study, half clustered strains that were sequevar 18 and a half strains that were sequevar 31. The high representation of sequevar 31 was also observed by Shutt et al. (2018) in South African strains and Chesneau et al. (2018) in the Mayotte population. Members of phylotype I and specifically sequevar 31 strains are known for their aggressiveness and ability to attack several Solanaceous hosts that include eggplant, sweet pepper, tomato, hot pepper, and black nightshade (Chesneau

et al., 2018). This is also attributed to the high recombinogenic ability of phylotype I strains (Wicker et al 2012). One of phylotype III strain was avirulent while the other one was virulent to RioGrande and sweet pepper and did not cause wilting in 'Hawaii 7996'. We could not establish the exact reason for the phylotype III strain to be avirulent, but we hypothesize the possibility of picking up avirulent colonies for inoculum or the pathogen itself has lost its virulence capacity during growth in culture medium following vigorous subculturing process. On the other hand, the failure of strains to cluster based on the area of origin within phylotypes suggests a horizontal exchange of genetic material among strains through transformation (Guidot et al., 2009). Sequevar characterization discloses more than 1% variability in *egl* sequences among strains in the same phylotype (Wicker et al., 2012).

In this study, four sequevars (18, 21, 22, and 31). were identified from only twelve representatives of Tanzanian phylotype I and III strains from Solanaceous crops. This suggests a high degree of genotypic variability among these strains. Future *egl* sequencing and sequevar assignment of the remaining 50 strains collected in the five tomato-producing regions will provide a more complete picture of genotypic variability within *R. pseudosolanacearum* in these regions. In this study, our phylotype 1 strains aligned with Guyana and strains from African countries such as South Africa, Ethiopia, Cameroon, and Mayotte. The similarity index scores also indicated that Tanzanian strains are similar to these reference strains. It is also suggestive that *R. pseudosolanacearum* strains may have been introduced into and from Tanzania from/to different parts of the world including neighboring countries. This can be possible because farmers in East Africa and neighboring

countries exchange planting materials such as tomato seeds and seed potato, which can aid in the dissemination of *R. solanacearum* through latently infected planting materials. Sequevar 31 strains from this study clustered with all South African reference strains, which demonstrates further that sharing planting materials among Southern African Development Community (SADC) countries if not well regulated may pose a danger of being a good source of pathogen dissemination in the region. With this remark, governments and responsible authorities should seek to improve phytosanitary measures, proper and effective diagnostic techniques to help farmers avoid disseminating *Ralstonia* spp. through planting materials.

Pathogenicity testing of 61 *R. pseudosolanacearum* strains against two tomato and one sweet pepper varieties revealed variation in host susceptibility; tomato 'RioGrande' was most susceptible, followed by sweet pepper 'Yolo wonder' and the highly resistant 'Hawaii 7996'. Similar findings were reported by Lewis Ivey et al. (2021), who also observed higher susceptibility in tomato than pepper. Lebeau et al. (2012) and Shutt et al. (2018) also reported on the higher susceptibility of tomato to bacterial wilt as compared to pepper and eggplant. Bacterial wilt resistance in tomato and pepper is dominant monogenic or polygenic control by major and minor genes (Grimault and Prior 1995, Wang et al 1998) while in eggplant major resistant genes segregate as single genes (Salgon et al., 2017). Mechanisms of resistance are explained by the ability to restrict colonization and infection by *Ralstonia* spp. (Nakaho et al., 2004; Lebeau et al 2011; Caldwell 2017). The high occurrence of latent infection in peppers indicates that peppers use mechanisms of restricting pathogen movement and colonization through the vascular

bundles, i.e., pathogen may be restricted onto the lower stem region of the latently infected seedling.

Tanzanian phylotype I and III strains were all capable of anaerobic growth in the presence of nitrate, which is typical of *Ralstonia* spp. strains as suggested by Prior et al. (2016) and Dalsing et al., (2015). The majority (96.7%) of strains tested in this study however did not complete the denitrification process, in contrast to the work of Prior et al. (2016), in which all phylotype I and III strains produced nitrogen gas after 96 hr of culture. The functioning of NosZ genes that code for N2O reductase demands a completely anaerobic environment (Dalsing et al., 2015). We don't fully understand the reasons for our results; however, we hypothesize that the instruments used to create were not completely closed, allowing for oxygen to enter the system. However, mutations in the gene of NosZ that regulate the reduction of N_2O to N_2 gas could be responsible for prematurely terminating the denitrification process, or the absence of upstream genes is required for nitrogen dissimilation may have not present in these strains. In fact, the reduction of only to nitrate is a common variant in the use of nitrogen axyanions and oxides as terminal electron acceptors (Zumft,1992). Lastly, the strains that grew anaerobically but did not produce N2 gas may be using an element other than N (i.e sulfur or iron) as terminal electron acceptor. Additional studies to understand the denitrification pathway in these isolates are needed.

In this study, the *R. solanacearum* strains infecting tomato and pepper from Tanzania were identified and characterized based on morphology, the reaction in a *Ralstonia*-specific ELISA, genotypic properties, pathogenicity on tomato and pepper, and

ability to grow anaerobically. The information obtained adds significantly to knowledge of the distribution, genetic diversity, and virulence of *R. pseudosolanacearum* strains in five key tomato-producing regions of Tanzania. While some of the phylotype I strains partially overcame the resistance of 'Hawaii 7996', this tomato line could be useful as a rootstock in Tanzania, especially where phylotype III strains predominate. However, a more extensive population study is needed to generate more genomic and virulence data that can inform extension recommendations for the management of bacterial wilt in Tanzania and neighboring countries.

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Table 2. 1: Origin, host, phylotype, sequevar and bacterial wilt incidence and latent infection for Tanzanian strains of *Ralstonia pseudosolanacearum* used in this study.

Strains [x,y]	Origin	Host	Phylotype / sequevar	Bacterial wilt							
				Incidence (%)				Latent Infection (%)			
				'Yolo wonder'	'RioGrande'	'Hawaii 7996'	Mean [z]	'RioGrande'	'Yolo wonder'	'Hawaii 7996'	Mean [z]
TZ4	**Lushoto, Tanga**	**Tomato**	**I/18**	**0**	**0**	**0**	**0.0 c**	**33**	**33**	**33**	**33.0 abc**
TZ10	**Lushoto, Tanga**	**Sweet pepper**	**I/18**	**9**	**67**	**0**	**25.3 abc**	**0**	**0**	**0**	**0.0 c**
TZ15	**Lushoto, Tanga**	**Tomato**	**I/18**	**20**	**30**	**17**	**22.2 abc**	**25**	**0**	**25**	**16.7 abc**
TZ19	**Lushoto, Tanga**	**Tomato**	**I/31**	**53**	**73**	**10**	**45.3 abc**	**0**	**33**	**66**	**33.0 abc**
TZ26	**Inyala, Mbeya**	**Tomato**	**I/31**	**20**	**67**	**0**	**29 abc**	**0**	**50**	**47**	**32.3 abc**
TZ30	**Kiwira, Mbeya**	**Tomato**	**I/18**	**33**	**33**	**0**	**22 abc**	**50**	**50**	**0**	**33.3 abc**
TZ32	**Image, Iringa**	**Tomato**	**III/22**	**17**	**73**	**0**	**30 abc**	**100**	**45**	**27**	**57.3 abc**
TZ33	**Inyala, Mbeya**	**Tomato**	**I/18**	**47**	**83**	**0**	**43.3 abc**	**0**	**0**	**43**	**14.3 abc**
TZ35	**Mshewe, Mbeya**	**Sweet pepper**	**I/31**	**67**	**83**	**27**	**58.9 a**	**0**	**0**	**13**	**4.3 bc**
TZ45	**SUA GH, Morogoro**	**Sweet pepper**	**I/31**	**0**	**40**	**0**	**13.3 abc**	**16**	**33**	**37**	**28.7 abc**
TZ54	**Image, Iringa**	**Tomato**	**III/20**	**0**	**0**	**0**	**0.0 c**	**67**	**67**	**29**	**54.3 abc**
TZ55	**Inyala, Mbeya**	**Tomato**	**I/31**	**33**	**33**	**0**	**22 abc**	**50**	**50**	**17**	**39.0 abc**
TZ2	Lushoto, Tanga	Tomato	I	0	33	0	11.0 bc	0	33	33	22.0 abc
TZ3	Lushoto, Tanga	Pepper	I	33	67	33	44.3 abc	100	50	50	66.7 ab
TZ5	Mlali, Morogoro	Tomato	I	37	83	33	51 .0ab	0	33	0	11.0 abc
TZ6	Lushoto, Tanga	Sweet pepper	I	40	57	0	32.3 abc	50	67	17	44.7 abc
TZ7	Mlali, Morogoro	Tomato	I	37	57	10	34.7 abc	75	75	17	55.7 abc
TZ8	Lushoto, Tanga	Sweet pepper	I	0	0	0	0 .0 c	33	67	33	44.3 abc
TZ9	Lushoto, Tanga	Tomato	I	53	83	17	50.9 ab	50	67	0	39.0 abc
TZ11	Lushoto, Tanga	Tomato	I	67	67	0	44.7 abc	100	100	33	77.7 a

TZ12	Lushoto, Tanga	Tomato	I	30	67	10	35.7 abc	25	33	17	25.0 abc
TZ13	Lushoto, Tanga	Tomato	I	47	83	17	48.9 ab	0	75	25	33.3 abc
TZ14	Lushoto, Tanga	Tomato	I	33	53	17	34.2 abc	83	80	25	62.7 ab
TZ16	Lushoto, Tanga	Tomato	I	33	73	17	40.9 abc	50	65	25	46.7 abc
TZ17	Lushoto, Tanga	Tomato	I	27	63	0	30.0 abc	25	38	0	21.0 abc
TZ18	Lushoto, Tanga	Tomato	I	0	33	0	11.0 bc	50	33	33	38.7 abc
TZ20	Lushoto, Tanga	Sweet pepper	I	37	83	10	43.3 abc	0	58	58	38.7 abc
TZ21	Image, Iringa	Tomato	I	0	0	0	0 .0 c	33	33	33	33.0 abc
TZ22	Image, Iringa	Tomato	I	0	67	0	22.3 abc	0	33	0	11.0 abc
TZ23	Image, Iringa	Tomato	I	0	0	0	0.0 c	33	33	33	33.0 abc
TZ24	Misufini, Morogoro	Tomato	I	57	57	10	41.3 abc	50	50	47	49.0 abc
TZ25	Misufini, Morogoro	Tomato	I	33	33	0	22.0 abc	100	50	33	61.0 ab
TZ27	Inyala, Mbeya	Tomato	I	40	50	0	30.0 abc	16	17	33	22.0 abc
TZ28	Image, Iringa	Tomato	I	0	33	0	11.0 bc	0	67	67	44.7 abs
TZ29	Lushoto, Tanga	Tomato	I	33	33	10	25.3 abc	0	10	54	21.3 abc
TZ31	Image, Iringa	Tomato	III	3	0	0	1.0 c	67	67	0	44.7 abc
TZ34	Mshewe,Mbeya	Sweet pepper	I	27	83	0	36.7 abc	0	25	27	17.3 abc
TZ36	Mshewe, Mbeya	Eggplant	I	67	0	0	22.3 abc	33	100	0	44.3 abc
TZ37	Lushoto	Tomato	I	33	67	0	33.3 abc	0	0	33	11.0 abc
TZ38	Lushoto, Tanga	Tomato	I	57	67	27	50.2 ab	25	25	25	25.0 abc
TZ39	SUA GH, Morogoro	Sweet pepper	I	0	57	0	19.0 abc	0	0	33	11.0 abc
TZ40	Lushoto, Tanga	Sweet pepper	I	33	33	0	22.0 abc	50	75	33	52.7 abc
TZ41	Image, Iringa	Tomato	III	10	83	0	31.0 abc	50	17	17	28.0 abc
TZ42	Mlali, Morogoro	Tomato	I	40	67	17	41.2 abc	25	17	35	25.7 abc
TZ43	Lushoto, Tanga	Tomato	I	30	67	67	54.6 ab	0	0	0	0.0 c
TZ44	Mlali, Morogoro	Tomato	I	0	67	0	22.3 abc	0	30	67	32.3 abc
TZ46	Mlali,Morogoro	Tomato	I	0	33	17	16.6 abc	0	67	55	40.7 abc

TZ47	SUA GH, Morogoro	Sweet pepper	I	57	67	0	41.3 abc	0	50	27	25.7 abc
TZ48	SUA GH, Morogoro	Sweet pepper	I	10	47	10	22.3 abc	0	67	33	33.3 abc
TZ49	SUA GH, Morogoro	Sweet pepper	I	10	50	0	20.0abc	16	67	17	33.3 abc
TZ50	Ntokela, Mbeya	Tomato	I	57	50	0	35.7 abc	16	75	17	36.0 abc
TZ51	SUA GH, Morogoro	Sweet pepper	I	0	0	0	0.0 c	20	0	47	22.3 abc
TZ52	Mlali, Morogoro	Tomato	I	37	67	0	34.7 abc	50	50	63	54.3 abc
TZ53	Mlali, Morogoro	Tomato	I	33	0	0	11.0 bc	33	50	63	48.7 abc
TZ56	Inyala, Mbeya	Tomato	I	67	33	0	33.3 abc	0	0	27	9.0 abc
TZ57	Inyala, Mbeya	Tomato	I	0	0	0	0.0 c	33	33	17	27.7 abc
TZ58	Mshewe, Mbeya	Sweet pepper	I	0	33	33	22.0 abc	50	33	50	44.3 abc
TZ59	Mshewe, Mbeya	Eggplant	I	73	73	10	52.0 ab	50	50	37	45.7 abc
TZ60	Lushoto, Tanga	Tomato	I	53	97	0	50.0 ab	50	100	33	61.0 ab
TZ67	Mlali, Morogoro	Tomato	I	57	0	10	22.3 abc	0	75	67	47.3 abc
							P> 0.0001				*P*>0.0001

[X] Twelve Tanzanian *Ralstonia pseudosolanacearum* strains (bolded) used for phylogenetic analysis in this study.

[y] Sixty-one Tanzanian *Ralstonia pseudosolanacearum* strains used in tomato and sweet pepper pathogenicity experiments.

[z] Mean incidence/latent infection caused by *Ralstonia pseudosolanacearum strain* in the two experiments conducted under similar greenhouse conditions.

Table 2. 2: Distribution of virulent *Ralstonia pseudosolanacearum* strains across four key tomato-producing regions of Tanzania and bacterial wilt incidence in inoculated tomato 'RioGrande' and 'Hawaii 7996' and pepper 'Yolo wonder'

	Bacterial wilt incidence (%)			
Region	**'Yolo wonder'**	**'RioGrande'**	**'Hawaii 7996'**	**Mean [x]**
Iringa	7.8 (4) [y]	34.0 (5)	2.2 (1)	14.7 (10) b
Mbeya	40.9 (10)	47.6 (10)	5.8 (3)	31.4 (23) a
Morogoro	25.5 (11)	45.3 (13)	6.7 (7)	25.8 (31) ab
Tanga	31.1(20)	56.4 (22)	10.8 (13)	32.8 (55) a
Mean [z] (total)	26.3 (45) b	45.8 (50) a	6.4 (24) c	
P value		0.0005		0.0532

[y] **N**umbers in parentheses are the number of virulent strains in a line.
[X] Mean bacterial wilt incidence in a region caused by virulent strains averaged from the two experiments conducted under similar greenhouse conditions; means with similar letters in a column are not significantly different from each other at $P<0.05$.
[Z] Mean bacterial wilt incidence in a variety caused by virulent strains averaged from the two experiments conducted under similar greenhouse conditions; means with similar letters in a row are not significantly different from each other at $P<0.05$.

Table 2. 2: Mean bacterial wilt incidence, area under the disease progress curve (AUDPC) and latent infection in tomato and sweet pepper varieties/line inoculated with 61 *Ralstonia pseudosolanacearum* strains from five key tomato producing regions of Tanzania.

Variety/line [x]	**Incidence (%)** [y]	**AUPDC** [y]	**Latent infection (%)** [y]
'RioGrande'	45.8 a	201.3 a	29.5 b
'Yolo wonder'	26.3 b	97.1 b	43.2 a
'Hawaii 7996'	6.4 c	26.5 c	29.5 b
P value	<0.0001	<0.0001	<0.0001
Tukey's LSD	5.8	32.1	7.8

x Tomato 'RioGrande' and 'Hawaii 7996' and sweet pepper 'Yolo wonder'.
[y] Means of three combined experiments conducted under the similar greenhouse conditions using 61 *R. pseudosolanacearum* strains collected from the key tomato producing regions of Tanzania; means with same letters within a column are not significantly different from each other at 5% alpha value

Table 2. 3: Distribution of phylotype and sequevar of 12 *Ralstonia pseudosolanacearum* strains from key tomato-producing regions of mainland Tanzania virulent on tomato 'Hawaii 7996' and 'RioGrande' and sweet pepper 'Yolo wonder'

Line Variety	**Phylotype-sequevar of *R. pseudosolanacearum***				
	I- 18	I-31	III-20	III-22	**Total**
'Hawaii 7996'	[X]1 (33.3%)	2 (66.6%)	0 (0%)	0 (0.0)	3 (14.28%)
'Yolo wonder'	4 (50.0%)	4 (37.5%)	0 (0%)	1 (12.5)	9 (38.09%)
'RioGrande'	4 (40.0%)	5 (50.0%)	0 (0%)	1 (10.0)	10 (46.9%)
Total/Mean	9 (40.9%) a	11(50.0%) a	0 (0%) b	2 (9.1%) b	22 (100.0%)
P value	Phylotype = 0.0004 Sequevar = 0.3888.				

[X] Number outside the brackets represents number of virulent strains in a phylotype; number in brackets indicate percentage of strains virulent on the variety. Means with the same letters within a row are not significantly different at P<0.05

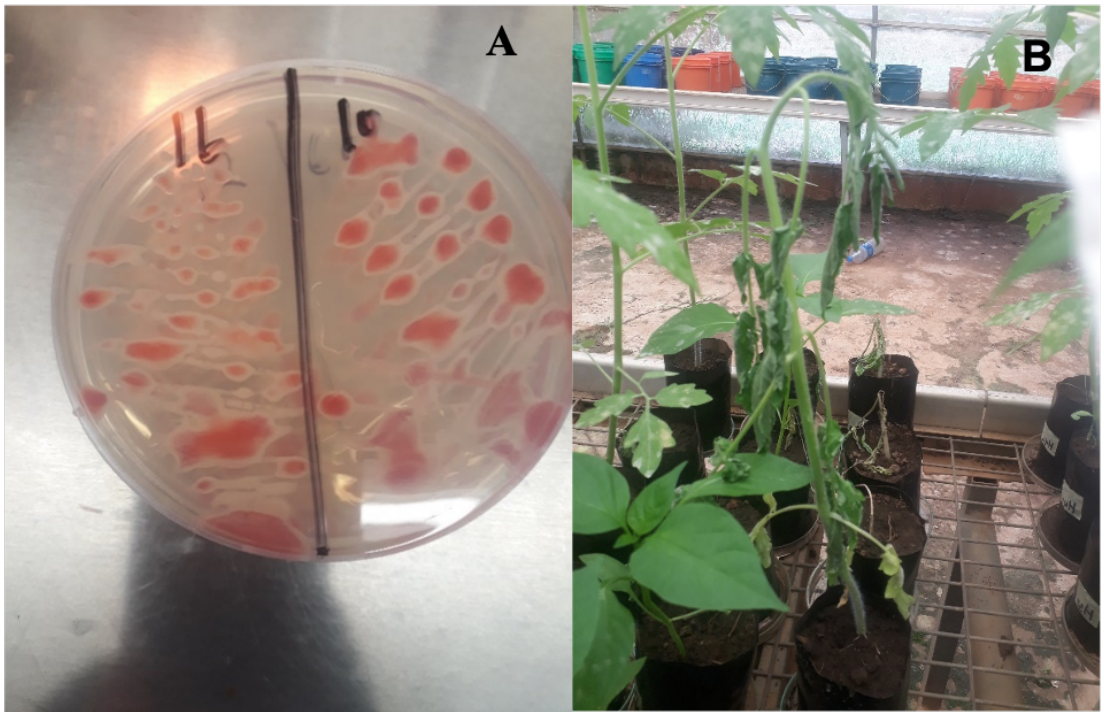

Fig 2. 1: A) Pure culture of *Ralstonia pseudosolanacearum* TZ 10 and TZ 16 growing on Tetrazolium chloride (TZC) medium and B) bacterial wilt symptomatic plants six days postinoculation with *Ralstonia pseudosolanasearum* strains collected from key tomato producing regions of Tanzania

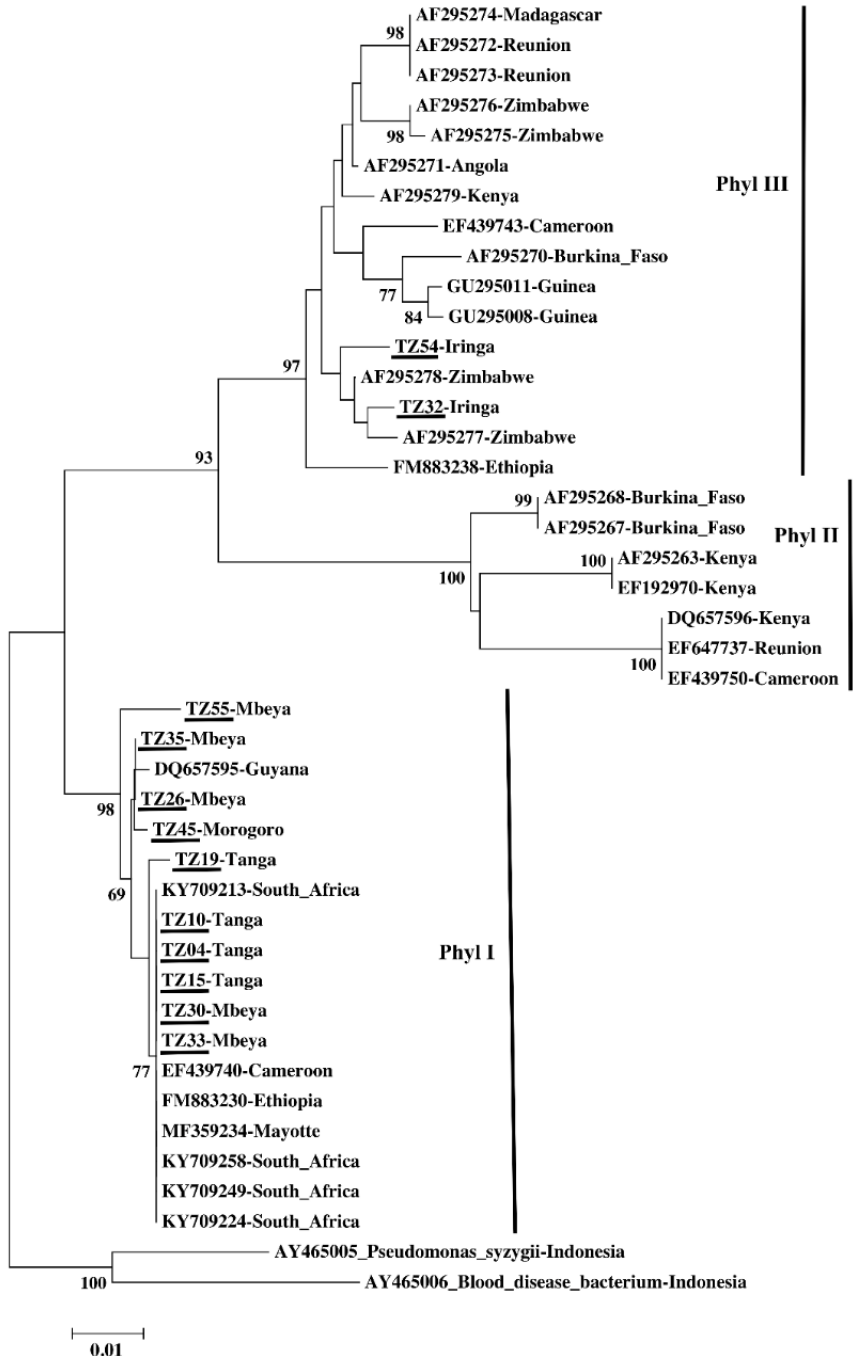

Fig 2. 2: Phylogenetic tree constructed from partial endoglucanase gene sequences of *Ralstonia pseudosolanacearum* strains collected from key tomato producing regions of Tanzania mainland using the neighbor-joining Jukes and Cantor algorithm. Bootstrap value (1000 resampling) is indicated on each node, scale represents one nucleotide substituted/100 nucleotides. Underlined strains are Tanzanian strains.

Chapter 3. Evaluation of Rootstocks and Efficacy of Grafting in the Management of Bacterial Wilt Disease of Tomato

Abstract

Bacterial wilt is a major bacterial disease that impacts tomato (*Solanum lycopersicum*) production in Tanzania. The disease is caused by members of the *Ralstonia solanacearum* species complex (RSSC), which attacks a wide range of host crops. Complex pathogen variability and a wide host range complicate the management of bacterial wilt disease. Host resistance is an environmentally friendly option for bacterial wilt management. However, strain specificity limits the durability of the host resistance. Therefore, we screened thirteen tomato and one eggplant *(Solanum melongena)* lines for resistance to a collection of Tanzanian and Asian *Ralstonia pseudosolanacearum* strains. From the preliminary screening, we selected tomato lines 'MT56' and WG120 and eggplant line 'EG190' for further evaluation for resistance against *R. pseudosolanacearum* strains collected from key tomato growing regions of Tanzania. All three lines were resistant to bacterial wilt with a significant difference in disease incidence scores (P<0.0001). Among these, 'EG190' was the most resistant to a wide range of tested strains. All lines supported latent infections of *R. pseudosolanacearum* with significant differences among them (P=0.0024). Lines 'MT56' and 'EG190' were selected as rootstocks for grafting with the susceptible tomato variety 'Moneymaker'.

During grafting process (propagation and weaning) the grafted seedlings had survived at a rate of 60% -75%. Grafted seedlings were challenged with a mixture of strains that were previously used in the evaluation of rootstocks. Grafted seedlings had significantly reduced bacterial wilt incidence compared to self-grafted ‘Moneymaker’. Bacterial wilt area under disease progress curves (AUDPCs) varied significantly among rootstock/scion combinations (P=0.0190). Area under the disease progress curve values was consistently low for self-grafted rootstock and rootstock/scion grafted seedlings. This study demonstrates the potential of using eggplant ‘EG190’ and tomato ‘MT56’ as rootstocks for grafting for bacterial wilt resistance in the key tomato producing regions of Tanzania.

Introduction

Tomato is amongst the most valuable horticultural crops in Tanzania contributing 64% of the total vegetable crop production in the country (De Putter et al., 2011; Luzi-Kihupi et al., 2015; Mutayoba and Ngaruko 2017). However, tomato production of 2.2 - 16.5 t/ha in Tanzania is far lower than the 27.5 t/ha global average (FAO, 2005, Maerere et al., 2006). Important factors such as deteriorating soil fertility, the use of vulnerable and low-yielding varieties, unreliable rainfall, diseases, pests, and poor farming practices contribute to reduced tomato production (Minja et al., 2011).

Bacterial wilt is the leading bacterial disease affecting tomatoes in Tanzania (Black et al., 1999; Baitani, 2017). The disease is caused by members of the *R. solanacearum* species complex (RSSC), the second-most recognized bacterial pathogen causing devastating crop and yield losses globally, especially in solanaceous crops (Champoseu and Momol, 2009; Mansfield et al., 2012; Meng, 2013). *Ralstonia* spp. are pathogenic soil ß-proteobacteria widely distributed in subtropical, tropical, and temperate areas (Hayward, 1991; Yabuuchi et al., 1995; Elphistone, 2005). Previously only tropical lowland areas with a warm climate were reported to support the perpetuation of members of the RSSC (Hayward, 1991). However, Elphinstone (2005) revealed the presence of RSSC race 3 biovar 2 (R3B2) strains that survive in cooler and high-altitude areas and cause southern wilt, bacterial wilt, and brown rot in geranium, tomato, and potato respectively (Champoseau and Mommol, 2009). The nomenclature of the RSSC has recently been revised (Safni et al., 2014); R3B2 strains are in phylotype II and have retained the name *R. solanacearum*, while phylotype I

and III strains are now designated *R pseudosolanacearum*. In Tanzania, phylotype I, II, and III strains have been found in association with solanaceous crops to date (Mwankemwa et al., 2015; Aloyce, 2020). As with many soilborne diseases, bacterial wilt management is complex and difficult to accomplish using popularly known disease management strategies. This is attributed in part to the ability of the pathogen to survive long term in the soil with favorable humidity and temperature conditions (French et al., 1998; Arwiyanto et al., 2015). Bacterial wilt management approaches range from phytosanitary measures, cultural practices, biological control, and chemical treatments to host resistance (Saddler, 2005; Elphinstone, 2005; Champoseau and Momol, 2009, Shutt et al., 2018). None of the single strategies has proven to be fully effective in controlling bacterial wilt. However, a combination of disease management strategies may reduce bacterial load in pathogen-infested soils (Priou et al., 2004; Champoseu and Momol, 2009).

Host resistance is the preferred means of bacterial wilt management, however most popular tomato varieties are susceptible to bacterial wilt (Opena et al., 1990; Wang et al., 1998; Huang et al., 2015). Host resistance has been explored in solanaceous crops that include pepper (*Capsicum* spp.), eggplant, and tomato (Wang et al., 1998; Oda, 1995; Lin et al., 2008; Du et al., 2016; Salgon et al., 2017). Currently available tomato varieties resistant to bacterial wilt have been bred using wild solanaceous species, namely *Solanum lycopersicum var. cerasiforme* (*Lycopersicon esculentum* var. *cerasiforme)* and *Solanum pimpinellifolium (L. pimpinellifolium*) (Scott et al., 2005). However, several factors limit breeders in the development of resistant tomato varieties (Rivald et al., 2012). Tomato bacterial wilt resistance can be polygenic or monogenic and in some cases strain- or

location-specific (Grimault and Prior, 1994; Hanson et al., 1998; Wang et al., 1998). In tomato line 'Hawaii 7996', bacterial wilt resistance is controlled by two quantitative trait loci (QTL) and resistance is durable in multiple environments (Wang et al., 2013). Strain-specific resistance is non-durable (Scott et al., 1996) and variety performance may be variable across multiple locations (Wang et al., 2013). Favorable conditions for disease occurrence that include soil type, soil microbiota, temperature, and moisture may also play a part in resistance failure. Besides, some resistant plants that do not show the wilting symptom exhibit latent infection, restricting the pathogen to the lower stem (Prior et al., 1998; Nakaho et al., 2004; Lebeau et al., 2011). Latently infected plants facilitate bacterial wilt pathogen dissemination.

Grafting has the potential to be a crucial tool in controlling bacterial wilt in tomatoes and other crops in the Solanaceae family. Grafting in vegetable crops makes use of host resistance to plant pathogenic microbes and environmental stresses, hence improving the yield and quality of grafted plants (Rivard et al., 2012). In grafting for disease resistance, the rootstock choice is based on its ability to resist or tolerate soilborne disease, whereas scion choice is based on fruit quality, yield potential, horticultural characteristics, and farmer or market preferences. The technique has been in practice since 1920 in Korea and Japan where initially it was used to control soil-dwelling pathogens and nematodes in cucurbits (Rivero et al., 2003). Likewise, in recent times grafting has been established as appropriate integrated pest management (IPM) technique for solanaceous crops that not only improves plant vigor, quality, and yield by facilitating plant nutrient and

water uptake but also decreases plant susceptibility to pests and root and foliar diseases (Black et al., 2003; Louws et al., 2010; Guan et al., 2012).

Many solanaceous rootstocks have been identified and explored for their performance in reducing bacterial wilt on susceptible horticulturally preferable varieties. Researchers in China deployed wild tomato species lines (CH-2-21, 25 and 26) as rootstocks for grafting fresh market tomato with 80-100% success in controlling bacterial wilt disease (Lu et al., 1992). Tomato breeding lines 'Hawaii 7996', Hawaii 7997, and Hawaii 7998 are known for reduced bacterial wilt incidence and severity in worldwide locations (Grimault et al. 1995, Hanson et al., 1996, Scott et al., 2005). Rahmawat and Arwiyanto (2020) reported about 40% bacterial wilt incidence when 'Hawaii 7996' was grafted to susceptible tomato scions in Indonesia. Rivard and Louws (2008) reported a 100% survival rate in heirloom tomato plants grafted onto 'Hawaii 7996' and CRA-66 rootstocks and exposed to *R. solanacearum*. Similar results were observed in Brazil using 'Hawaii 7996' as rootstock for commercial tomato varieties (Cardoso et al., 2012) and Louisiana, USA against phylotype I and II isolates (Lewis Ivey et al., 2021). In the same context Scott et al. (1995) crossed a susceptible tomato variety with Hawaii 7997 to obtain the variety Neptune with bacterial wilt resistance. However, a limited spectrum of resistance was observed and in 2009 they released lines Fla8109 and Fla8109b that had similar pedigrees as Hawaii 7997 (Scott et al., 2009). Other commercial tomato rootstocks Cheong Gang (Seminis), Shield (Rijk Zwaan), and RST-04-106-T (DP Seeds) were reported to fully control bacterial wilt disease when grafted to susceptible tomato scions (Suchoff et al., 2019).

Eggplant is preferred to tomato as a rootstock because of eggplant's durable resistance to *Ralstonia* spp. and ability to survive in flooded soils (Lee et al., 2013). In eggplant, bacterial wilt resistance genes segregate as single genes (*ERs1* and *RE-bw*) (Salgon et al., 2017). The eggplant rootstock EG203 (AVRDC The World Vegetable Center) was reported to survive at a rate of >95% in bacterial wilt-infested soils. Fresh market wilt-susceptible tomato varieties TStarE and Victoria grafted onto five eggplant rootstock accessions VI041979A, VI041809A, VI041984, VI041945, and VI041943 from AVRDC exhibited 0-20% bacterial wilt incidence (Manickam et al., 2021). Other eggplant lines or varieties including SM164, SM6, Surya, and AF9125 exhibited promising resistance to phylotype I and II *Ralstonia* spp. strains (Lewis Ivey et al., 2021).

Tanzanian tomato farmers consider qualities such as fruit shape, size, yield, flavor, and market preferences in choosing varieties (Testen et al., 2016). The lack of availability of varieties resistant to bacterial wilt and/or soilborne diseases and having preferred horticultural traits curtails tomato production, especially on smallholder farms in Tanzania and other East African countries (Luzi Kihupi et al., 2015; Akemo et al., 2002). The inadequate supply of quality seeds and breeding programs for developing locally adapted varieties (Minja et al., 2011; Testen et al., 2016) limits the availability of disease resistant cultivars in Tanzania. Grafting farmer-preferred tomato varieties onto locally adapted, bacterial wilt-resistant rootstocks would provide an effective means of disease management while preserving farmer preferences. However, commercial rootstock varieties tested and deployed in other countries are not available or prohibitively expensive

for smallholder farmers in Tanzania. Affordable open-pollinated (non-hybrid) rootstock varieties or lines that enable farmers to save and share seeds are needed.

This study was focused on identifying bacterial wilt-resistant rootstocks for use in grafting susceptible farmer-preferred tomato varieties in Tanzania. We hypothesized that the impact of bacterial wilt on susceptible tomato varieties will be reduced significantly when they are grafted to rootstocks with host resistance to *R. pseudosolanacearum* strains collected from Tanzania's key tomato producing regions. We aimed to identify eggplant and tomato breeding lines resistant to *R. pseudosolanacearum* for use as rootstocks for grafting with the most horticulturally preferred tomato varieties identified during the tomato farmers' survey (Chapter 5).

The following were the specific objectives of this study:

A. Screen potential wilt-resistant rootstocks using strains of *R. pseudosolanacearum* collected from key tomato producing regions of Tanzania.
B. Graft resistant rootstocks with farmer/market preferred lines.

Assess the performance of grafted plants using representative *R. pseudosolanacearum* strains from key tomato producing regions of Tanzania.

Materials and methods

Preliminary rootstock evaluation

Bacterial strains. Strains used in preliminary rootstock evaluations were selected from among *R. pseudosolanacearum* strains collected in Tanzania during the 2017 farm survey and previously in South Asia (Subedi, 2015) as summarized in Table 3.1. Three strains from Tanzania and two from South Asia were used to screen 11 Ohio tomato rootstocks

lines for resistance. Ten strains from Tanzania (2017 collection) and five from Asia were used to screen 'MT56' and 'EG190' for resistance to bacterial wilt. Six strains collected during the second survey in Tanzania in 2019 were used in the selected rootstock evaluations conducted in Tanzania.

Rootstocks. Tomato breeding lines (N=11) developed for use as rootstocks with soilborne disease resistance provided by Dr. David Francis (Dept. of Horticulture and Crop Sciences, The Ohio State University), tomato line 'MT56' (Makerere University, Kampala, Uganda), and eggplant line 'EG190' (AVRDC, the World Vegetable Center, Tainan, Taiwan) were screened for bacterial wilt resistance (Table 3.2).

Preliminary rootstock screening. Eleven tomato breeding lines from the OSU Tomato Breeding Program, and tomato 'MT56' and eggplant 'EG190' were screened for resistance to bacterial wilt in two separate experiments conducted in Ohio in 2017-2018. A split plot randomized block design was used for these experiments in which rootstock lines were the main plots and *R. pseudosolanacearum* strains were subplots. Each of the four blocks contained three plants (one plant per pot). Plants of each test line were inoculated separately with each of the test strains or mock-inoculated with sterile distilled water as a negative control. The bacterial wilt-susceptible tomato variety 'Moneymaker' was used as a control in this experiment. The experiments were replicated twice and blocked by time.

Seedlings, inoculum preparation, and inoculation. Tomato and eggplant seeds were sown in fertilizer amended (NPK 20:20:20) autoclaved muck soil collected from the OSU CFAES Muck Crops Experiment Station in Willard, OH. Seedlings were grown in 15cm-diameter plastic pots in a greenhouse set at 28°C, with 16 hrs of light, and average relative

humidity of 80% for 4 weeks. Seedlings were inoculated by drenching the soil in each pot with 50ml of 10^8 CFU/ml *R. pseudosolanacearum* cell suspension prepared from 48 hr cultures grown on Casamino acid-Peptone-Glucose (CPG) medium. The inoculum was prepared by flooding pure cultures with sterile distilled water, scraping the cells with a sterile spreader, and pouring the resulting suspension into a flask containing a known amount of sterile distilled water. The concentration of inoculum was adjusted to 10^8 CFU/ml (OD_{600}= 0.1). Control plants were drenched with 50 ml sterile water. Control and inoculated plants were arranged in a split plot RCBD described above with three replications and maintained in a BSL2 greenhouse room set at 28°C ± 2°C for eight weeks. Plants were watered once daily and fertilized with NPK 20:20:20 once every two weeks. Pots with inoculated plants were placed on 15cm Petri plate bottoms and maintained in double plastic trays to trap overflowing water and prevent cross contamination between inoculated plants. At the end of the experiment, all plant tissues and soil were autoclaved while pots, tables, and floor were washed and disinfected with 2% sodium hypochlorite.

Disease assessment: Plants were assessed weekly for incidence and severity of wilting symptoms. Disease incidence was assessed by counting the total number of plants (N) and the number of plants with bacterial wilt symptoms (n) for each line and isolate inoculated. The incidence of bacterial wilt was calculated using the equation

$$Incidence\ of\ bacterial\ wilt = \frac{n}{N} * 100$$

Disease severity values were obtained by rating diseased plants for each line x strain combination using a 1-5 scale (Horita and Tsuchiya, 2001) in which 1=asymptomatic, 2=two leaves withered, 3=three leaves wilted, 4=at least four leaves wilted, and 5=dead

plant. Each severity score was converted to a leaf damage scale (0-100 %), where 0 represented asymptomatic leaves and 1, 2, 3, 4, and 5 represented 20, 40, 60, 80, and 100% wilted leaves, respectively. Mean disease severity was expressed as the mean of all leaf damage percentages for each line x isolate combination. The area under the disease progress curve (AUDPC) was calculated according to the Excel formula (Madden et al., 2007).

$$\text{AUDPC} = \sum_{i=1}^{n} \left(\left(\frac{y_i + y_{i+1}}{2} \right) + (t_i + 1 - t_i) \right)$$

Where y_i = measures of disease level at ith observation and t_i= time of disease measure at ith observation.

To assess latent infection, plants remaining asymptomatic 8 weeks after inoculation were sampled by cutting a 2 cm stem section from the base of the plant and placing it in a tube containing 2.5 ml of sterile distilled water to allow for bacterial streaming for one hour. Suspensions (100µl) were pipetted into wells of a 96-well microtiter plate and an enzyme-linked immunosorbent assay (*R. solanacearum* ELISA kit; Agdia Inc. Elkhart, IN, USA) was conducted according to manufacturer instructions. Negative and positive controls provided with the kit were used as color change guides to score positive or negative results. Wells with blue color visibly darker than the negative control were scored as positive, whereas wells with no color or lighter blue than the negative control were scored as negative for latent infection. The percentage of plants with latent infection was calculated by dividing the number of plants that tested positive *for R. pseudosolanacearum* by the total number of plants sampled for latent infection x 100.

Selected rootstock evaluation in Tanzania. From the preliminary rootstock evaluation, bacterial wilt-resistant rootstocks WG120, 'EG190', and 'MT56' were selected for further evaluation at the Department of Horticulture and Crop Science, Sokoine University of Agriculture, Morogoro, Tanzania from November 2019 to June 2020. The protocol for the evaluation of bacterial wilt was modified from Lebeau et al. (2011) by changing the experimental design to split plot to accommodate time and resource limitations and the inoculation method to soil drenching. A split plot in a completely randomized block design (RCBD) with four replications (blocks) was used to assess the resistance of tomato 'MT56' and WG120 and eggplant 'EG190' against six selected virulent *R. pseudosolanacearum* strains collected from the five key tomato producing regions of Tanzania during a 2019 farm survey (Table 3.1). In the experiment, *R. pseudosolanacearum* strains were the main plots and lines were subplots randomized within the main plots, with five seedlings per line in separate pots per block. Tomato variety 'Tanya' F1 (Seminis, Holland) was used as a susceptible control. Tomato seedlings and the inoculum were prepared as described in the preliminary rootstock evaluation except for the use of pre-autoclaved forest soil, polythene bags (0.5kg) instead of pots, and NPK fertilizer (YaraMila Winner 15-9-20). Plants were inoculated by drenching soil with 50ml of inoculum. Inoculated plants were maintained in a screenhouse with a daily average temperature of 30°C and nighttime of 22°C and plants were watered once daily and fertilized once every two weeks (Fig. 3.1A). Disease scoring and latent infection assessments were as described in the preliminary evaluation experiment. The experiment was conducted twice.

Rootstock-scion graft compatibility evaluation in Tanzania. The grafting experiment was conducted in a Sokoine University of Agriculture screenhouse with a daily average temperature of 30°C and nighttime of 22°C in February and June 2020. Tomato line 'MT56' and eggplant line 'EG190' were selected as rootstocks and the tomato variety 'Moneymaker' was the scion. Fifty seeds each of the rootstocks and scion were sown into 0.5kg polythene bags filled with pre-sterilized (autoclaved) moist field soil and kept for 3 to 4 weeks on the screenhouse bench. Fifteen seedlings that had the same stem diameter from each of the lines were selected for each grafting treatment. The treatments were self-grafted plants of 'MT56', 'EG190', and 'Moneymaker', and 'Moneymaker' grafted to 'MT56' and 'EG190'. Seedlings were grafted using the tube grafting method by joining the scion to the rootstock cut above the cotyledon with silicone clips to support the graft union (Black et al., 2003). Grafting was conducted in a screenhouse room precleaned by mopping with soapy water and wiped with a solution of sodium hypochlorite (2% sodium hypochlorite). All the grafting tools and the grafter's hands were cleaned and disinfected using 70% alcohol before and during grafting to minimize contamination. Tools and hands were disinfected with 70% ethanol after each graft. Immediately after grafting seedlings were placed in a healing chamber (Fig. 3.1B) on the benchtop of a screenhouse room. The chamber consisted of a rectangular frame constructed of polyvinyl chloride (PVC) tubing covered with transparent plastic sheeting for moisture retention. A black horticultural net was placed on top to reduce the light intensity during the first week of graft healing. The chamber was misted twice daily with a hand sprayer to maintain high relative humidity. Plants were removed from the healing chamber two weeks after grafting and arranged on

benches of a screenhouse with a daily average temperature of 30°C and a night temperature of 26°C for four weeks. Plants were watered once daily and fertilized with Yara NPK fertilizer once every two weeks. A randomized complete block design (RCBD) was used to lay 75 seedlings (Fig. 3.2A), five from each of the self-grafted rootstocks and scions, and rootstock-scion combinations in three blocks. The number of live and dead plants was recorded twice weekly for two weeks. The experiment was conducted twice.

Resistance of grafted plants to bacterial wilt. Fully recovered 4-week-old grafted seedlings from the grafting experiment were tested for resistance to bacterial wilt. The experimental design was a RCBD with three blocks each containing five seedlings from each treatment of grafted plants inoculated with 10^8 CFU/ml *R. pseudo solanacearum* cell suspension from 48 hr cultures prepared as described above for rootstock screening. The inoculum was a combination of the six *R. pseudosolanacearum* strains (Table 3.1) used to screen rootstocks. Plants were inoculated by drenching 50ml of inoculum directly into pots with grafted seedlings. Each inoculated seedling was placed on an open Petri dish and maintained in a screenhouse as described above for eight weeks (Fig 3.2A). Disease incidence was assessed by counting wilting plants twice a week for eight weeks, then stem tissue was sampled from asymptomatic seedlings by cutting a 2cm portion from the lower stem (crown) and tested for latent infection using the Agdia *Ralstonia* ELISA kit as described above. The experiment was conducted twice.

Statistical analysis. Statistical analyses were carried out using SAS statistical software (SAS 94.4.4 2017) with Proc GLM (SAS Institute). All data were tested for equality of variance with Levene's test for treatments and experiment as fixed effects. When no

significant difference was observed between treatment and replications and their interaction the two experiments were combined for analysis. Analysis of variance (ANOVA) was used to compare variety responses (rootstocks and scion) and isolates used for evaluation using F and t-tests where applicable. Means were separated using the Fisher's Tukey's Least Significant Difference (LSD) test in SAS using Proc GLM.

Results

Preliminary rootstock screening. There were significant differences between tomato lines and between strains in bacterial wilt incidence (Table B.1), severity (Table B.2), and latent infection (Table B.3) and a significant interaction ($P<0.0001$) between tomato lines and strains for all three variables. None of the eleven rootstocks were completely resistant to the five Tanzanian and Asian strains tested (Table 3.3). Bacterial wilt incidence was significantly ($P<0.0001$) lower in nine rootstock lines, higher in one and not different in one line than in the bacterial wilt-susceptible variety 'Moneymaker' (66%). Disease severity was significantly ($P<0.0001$) lower in eight rootstock lines than in 'Moneymaker'. Latent infections ranged from 2.5% to 90% of surviving plants at the end of the experiment. Of the eleven lines screened, WG12-120 exhibited amongst the lowest wilt incidence (36%) and latent infection (2.5%), and the lowest disease severity (31%). Averaged across all tomato lines, the South Asian *R. pseudosolanacearum* strains SM747 and SM716 were more aggressive than the Tanzanian strains TZ48, TZ130 and TZ9, causing significantly higher disease incidence ($P<0.0001$) and severity ($P<0.001$) (Table 3.4). However, latent infection was significantly ($P=0.008$) higher in surviving tomato plants inoculated with

TZ9 than with SM747, SM716 or TZ48. The significant line*strain interaction was evident as the South Asian strains caused on average higher disease incidence than the Tanzanian strains on all lines except SGH06-216, FGH06-304 and FGH06-301 (Table B.4). Tanzanian strain TZ48 was highly aggressive (100% incidence) on tomato lines FGH06-304, FGH06-220, FGH06-215, WG 211 and 'Moneymaker'. For the lines FGH06 -302 and WG-110, moderately low bacterial wilt incidence (< 60%) was observed after inoculation with both South Asian and Tanzanian strains. Similar interaction patterns were observed for latent infection and disease severity.

In the second experiment there were significant differences between tomato/eggplant lines in bacterial wilt incidence (Table B.5), severity (Table B.6), and latent infection (Table B.7) and a significant line*strain interaction for all three variables (P<0.0001). Neither eggplant 'EG190' nor tomato 'MT56' were completely resistant to all 15 *R. pseudosolanacearum* strains used to screen them (Table 3.5). Averaged across all 15 strains, disease incidence, severity and latent infection were significantly (P<0.0001) lower in 'EG190' and 'MT56' than in the susceptible variety 'Moneymaker'. Disease incidence was lowest in 'EG190' (5.9%) followed by 'MT56' (22.9%) and high in 'Moneymaker' (56.4%). Bacterial wilt disease severity and latent infection were statistically similar in 'EG190' and 'MT56', but lower than in 'Moneymaker'. Averaged across all three plant lines, there were significant differences in bacterial wilt incidence (P=0.0099) and severity (P<0.0001), but not latent infection (P=0.0927) among plants inoculated with different *R. pseudosolanacearum* strains (Table 3.6). South Asian strains SM747 and SM727 were significantly more aggressive than the least aggressive strain

TZ22 in terms of bacterial wilt incidence, but there were no other differences among strains. Disease severity was significantly higher in plants inoculated with SM747 and SM727 than in those inoculated with a majority of the Tanzanian strains. Tanzanian strains did not cause disease on 'EG190' rootstock (0% incidence) but varied in aggressiveness (0 - 47% incidence) on 'MT56' 42.2(Table B.8). Disease incidence was moderately high in 'Moneymaker' seedlings inoculated with South Asian (mean incidence = 63.5%) and Tanzanian (mean incidence = 42.2) strains. Similar line*strain interaction trends were observed for disease severity and latent infection.

Selected rootstock evaluation in Tanzania. None of the rootstocks were completely resistant to the six *R. pseudosolanacearum* strains tested (Table 3.7). There were significant (P<0.0001) differences between rootstocks and between strains in bacterial wilt incidence, but no significant line*strain interaction (P=0.107) (Table B.9). There was, however, a significant (P< 0.0001) rep*line interaction. Bacterial wilt incidence eight weeks after inoculation was significantly lower in all three rootstock lines averaged across *R. pseudosolanacearum* strains than in the wilt-susceptible variety 'Tanya' (64.4%). Disease incidence was lowest (8.9%) in 'EG190', followed by WG120 (28.9%), and 'MT56' (43.9%). Similar results were observed for disease progress (P<0.0001), except that AUDPC values for wilt incidence over eight weeks post-inoculation did not differ significantly for tomato lines 'MT56' and WG120. Bacterial wilt AUDPC was lowest in line 'EG190' (44.1) followed by WG120 (150.7) and 'MT56' (166.93) and highest in the susceptible control 'Tanya' (368.3). Many surviving plants exhibited latent infection, ranging from 62.7% to 80.3% of the plants testing positive by *Ralstonia* ELISA. There

were no significant differences in latent infection between rootstocks and the scion variety 'Tanya' (P=0.1161) or among *R. pseudosolanacearum* strains (P=0.1428) (Supplementary Tables 3.9, 3.10). Rep*line (P=0.1221) and line*strain (P=0.3419) interactions were not significant.

There were significant differences in aggressiveness between Tanzanian *R. pseudosolanacearum* strains averaged across tomato 'MT56' and eggplant 'EG190' rootstock lines and tomato variety 'Tanya' (Table 3.8). Mean disease incidence (P<0.0001) and AUDPC (P=0.0002) differed significantly by strain but there were no differences (P=0.1428) among strains in the ability to cause latent infection in these plants (Supplementary Tables 3.9, 3.10, 3.11). Mean disease incidence across lines/variety was significantly higher in plants inoculated with TZ 24 (55%) or TZ 25 (40.8%) than in plants inoculated with any of the other strains. Bacterial wilt AUDPC was highest in plants inoculated with TZ 24, TZ 25, and 73 followed by TZ 71 and TZ 95 and lowest in those inoculated with TZ 72. The line*strain interaction for AUDPC was marginally insignificant (P=0.0553) but the effect of rep (P=0.0075) and the rep*line interaction (P=0.0028) were significant (Table B.10). Tanzanian strains TZ 24 and TZ 25 were consistently highly aggressive against the three tomato lines, while TZ 95 was among the most aggressive on 'Tanya' and 'MT56' and least aggressive on WG120 (Table B.12). Line*strain interactions related to latent infection and severity followed similar trends.

Grafted plant survival and response to *R. pseudosolanacearum* inoculation. Survival of grafted plants prior to inoculation by *R. pseudosolanacearum* strains and subsequent bacterial wilt incidence, AUDPC, and latent infection after inoculation are summarized in

Table 3.9. There were no statistically significant differences (*P*=0.5535) in the percentage of surviving plants among any of the rootstock/scion or self-grafting combinations four weeks after grafting. Survival of grafted seedlings ranged from 60-75%. There were significant differences between seedlings of 'Moneymaker' scion grafted onto either rootstock and self-grafted 'Moneymaker' seedlings in bacterial wilt incidence (*P*= 0.0024) and AUDPC (*P*=0.019) after challenge with six combined Tanzanian *R. pseodosolanacearum* strains. Disease incidence percentage eight weeks post-inoculation did not differ significantly for plants that were grafted onto tomato 'MT56' and eggplant 'EG190' rootstocks and was significantly higher in self-grafted 'Moneymaker' (70%). Similar results were observed for disease progress where AUDPC values over eight weeks post-inoculation did not differ significantly for tomato lines seedlings grafted to 'MT56' and 'EG190' and were significantly higher in self grafted 'Moneymaker'. Latent infection by *R. pseudosolanacearum* was detected using ELISA at the termination of the experiment eight weeks after inoculation and no significant differences (*P*=0.4533) were observed between graft types.

Discussion

Selected tomato and eggplant lines were evaluated for resistance to *R. pseudosolanacearum* strains collected from Tanzania's main tomato producing regions for use as rootstocks for grafting farmer-preferred susceptible tomato varieties. From the preliminary screening of one eggplant and twelve tomato lines with Tanzanian and South Asian *R. pseudosolanacearum* strains, we selected three rootstocks for further evaluation.

The three rootstocks exhibited a moderate (<40% incidences) to high degree of resistance (<10%) disease incidence to the selected strains, of which 93% were phylotype I and 7% were phylotype III. Eggplant line 'EG190' was highly resistant (< 10% incidence) while tomato lines 'MT56' and WG 120 (25-45% incidence) were moderately resistant to the *R. pseudosolanacearum* strains tested. The pedigree of line WG120 includes Hawaii 7997 (Dr. D. Francis personal communication), a tomato breeding line resistant to bacterial wilt in multiple locations with resistance presented by a single dominant gene (Grimault and Prior, 1995; Wang et al., 1998). Line 'MT56' exhibited moderate to high resistance to bacterial wilt when tested in various agroecological zones of Uganda (Asiimwe et al., 2013). The response of rootstocks to bacterial wilt can be variable depending on the source of resistance genes and edaphic soil and environmental factors (Scott et al., 2005, Lebeau et al., 2011). Complexity and variability *R. pseudosolanacearum* may lead to non-durable resistance and limitation of global use of identified resistant lines or varieties (Wang et al., 1998). Therefore, screening with local populations of *R. pseudosolanacearum* is important to identify reliable rootstocks for use in Tanzania or areas with similar soil and *Ralstonia* population characteristics. In this study we also observed variability in the aggressiveness of *R. pseudosolanacearum* strains against the lines tested. This either describes the reaction of resistance genes against the strains or virulence of the strains. Rootstocks such as 'Hawaii 7996' were observed to restrict the movement of the pathogen beyond the lower stem (Grimault and Prior, 1995, Nakaho et al. 2004; Caldwell et al 2017). Restriction of pathogens to protoxylem tissue lowers bacterial wilt disease intensity (Nakaho et al., 2004) hence reducing disease in susceptible scion grafted onto them. Variability in resistance of

rootstocks was also reported by MacAvoy et al. (2012) and Scott et al. (2005, 2009). Variety response to strains may vary with variety depending on the virulence of strains. Strain virulence is highly influenced by population density as well as the expression of virulence genes that directly determine the aggressiveness of the pathogen (Grimault and Prior, 1995; Shutt et al., 2018; Manickam et al., 2021). Variability in pathogen virulence was also reported by Li et al., 2016 and Shutt et al., 2018). Thus, Lebeau et al., (2011) described resistance based on surviving plants because it is almost impossible to achieve 100% survival when screening for bacterial wilt resistance, especially in disease hotspots.

Our results indicated compatibility of selected rootstocks and scions; however, the successful survival of grafted plants was only 60-75% during the grafting and weaning process which is low for the grafting between these species (Msogoya and Mamiro 2016). The grafting process requires optimal conditions i.e., relatively high humidity (>85%) and moderately warm conditions to facilitate the healing process (Scott et al., 2005). We achieved >60% survival of grafted plants in this study, but better results could be achieved with more precise and monitoring and adjusting relative humidity and temperature. We used a non-automated screenhouse and hand sprayer to humidify the healing chamber used in this study. High and fluctuating daily temperatures sometimes reaching 35°C in the afternoon and >16°C during the night was a major challenge. Grafting for agronomic characteristics in Tanzania (Shipepe 2018; Msogoya and Mamiro, 2016) as well as resistance to soilborne diseases and bacterial wilt has been studied in other East African countries (Kenya and Uganda) (Akemo et al., 2002; Waiganjo et al., 2011; Erbaugh et al., 2011; Onduso, 2014; Kanyua, 2018; Kanyua et al., 2020). Despite promising results from

all these studies, the adoption of grafting technology by local farmers is low. The main reasons for poor adoption could be related to limited grafting knowledge, resources, facilities, and time by smallholder lower resource farmers in addition to the availability of seeds or commercial resistant rootstocks. It is important that during the first 14 days after grafting, plants be maintained at adequate relative humidity (>85%) and temperature (22-32°C) (Scott et al., 2005) to achieve maximum healing. Maintenance of these conditions by smallholder farmers could be challenging as possession of special healing chambers and prior knowledge of grafting technology. Government and stakeholders in tomato value chain should work together with non-governmental organizations and development partners to facilitate training and promote awareness among extensionists and farmers to promote grafting technology and its adoption (Anonymous, 2016). Thus, dissemination of grafting techniques, as well as experimentation on low-cost and effective growth chambers to help poor resource farmers adopt grafting as a disease management strategy, should be a priority (Manickam et al 2021). Use of materials such as PVC pipes or wooden sticks and transparent polythene sheets in building healing chambers and use of readily available resistant seeds from wild varieties have been promoted in developing countries in Southeast Asia, South Asia, East Africa, West Africa, Central Asia and Central America to help farmers adopt grafting (Manickam et al., 2021). In addition, grafting can be adopted by small nursery operators and women's groups. In Nepal, Bangladesh and Kenya entrepreneurs produce seedlings and sell them to smallholder farmers (Waiganjo et al., 2011; Manickam et al., 2021)

Tomato plants grafted onto resistant tomato and eggplant rootstocks have shown high potential to resist bacterial wilt (Rivard and Louws, 2008; Waiganjo et al., 2011; Kanyua, 2018; Lewis Ivey et al., 2021). Our findings indicated 100% survival of seedlings grafted with resistant rootstock 'EG190' after a challenge by strains of *R. pseudosolanacearum* collected from the major tomato growing regions of Tanzania. Rivard and Louws (2008) had similar observations with CR66, and 'Hawaii 7996' grafted to heirloom tomatoes. Rivard et al. (2012) also demonstrated the potential of Dai Honmei rootstock to resist bacterial wilt disease. However, they also reported that grafted plant survival was inconsistent across different locations. We also observed differences in aggressiveness of Tanzanian strains to the rootstocks 'EG190', 'MT56' and WG120 but in our study strain*line interactions were lacking among the rootstocks. Strain virulence is highly influenced by population density as well as the expression of virulence genes that directly determine the aggressiveness of the pathogen (Grimault and Prior, 1995; Shutt et al., 2018; Manickam et al., 2021). We observed differences in bacterial wilt incidence with a significant strain*line interaction in the prescreening experiments where South Asian strains were more virulent than Tanzanian strains. Although the Tanzanian strains were also phylotype I little is known about their ability to attack multiple solanaceous crops as our results indicated they were virulent on tomato but not eggplant. Asian phylotype I members are known for their high degree of virulence and ability to attack several solanaceous hosts including eggplant, sweet pepper, tomato, hot pepper, and black nightshade (Chesneau et al., 2018). All South Asian strains tested in this study were virulent to eggplant and tomato lines with variation in the percentage of surviving plants

(incidence) but no variation in latent infection or disease severity. Significant interactions are not only subject to change with pathogen variability but also the environmental conditions of the test (Lebeau et al., 2011). We hypothesize that controlled environmental conditions may have contribution to the behavior of the strains as well as the lines used. However, more experiments are needed to establish how environmental conditions contribute to the interaction.

Like other reports (Grimault and Prior, 1990; Lebeau et al., 2011), in this study bacterial wilt-resistant rootstocks and susceptible scions grafted onto these rootstocks supported latent infection by *R. pseudosolanacearum*. The rootstocks 'EG190', 'MT56', and WG120 can be regarded as potential candidates for grafting for bacterial wilt resistance in Tanzania. Lebeau et al. (2011) described good rootstocks as ones that can adapt to the pathogen (tolerance) or restrict the movement of *R. pseudosolanacearum* to the lower part of the seedlings. Resistant rootstocks enhance plant survival in pathogen-infested soils by inhibiting plant colonization or restricting pathogen movement in xylem (Nakaho et al., 2004; Lebeau et al., 2012, Caldwell et al 2017). Reports have proven recovery of bacterial wilt pathogen above the graft union (Nakaho et al 2004) however, restriction of pathogen movement or multiplication by latently infected resistant rootstock may lower the population density of bacterial wilt pathogen to the threshold that cannot cause plant wilting (Nakaho et al. 2004).

Research with bacterial wilt-resistant eggplant and/or tomato rootstocks demonstrated improved plant vigor, increased fruit yield, size, and weight of the grafted plants (McAvoy et al., 2012; Rivard and Louws, 2008; Manickam et al., 2021) through

robust root systems as well as their strong disease resistance (Salgon et al., 2017; Manickam et al., 2021). The robust root system improves nutrient and water uptake and enhances the signaling and translocation of defense hormones and proteins (Kumar et al., 2017, Manickam et al., 2021). With these findings, the main challenge remains to the availability of seeds from the three candidates identified in this study. Seeds for these experiments were obtained direct from breeders via special requests. Thus, breeding programs should focus on increasing the seeds for community use because currently, only breeders volunteer to provide seeds for research purposes. In addition to seed availability, this work has been done in a controlled greenhouse environment and large-scale and field testing is required. Future research should concentrate on field experiments to assess the performance of grafted plants with a broad representation of strains and natural environmental factors for better rootstock recommendations based on ecological characteristics or individual regions.

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Table 3. 1: *Ralstonia pseudosolanacearum* strains used in these studies.

Strain	Origin	Host	Phylotype	Year of collection	Experiment	Reference
TZ 9	Rungwe, Mbeya	Tomato	III	2017	Preliminary	This study
TZ 130	Rungwe, Mbeya	Tomato	III	2017	Preliminary	This study
TZ 48	Mbeya rural, Mbeya	Eggplant	III	2017	Preliminary	This study
SM 716	Comilla, Bangladesh	Pepper	I	2012	Preliminary	Subedi, 2015
SM 747	Chitwan, Nepal	Eggplant	I	2012	Preliminary	Subedi, 2015
TZ 55	Kilolo, Iringa	Tomato	III	2017	Preliminary	This study
TZ 57	Iringa rural, Iringa	Tomato	III	2017	Preliminary	This study
TZ 58	Mvomero, Morogoro	Soil	III	2017	Preliminary	This study
TZ 70	Arumeru, Arusha	Potato	III	2017	Preliminary	This study
TZ 71	Arumeru, Arusha	Tomato	III	2017	Preliminary	This study
TZ 80	Lushoto, Tanga	Potato	III	2017	Preliminary	This study
SM 727	Tangail, Bangladesh	Eggplant grafted on *S. sisymbriifolium*	I	2012	Preliminary	Subedi, 2015
SM 732	Tangail, Bangladesh	Eggplant grafted on *S. sisymbriifolium*	I	2012	Preliminary	Subedi, 2015
SM 738	Bogra, Bangladesh	Eggplant	I	2012	Preliminary	Subedi, 2015
TZ 22	Mvomero, Morogoro	Tomato	I	2017	Preliminary	This study
TZ 71	Misufini, Morogoro	Tomato	I	2019	TZ rootstock; grafted plants	This study
TZ 72	Misufini, Morogoro	Tomato	I	2019	TZ rootstock; grafted plants	This study
TZ 73	Misufini, Morogoro	Tomato	I	2019	TZ rootstock; grafted plants	This study
TZ 25	Mlali, Morogoro	Tomato	I	2019	TZ rootstock; grafted plants	This study
TZ 24	Mlali, Morogoro	Sweet pepper	I	2019	TZ rootstock; grafted plants	This study
TZ 95	Mlali, Morogoro	Sweet pepper	I	2019	TZ rootstock; grafted plants	This study

Table 3. 2: Tomato and eggplant lines/varieties screened for resistance to *Ralstonia pseudosolanacearum* in these studies.

Line/Variety	Source	Experiment
SGH06 220(WG12-128A)	D. Francis, OSU	Preliminary
WG2 121	D. Francis, OSU	Preliminary
SGH06 215(WG12-108A)	D. Francis, OSU	Preliminary
SGH06-211(WG12-139A130)	D. Francis, OSU	Preliminary
SGH06-216(FG12-608A)	D. Francis, OSU	Preliminary
WG12-110	D. Francis, OSU	Preliminary
WG12-130	D. Francis, OSU	Preliminary
WG12-120	D. Francis, OSU	Preliminary and Tanzania rootstock evaluation
FGH06- 304	D. Francis, OSU	Preliminary
FGH06- 301	D. Francis, OSU	Preliminary
FGH06-302	D. Francis, OSU	Preliminary
'MT56'	Makerere University, Uganda	Preliminary and Tanzania rootstock evaluation
'EG190'	AVRDC, Taiwan	Preliminary and Tanzania rootstock evaluation
'Moneymaker'	Growseed, Bristol, UK	Preliminary
'Tanya'	Seminis, Holland	Tanzania rootstock evaluation

Table 3. 3: Bacterial wilt incidence, severity, and latent infection in Ohio tomato rootstock breeding lines and scion variety ‘‘Moneymaker’’ during preliminary screening with five selected Tanzanian and South Asian *Ralstonia pseudosolanacearum* strains

Tomato line	**Incidence (%)**	**Severity (%)**	**Latent infection (%)**
‘Moneymaker’	66 bc	66.8 a	25.0 c
SGH06-211	40 ed	44.4 g	17.5 dc
SGH06-215	80 a	50.4 a	20.1 dc
SGH06-216	40 ed	58.4 bc	7.5 dc
SGH06-220	78 ab	64.4 a	17.5 dcd
WG12- 110	40 ed	62.4 ab	46.0 b
WG12- 120	36 e	31.0 h	2.5 d
WG12- 130	50 d	52.4 dfe	55.8 b
WG12- 121	64 c	57.1 dc	5.8 b
SGH06-301	40 ed	56.0 dce	90.0 a
SGH06-302	36 e	42.0 g	60.0 b
SGH06-304	48 ed	52.0 fe	5.0 d
P value	<0.0001	<0.0001	<0.0001
LSD	13.4	5.1	18.8

Values are means across individual inoculations of three Tanzanian and two South Asian strains of *R. pseudosolanacearum.* Means of two combined experiments conducted under similar greenhouse conditions; means with the same letters in a column are not significantly different at 5% alpha value

Table 3. 4: Mean bacterial wilt incidence, severity, and latent infection across 11 Ohio tomato rootstock lines and tomato variety ‘‘Moneymaker’’ eight weeks after inoculation with South Asian and Tanzanian strains of *Ralstonia pseudosolanacearum* under greenhouse conditions during a preliminary rootstock evaluation.

Strain	**Incidence (%)** [x]	**Severity (%)** [x]	**Latent infection (%)** [x]
SM747	66.7 a	57.3 b	32.7 bc
SM716	63.3 a	56.8 b	27.1 bc
TZ48	51.7 b	52.4 c	24.3 c
TZ130	43.3 b	37.7 d	37.6 ab
TZ9	32.5 c	61.6 a	45.5 a
P value	<0.0001	<0.001	0.008
LSD	8.6	3.3	12.1

[x] Values are means across 11 Ohio lines inoculated with individual inoculations of three Tanzanian and two South Asian strains of *R. pseudosolanacearum* Means of two combined experiments conducted under similar greenhouse conditions; means with the same letters in a column are not significantly different at 5% alpha value.

Table 3. 5: Bacterial wilt incidence, severity, and latent infection in tomato ‘MT56’ and eggplant ‘EG190’ rootstock lines and the susceptible variety ‘‘Moneymaker’’ inoculated individually with 15 selected Tanzanian and South Asian *Ralstonia pseudosolanacearum* strains.

Variety / line	**Incidence (%)** [x]	**Severity (%)** [x]	**Latent infection (%)** [x]
‘Moneymaker’	56.4 a	40.1 a	51.3 a
‘MT56’	22.9 b	16.2 b	23.3 b
‘EG190’	5.9 c	11.1 b	16.6 b
P value	<0.0001	<0.0001	<0.0001

[x] Averaged across all ten Tanzanian and five South Asian R. *pseudosolanacearum* strains used as inoculum. Means of two combined experiments; means with same letters in a column are not significantly different at 5% alpha value.

Table 3. 6: Mean bacterial wilt incidence, severity, and latent infection across eggplant 'EG190' and tomato 'MT56' rootstock lines and tomato variety ''Moneymaker'' eight weeks after inoculation with South Asian and Tanzanian strains of *Ralstonia pseudosolanacearum* under greenhouse conditions during a preliminary rootstock evaluation.

Strain	**Incidence (%)** [x]	**Severity (%)** [x]	**Latent infection (%)** [x]
TZ55	29.7 ab	20.1 dc	47
SM747	51.8 a	47.8 a	44.4
TZ48	18.6 ab	16.8 d	42.4
SM727	51.8 a	44.4 ab	40
TZ70	29.6 ab	23.7 bcd	38.9
TZ58	29.7 ab	24.6 abcd	33.3
TZ22	14.8 b	11.9 d	33.3
TZ9	26.0 ab	15.6 d	33.3
TZ130	33.6 ab	23.8 bcd	28.8
SM716	29.4 ab	40.0 abcd	27.8
SM732	18.4 ab	11.1 d	26.7
TZ57	29.6 ab	18.9 dc	22.2
TZ80	26.0 ab	11.1 d	22.1
TZ738	18.6 ab	18.9 dc	14.8
TZ71	18.4 ab	10.4 d	0
P value	0.0099	<0.0001	0.0927
LSD	36.-0	23.5	

[x] Values are means across 'EG190', 'MT56' and 'Moneymaker' inoculated with individual inoculations of ten Tanzanian and five South Asian strains of *R. pseudosolanacearum*. Means of two combined experiments conducted under similar greenhouse conditions, means with the same letters within a column are not significantly different from each other at a 5% alpha value.

Table 3. 7: Bacterial wilt incidence, area under the disease progress curve (AUDPC), and latent infection in tomato 'MT56' and WG120 and eggplant 'EG190' rootstock lines and tomato variety ''Tanya'' eight weeks post-inoculation with six *Ralstonia pseudosolanacearum* strains collected from key tomato producing regions of Tanzania.

Variety/line	**Incidence (%)** [x]	**AUPDC** [x]	**Latent infection (%)** [x]
'Tanya'	64.4 a	368.3 a	73.6
'MT56'	43.9 b	166.7 b	72.6
WG120	28.9 c	175.9 b	62.7
'EG190'	8.9 d	41.1 c	80.3
P-value	<0.0001	<0.0001	0.1161
LSD	7.3	72.3	-

[x] Means of two combined experiments across individual inoculations of six Tanzanian strains of *R. pseudosolanacearum*. Means with the same letter in a column are not significantly different at $P \leq 0.05$.

Table 3. 8: Bacterial wilt incidence, area under the disease progress curve (AUDPC), and latent infection averaged across tomato 'MT56' and WG120 and eggplant 'EG190' rootstock lines and tomato variety ''Tanya'' eight weeks post-inoculation with six *Ralstonia pseudosolanacearum* strains collected from key tomato producing regions of Tanzania.

Strain	incidence (%) [x]	AUDPC [x]	Latent infection (%) [x]
TZ 71	28.3 cd	125.0 bc	82.6
TZ 72	25.0 d	100.0 c	72.9
TZ 73	33.3cd	217.5 a	80.6
TZ 25	40.8b	221.7 a	67.8
TZ 24	55.0 a	241.7 a	68.6
TZ 95	36 cd	184.2 ab	61.3
P value	<0.0001	0.0002	0.1428
LSD	8.9	65.1	-

[x] Means across 'EG190', 'MT56', WG120 and 'Tanya' individually inoculated with six Tanzanian strains of *R. pseudosolanacearum* (all phylotype I). Means of two combined experiments conducted under similar greenhouse conditions; means in a column with same letters are not significantly different at 5% alpha value.

Table 3. 9: Grafted tomato seedling survival and bacterial wilt incidence, area under the disease progress curve (AUDPC), and latent infection in response to challenge with a combination of six Tanzanian *Ralstonia pseudosolanacearum* strains. The seedlings were self-grafted or grafted using tomato 'MT56' and eggplant 'EG190' rootstocks and 'Moneymaker' (MM) scion and tested under screenhouse conditions.

Graft type (rootstock/scion)	**Survival (%) [z]**	**Incidence (%) [x]**	**AUDPC [x]**	**Latent infection (%) [x]**
'Moneymaker'/'Moneymaker'	75	70 a	446.9 a	30
'MT56'/'MT56'	60	16 b	121.9 b	83.5
'EG190'/'EG190'	65	0 b	0.0 b	37.5
'MT56'/'Moneymaker'	65	10 b	81.3 b	66.5
'EG190'/'Moneymaker'	70	0 b	0.0 b	44
P value	0.5535	0.0024	0.019	0.4533
LSD	-	19.7	216.6	-

[x] Means across grafts from 'EG190', 'MT56' rootstocks and 'Moneymaker' scion inoculated with combined six Tanzanian strains of *R. pseudosolanacearum*. Means of two combined experiments conducted under similar greenhouse conditions; means in a column with same letters are not significantly different at 5% alpha value.

[z] Percentage grafted seedlings that survived healing chamber and weaning process for four weeks post grafting.

Fig. 3. 1: A) Wilting eggplant seedling during rootstocks evaluation; B) Healing chamber for tomato seedlings grafted onto tomato or eggplant rootstocks lined with wet paper towels and covered by plastic sheeting and shade cloth; the healing chamber was maintained in a screenhouse at Sokoine University of Agriculture, Morogoro, Tanzania.

Fig. 3. 2: A) Describe; B) Tomato seedlings grafted onto tomato or eggplant rootstocks inoculated with *R. pseudosolanacearum* strains in a screenhouse at Sokoine University of Agriculture, Morogoro, Tanzania.

Chapter 4. Anaerobic Soil Disinfestation for Bacterial Wilt Management in Tomato

Abstract

On-farm trials and bioassays were conducted to determine the efficacy of anaerobic soil disinfestation (ASD) with wheat bran, rice bran, molasses, and cow manure carbon sources in suppressing bacterial wilt disease of tomato in Tanzania. We established randomized complete block design (RCBD) experiments in nine farm fields located in Misufini, Mlali, and Image villages in the Morogoro and Iringa regions of mainland Tanzania. The bioassay experiment was also laid in a RCBD and conducted using soil naturally infested with *Ralstonia pseudosolanacearum* collected from the same nine fields. We observed a significant difference in pH and paint removal (P>0.0001) and temperature (P =0.01) between our carbon source-amended treatments and non-amended controls in both field and greenhouse (bioassay) environments. The treatments reduced bacterial wilt incidence in tomatoes grown in ASD-treated soils compared to non-treated control soils in field trials at Misufini 1 (P=0.0205), Misufini 2 (0.0061), and Mlali 2 (P =0.019). There were no significant differences (P<0.0001) in mean incidence among ASD treatments with different carbon sources. This trend was also observed in the bioassays in which bacterial wilt incidence and area under disease progress curves in tomato seedlings grown in field-treated soils from Image, Mlali, and Misufini were significantly lower than in non-treated controls (P <0.0001). A significant difference (P <0.05) was observed in latent infection among the treatments. However, ASD treatments varied significantly in reducing bacterial

wilt incidence among carbon sources for eight of nine soils, but results were inconsistent between fields for all carbon sources. Yield was assessed on three farms with low (<10%) wilt incidence and was not significantly affected by ASD treatment compared to controls.

Multivariate analysis of variance revealed non-significant (P>0.05) relationship in most of ASD independent factors with disease incidence and yield except for few significant positive or negative relationships observed for pH, reducing conditions, and temperature (P>0.05) in on-farm trials. Similarly, there were very few significant correlations between disease incidence, area under the disease progress curve (AUDPC), and latent infection to pH and reducing conditions in the bioassay. These experiments support the efficacy of ASD treatment in reducing bacterial wilt disease incidence, suggesting that ASD can be an important tool for bacterial wilt management on smallholder farms in Tanzania.

Introduction

Tomato is among the most widely cultivated horticultural crops in Tanzania for local consumption and export to neighboring countries (Maerere et al., 2006; de Putter et al., 2011). Tomato farmers consider qualities such as shape, size, yield, and market preferences in choosing varieties (Minja et al., 2011; Testen et al., 2016). However, the most preferred varieties are highly susceptible to diseases and pests that cause damage and reduce yield. Pests and diseases account for 56% and 88%, respectively, of tomato yield losses in Tanzania (UMADEP, 2003; CABI, 2004). Up to 100% yield losses have been reported in areas with high disease and pest pressure (UMADEP, 2003). Bacterial wilt is among the most damaging soilborne diseases of tomatoes, leading to total wilting of plants especially at the flowering and fruiting stage, and large yield losses (Elphinstone, 2005). The disease is caused by members of the *Ralstonia solanacearum* species complex (RSSC), the second most recognized bacterial pathogen causing devastating crop and yield loss globally, especially in Solanaceous crops (Champoseu and Momol, 2009; Mansfield et al., 2012; Meng, 2013). Members of the RSSC are widely distributed in subtropical, tropical, and temperate areas worldwide (Hayward, 1991; Yabuuchi et al., 1995; Elphistone, 2005). In Tanzanian tomato producing regions, *Ralstonia* spp phylotype I and III strains have predominated to date, which are designated *R. pseudosolanacerum* (Chapter 2; Remenant et al., 2011; Safni et al., 2014; Prior et al., 2016; Aloyce, 2020)

Bacterial wilt management approaches range from phytosanitary measures, cultural practices, biological control, and chemical treatments to host resistance (Saddler, 2005; Elphinstone, 2005; Champoseau and Momol, 2009, Shutt et al., 2018). Phytosanitation approaches may be effective in areas where the pathogen has not yet been introduced. In

these areas, quarantines and removal of infected plants can minimize disease introduction or spread. Cultural approaches including intercropping, crop rotation, delayed planting periods, and solarization has been shown to reduce bacterial wilt (Saddler, 2005; Kinyua et al., 2001). However, in the African environment, insufficient land availability and the short period between crops limit these approaches. In typical African tomato farming systems, tomato is planted after main crops (maize or rice) that are cultivated twice annually (Luzi -Kihupi et al., 2015), thus a very short time separates the rotation programs. Besides, intercropping is rarely practiced especially in areas with land scarcity where main crops are given priority with limited spacing. Biocontrol is not commonly used in African farming systems as research on their local adaptability, efficacy, dosage, and species recommendations is currently insufficient. The use of disease-resistant tomato varieties is constrained in lower-income countries due to their lack of availability, high cost, and/or failure to match local market preferences.

Among soil treatments to suppress bacterial wilt, solarization has been effective only for *Ralstonia* biovar 2 strains that inhabit cool areas, as strains from other biovar groups easily adapt to higher temperatures (French, 1994). Anaerobic soil disinfestation (ASD) has been shown in numerous studies to reduce *R. pseudosolanacearum* populations to undetectable levels or symptoms have been delayed or reduced in susceptible plants (Blok et al., 2000; Momma et al., 2006; Messiha et al., 2007; Van Overbeek et al., 2013). No ASD studies have been reported in Eastern Africa or Tanzania in particular.

Anaerobic soil disinfestation is a soil rejuvenation process that involves the creation of an anaerobic environment in water-saturated soil amended with high carbon-based organic materials. Anaerobic conditions are generated by flooding amended soil and

covering it with plastic sheeting to limit exchanges of gases for 2-15 weeks (Blok et al., 2000; Momma et al., 2006; Messiha et al., 2007). The addition of organic matter promotes the multiplication of soil microbial flora that produce compounds with antimicrobial activity and create an anaerobic environment harmful to aerobic microorganisms, including most plant pathogens (Momma, 2008; Runia et al., 2014; Huang et al., 2016; Testen and Miller, 2018). Anaerobic organic matter consumption by soil microbes leads to the release of abundant acetic and n-butyric acids among other organic acids (Momma et al., 2006; Sanabria et al., 2020) into the soil (Momma, 2008; Butler et al., 2012). Other organic acids include isovaleric, isobutyric, and propionic acids that are released in small quantities (Sanabria et al., 2020).

The efficacy of carbon sources used in ASD is a function of the quantity and types of metabolic products that are produced as they are broken down (Shrestha et al., 2016). The quantity and type of carbon source selected determine the type of organic acids and other toxic products released during the ASD (Hewavitharana et al., 2014). In some cases, availability may also guide the choice of carbon source used in ASD. Anaerobic soil disinfestation with cover crops such as mustard greens, plant by-products including rice husk, grape pomace, and wheat bran, molasses, and animal manure as carbon sources has been reported to effectively reduce diseases caused by soilborne pathogens (Blok et al., 2000; Momma, 2008; Testen and Miller 2019; Testen et al., 2021; Khadka and Miller 2021). Acetic and n-butyric acids reduced *Ralstonia* spp. populations in ASD-treated soils amended with wheat bran and other organic carbon sources with subsequent decreases in bacterial wilt incidence to below measurable rates in tomato (Momma et al., 2006; Momma, 2008). Fresh grass, commercial media containing plants with high protein

content, and potato haulms have been used as ASD carbon sources to treat soils contaminated by *Ralstonia* spp. with great success (Messiha et al., 2007; Van Overbeek et al., 2013). Messiha et al. (2007) reported *Ralstonia* spp. population reductions of over 90% after ASD treatment.

Ralstonia spp. strains can grow anaerobically as they invade and colonize plants by using nitrate respiration to generate energy, transforming inorganic nitrate into gaseous form (Chapter 2; Dalsing et al., 2015; Prior et al., 2016). However, a combination of factors and mechanisms in addition to soil anaerobicity contributes to the efficacy of ASD in suppressing soilborne pathogens (Momma et al., 2006; Runia et al., 2014). Microbial metabolic activities responsible for the accumulation of organic acids in ASD-treated soil also result in the release of toxic gases such as methane, nitrous oxide, ammonia, and hydrogen sulfide that have antimicrobial effects and help to reduce soil pathogen populations (Runia et al., 2014). Also, the incorporation of high-carbon amendments and limited oxygen promotes shifts towards anaerobic microbial populations that include *Clostridia*, *Klebsiella*, *Enterobacter* species (Huang et al., 2016; Testen and Miller, 2018). Members of these genera and others act as natural biocontrol agents through the production of soil anaerobic conditions, reduced soil pH, and formation of toxic compounds (Hewavitharana et al., 2014; Choi et al., 2018). They also out-compete and suppress the growth of soilborne pathogens by their rapid multiplication and adaptation to changes in soil conditions.

This study was focused on using ASD as a potential tool in reducing bacterial wilt in tomatoes grown in naturally infested soils using locally available carbon sources. We aimed at determining the efficacy of ASD treatment in delaying or reducing disease

symptoms in farmer-preferred tomato varieties that are highly susceptible to bacterial wilt. We hypothesized that the impact of bacterial wilt on susceptible tomato varieties will be reduced significantly with soil treatment using the appropriate carbon sources. The following were the specific objectives of this study:

A. Compare the efficacy of ASD treatments with different locally available carbon sources in reducing bacterial wilt in a wilt-susceptible tomato variety.
B. Assess the efficacy of ASD treatments in reducing tomato bacterial wilt incidence and severity in on-farm participatory field trials in selected bacterial wilt disease hotspots of Tanzania.

Materials and Methods

Study site. Three villages in the Morogoro and Iringa regions of Tanzania mainland (Fig. 4.1) were selected for conducting the ASD trials: Mlali and Misufini (Morogoro region) and Image (Iringa region). The villages are characterized by different soil types and rainfall patterns, but tomatoes are widely produced throughout these areas.

Morogoro: Misufini and Mlali villages. Misufini is located at Mvomero district Morogoro region and lies between 6°17’ 29.16°S, 37°28 19° 92’E approximate 400m absl. The area lies in the flat Wami river plain with characteristic fertile clay loamy soil. The area receives heavy rainfall (March-June) and shorter rains (October-December). Tomato is normally planted after the main crop (rice or maize) season together with other vegetables that include eggplant, melons, onions, and cucumber**.** Mlali lies between 6°57’ 39.60’ S, 37°3’ 11°64’ E6° 57' 0" S, 37° 32' 0" E. The area is a flat (599m absl) river plain that is dependent on irrigation and vegetables are produced all year round. Tomato is

always rotated with sweet pepper, eggplant, and maize (Chapter 5). The area is characterized by sandy loamy and moderately high temperatures throughout the year. It has long (March-June) and short (October-December) rainy seasons.

Iringa: Image village. Image is in Kilolo district Iringa region. Image is located at an altitude of 1500m and is characterized by sandy loam soil and cool temperatures as low as 10°C in cooler months. The main rainy season is November to April and the short rainy season is February to April. Many farms are irrigated, and tomato is rotated with the main crops' maize and sunflower throughout the year. Tillage in vegetable and tomato fields is by tractors and ox-drawn plow.

On-farm field ASD treatment. ASD experiments were conducted in nine farm fields (0.25 ha) located in Morogoro (six) and Iringa (three), in October 2019 and February 2020 to evaluate the efficacy of ASD with four different carbon sources in reducing *R. pseudosolanacearum* load in naturally infested soil. The soils were amended with rice bran or wheat bran (sourced from mills in rice and wheat farming areas), liquid molasses (Kilombero Sugar Company, Illovo, Morogoro), or cow manure (collected from Sokoine University of Agriculture animal farm) at the rate of 20.2 Mg/ha fresh weight. Controls were saturated soils that were neither amended nor covered (aerobic control) and saturated and covered but not amended (anaerobic control). The experiment was set in a completely randomized block design with four blocks. Each block had all six treatments in six plots of 1m × 2.5 m. The field had four rows with 0.5-m spacing within and 1-m between each of the four blocks (Fig. 4.2 A, B). Carbon sources were mixed into the 15 cm top of soil manually using a hoe. Indicator of Reduction in Soils (IRIS) tubes were prepared as described by Castenson and Rabenhorst (2006) by painting 2 x 30 cm polyvinyl chloride

(PVC) tubes with iron III oxide paint. IRIS tubes were inserted into the soil in each plot through a guide hole created by a 2 x 30cm PVC tube. The plots were saturated with water using a watering can or hose with a pump, then covered with black plastic sheeting 2m wide 3.5mil thick. The edges of the plastic sheeting were covered with soil to limit the exchange of air. The ASD treatments lasted 3 weeks. Soil temperatures were measured using a probe field digital thermometer (Fisherbrand^{TM}, Thermo Fisher Scientific Inc., UK). Immediately after removal of the plastic sheet, the thermometer probe was inserted into the soil in the four corners and center of each plot to a depth of 10cm and average temperature computed for each treatment in a field. IRIS tubes were removed from the soil immediately after the removal of the plastic sheet, rinsed, and allowed to dry. Percentage paint removal (PR) was estimated by the grid method, in which 1mm squares were drawn all over the tubes. The total number of squares was counted, followed by counting the number of squares from which the paint was removed. The percent of paint removal was calculated using the formula (Sanabria et al. 2020):

Percentage IRIS tube paint removal = 100% - ((PA - PR)/PA×100)

where (PA) = painted area and (PR) = area with paint removed. From each plot, 10g of soil was sampled in separate 10cm Falcon tubes for pH analysis at Sokoine University of Agriculture (SUA) soil science laboratories. Soil PH was determined by mixing the sampled soils (1:1) with distilled water, agitating vigorously on a shaker for 30 minutes, and centrifuging at 30,000 revolutions per minute (rpm) for 10 minutes. The pH meter electrode Cyberscan pH 510 (Eutech Instruments, Singapore) was inserted into the supernatant and the displayed pH value was recorded. Measurements of pH, temperature and PR are reported as the average of measurements of four reps/treatment.

On-farm evaluation of ASD efficacy. The efficacy of ASD with four different carbon sources was assessed in on-farm plots located in bacterial wilt disease hotspots. The ASD-treated plots were left for 7 days to aerate after removal of plastic sheets before tilling them with a hand hoe at approx. 15 cm depth. Ten holes approx. 10X10 cm were dug in each plot, in two rows (five holes each) separated by 100cm spacing and 50cm between holes. A 20-day-old tomato seedling (bacterial wilt-susceptible variety Assila (Seminis, Holland) raised by the farm owner was planted in each hole. The seedlings were raised in 0.5kg polythene bags filled with soil heated for 5-6 hrs in firewood-fueled local stoves and amended with cow manure before seed sowing. The bags were kept in nurseries near fields and watered once daily. Each farmer planted additional seedlings in areas surrounding the ASD-treated plots for comparison. The plants received the same fertilization and pesticide applications as the test plants. Seedlings were fertilized with NPK (15-9-20) fertilizer (Yaramila Winner, Yara TZ) 2 and 6 weeks after planting at the rate of 5g/seedling, followed by UREA and sulfur (40N, 5.6S) (Yaravera AMIDAS, Yara TZ) at 8 weeks after planting and Nitrabor with nitrogen, calcium, and boron (YaraLiva Nitrabor, Yara) at fruit set. The plots were watered twice weekly on dry days, otherwise, they were rainfed. The plants also received applications of fungicides (Linkmill WP; metalaxyl-M 40g/Kg, mancozeb 640g/Kg) and insecticide (Abamectin 5% EC) every two weeks. Weeding was done manually as needed to maintain the plots weed-free. Disease scores for bacterial wilt incidence were taken every two weeks by counting dead and wilting plants. The plants were raised for 3 months. The yield was determined in two trials from Image and one from Mlali by weekly harvesting and weighing of mature tomato using a digital weighing

balance for one month. All yield measurements were made by *farmers* under the supervision of the researcher.

Bioassay of soils naturally infested with *R. pseudosolanacearum*. Soil naturally infested with *R. pseudosolanacearum* was collected from the nine fields that were used for the above on-farm ASD trials. Experiments were conducted in Department of Crop Science and Horticulture screenhouses from November to February 2019 and repeated from March to June 2020. Wheat bran, molasses, rice bran, and cow manure were used as carbon sources at the rate of 20.2 Mg/ha. Controls were neither amended nor covered (aerobic control) and saturated and covered but not amended (anaerobic control). The experiment was set in a completely randomized block design with four blocks each containing all six treatments arranged similar to the field setup for each of the nine soil samples collected from the aforementioned fields. Carbon sources were added to 300g fresh weight of infested soil packed in 500g ziplock bags and mixed thoroughly by hand. Soils were saturated with 30ml of distilled water. IRIS tubes (0.5cm x 10-cm) were inserted into each soil before sealing the bags to limit air exchange. The bags were arranged in RCBD on plastic trays (one for each site). The ASD treatment lasted 3 weeks. Screenhouse temperature was recorded during the ASD experiment using a thermometer and averaged to report as ASD treatment temperature. IRIS tubes were removed immediately after opening the ziplock bags and paint removal was estimated as described previously. From each plot, 10g of soil was sampled in separate 10cm falcon tubes for pH analysis. Soil pH was determined as explained in the previous section.

The bags were opened to aerate the soil for 7 days, then the soil was pulverized by hand and placed in 250ml disposable cups. A 20-day-old tomato variety Assila seedling

was transplanted into each cup. The tomato seedlings were previously raised in plastic trays with 50x50 mm holes filled with autoclaved soil amended with cow manure. The cups were arranged in RCBD within each field collection (Fig. 4.3). The tomato seedlings received the same fertilization and pesticide applications as field plants except for the amounts of fertilizer applied adjusted to small-sized pots. The seedlings were raised for 8 weeks and disease assessments were done twice weekly.

Plants were assessed weekly for the incidence and severity of wilting symptoms. Disease incidence was assessed by counting the total number of plants (N) and the number of plants with bacterial wilt symptoms (n) for each replicate. The incidence of bacterial wilt was calculated using the equation

$$Incidence\ of\ bacterial\ wilt = \frac{n}{N} * 100$$

Disease severity values were obtained by rating diseased plants for each treatment replication using a 1-5 scale (Horita and Tsuchiya, 2001) in which 1=asymptomatic, 2=two leaves withered, 3=three leaves wilted, 4=at least four leaves wilted, and 5=dead plant (5). Each severity score was converted to a leaf damage scale (0-100 %), where 0 represented asymptomatic leaves and 1, 2, 3, 4, and 5 represented 20, 40, 60, 80, and 100% wilted leaves, respectively. Mean disease severity was expressed as the mean of all leaf damage percentages for each treatment. The area under the disease progress curve (AUDPC) was calculated according to the Excel formula (Madden et al., 2007).

$$\text{AUDPC} = \sum_{i=1}^{n} \left(\left(\frac{y_i + y_{i+1}}{2} \right) + (t_i + 1 - t_i) \right)$$

Where y_i = measures of disease level at ith observation and t_i= time of disease measure at ith observation.

To assess latent infection, plants remaining asymptomatic 8 weeks after inoculation were sampled by cutting a 2 cm stem section from the base of the plant and placing it in a tube containing 2.5 ml of sterile distilled water to allow for bacterial streaming for one hour. Suspensions (100µl) were pipetted into wells of a 96-well microtiter plate and an enzyme-linked immunosorbent assay (*R. solanacearum* ELISA kit; Agdia Inc. Elkhart, IN, USA) was conducted according to manufacturer instructions (Fig. 4.4). Negative and positive controls provided with the kit were used as color change guides to score positive or negative results. Wells with blue color visibly darker than the negative control were scored as positive, whereas wells with no color or lighter blue than the negative control were scored as negative for latent infection. The percentage of plants with latent infection was calculated by dividing the number of plants that tested positive for *R. solanacearum* by the total number of plants sampled for latent infection x 100.

Statistical analyses. Statistical analyses were carried out in SAS 9.4.4 (2017) using the statistical package Proc GLM. All data were tested for normality using the Shapiro test and equal variance using Bartlett's test; when needed data were transformed using arcsin (n) or $\log_{10}(n)$. Treatment means were analyzed and compared using the Tukey Kramer test at $P = 0.05$. Multivariate Analysis of Variance (MANOVA) was used to determine the correlation between ASD predictor variables (soil pH, temperature, and % paint removal) and bacterial wilt incidence for both on-farm and bioassay experiments. Area under the disease progress curve means were compared in SAS using Proc GLM.

Results

On-farm evaluation of ASD efficacy

Indicators of ASD (soil pH, temperature, and paint removal): There were significant differences ($P<0.05$) in soil pH among ASD-treated and control soils in plots in Image 2 and 3, Misufini 1 and 3, and Mlali 1, 2, and 3 (Table 4.1). Significant variation was also observed in IRIS tube paint removal for all but one trial in Misufini and all trials in Image and Mlali. Soil temperature varied significantly in Image 2, Misufini 1 and 3, and Mlali 1 and 3. Low values of soil pH were observed with carbon source-amended treatments as compared to aerobic control. Soil pH in the aerobic controls ranged from 5.6 to 7.1 amongst the nine fields, and in the anaerobic controls ranged from 5.5 to 7.1. In five of nine trials, soil pH was significantly lower in the anaerobic control than in the aerobic control. The effect of ASD carbon sources on soil pH was inconsistent across the nine fields. Soil pH was significantly lower than in the aerobic controls in five fields in ASD-treated plots amended with wheat bran, rice bran, or molasses, and four amended with cow manure. Percentage IRIS tube paint removal, an indicator of soil reducing conditions, was low (<10%) in aerobic controls in five of nine fields, and significantly lower than in anaerobic control soils in three of nine fields. The percentage of IRIS paint removal in carbon source-amended ASD-treated soils ranged from 14.8% to 53.8%. Soil reducing conditions were significantly higher in 27 of 36 carbon source-amended ASD-treated field plots than in their corresponding aerobic control plots. Soil temperature in aerobic control plots ranged from 24°C to 34.7°C and was significantly lower than in anaerobic control plots in five of nine fields. Significant temperature increases were observed in 19 of 36 ASD-treated field plots compared to their corresponding aerobic controls.

Effect of ASD treatment with different carbon sources on bacterial wilt disease incidence and tomato yield. Bacterial wilt incidence was low (<10%) in tomatoes in the three Image village fields and Mlali 1, and differences in disease incidence between the aerobic and anaerobic controls and ASD treatments were significant only in the three fields with higher disease pressure: Mlali 2 (P=0.0079), Misufini 1 (P=0.0205) and (P=0.0061) Misufini 2 (Fig. 4.5). In the Misufini 1 and 2 fields, wilt incidence in the aerobic control was high (40% and 55%, respectively) and did not differ from disease incidence in the anaerobic control. Wilt incidence was significantly lower in ASD-treated plots than in the aerobic control in these two fields except for the molasses-amended plots in Misufini 1. Similarly, although bacterial wilt incidence was lower in Mlali 2 (23%) than in Misufini and disease incidence was significantly lower in the anaerobic than aerobic control, wilt incidence was lower in all ASD-treated plots regardless of carbon source.

There were no significant differences in tomato yield between ASD-treated and control plots in any of the three farms selected to collect yield data: Image 1 and 2 and Mlali 1 (Table C.1).

Comparison between ASD predictor variables (pH, PR, and temperature) and response variables (incidence and yield) in on-farm trials: pH was a significant (P = 0.0329) predictor of bacterial wilt incidence only in Image 3, with a weak (r^2 = 0.536) positive correlation (Table 4.2). A stronger positive correlation between disease incidence and percentage point loss from IRIS tubes was observed in Mlali 2 (r^2 =0.6719, P = 0.0044) but there were no significant correlations between paint loss and disease incidence at any other sites. Furthermore, the temperature was weakly positively correlated only in Misufini

2 (r^2=0.46663, P = 0.025). There were no significant correlations between yield as a dependent variable and pH, paint removal, or temperature.

Bioassay evaluation of ASD efficacy

Indicators of ASD (soil pH and paint removal). There were significant differences (P<0.05) in pH among ASD-treated and control soils for all samples collected from Image, Misufini, and Mlali except for soil collected from Mlali 2 and Image 3 fields (Table 4.3). Significant variation was also observed in IRIS tube paint removal for all soils collected from Image, Misufini, and Mlali fields. There were significant differences in soil pH among ASD-treated and control soils from Image 1 and 2, Misufini 1, 2, and 3, and Mlali 1 and 3 (Table 4.3). Soil pH in the aerobic controls ranged from 6.7 to 7.0 amongst the soils collected from the nine fields, and in the anaerobic controls ranged from 6.0 to 6.8. In seven of nine soils, pH was significantly lower in the anaerobic control and most ASD-treated soils than in the aerobic control. Differences in soil pH between ASD-treated soils amended with the four carbon sources were not consistent across the seven fields. Percentage IRIS tube paint removal was low (0.9-12%) in aerobic and anaerobic controls in all soils from the nine fields. The percentage of IRIS paint removal in carbon source-amended ASD-treated soils ranged from 3.8% to 40.5%. Soil reducing conditions were significantly higher in 26 of 36 carbon source-amended ASD-treated field plots than in their corresponding aerobic and anaerobic control plots.

Efficacy of ASD with different carbon sources in reducing bacterial wilt in tomato seedlings. There was a significant difference (P<0.0001) in disease incidence and AUDPC among Assila tomato seedlings grown in ASD-treated soils collected from the nine fields (Fig 4.6). Bacterial wilt incidence among tomato variety Assila seedlings grown in aerobic control soils ranged from 25% to 50%. Bacterial wilt incidence was significantly lower among seedlings planted in ASD-treated soils from all nine fields than in aerobic control soils regardless of carbon source. Disease incidence was also lower in seedlings grown in anaerobic control than aerobic control soils from eight of nine fields. Significant variations were observed in disease incidence among seedlings grown in ASD-treated soils amended with different carbon sources for each field. However, the effect of carbon source on ASD efficacy varied across the nine field soils. Bacterial wilt disease progress throughout the experiment as measured by AUDPC also varied significantly among seedlings planted in ASD-treated and control soils from all nine fields (Fig. 4.7).

The presence of latent infection varied significantly (P<0.05) among tomato seedlings surviving 8 weeks after planting in ASD-treated and control soils from all nine *P. pseudosolanacearum*-infested tomato fields (Table 4.4). Percentage latent infection in tomato seedlings planted in aerobic control soils varied from 0% to 100% across the nine field samples.

Comparison between ASD predictor variables (pH and PR) and response variables (incidence, AUDPC, and latent infection). Weak but significant positive correlations were observed between soil pH and bacterial wilt incidence (Image 3, $r^2 = 0.3495$, $P = 0.0292$), AUDPC (Misufini 1, $r^2 = 0.4325$, $P = 0.006$) and latent infection (Misufini 3, $r^2 = 0.3145$, $P = 0.0481$; Image 1, $r^2 = 0.4283$, $P = 0.0065$) (Table 4.5). Significant weak

negative correlations were observed between the percentage paint loss from IRIS tubes and disease incidence for soils from two fields in Misufini (Misufini 1, $r^2 = -0.4288$, $P = 0.0064$; Misufini 2, $r^2 = -0.4452$, $P = 0.0045$), but no significant correlations between PR and AUDPC were detected. Weak but significant positive (Misufini 1, $r^2 = 0.3713$, $P = 0.02$; Misufini 3, $r^2 = 0.4901$, $P = 0.0013$) and negative (Misufini 2, $r^2 = -0.5772$, $P = 0.0001$) correlations were found between PR and latent infection in tomato seedlings grown in these soils.

Discussion

Experiments were carried out to evaluate the efficacy of ASD with four different carbon sources (Wheat or rice bran, cow manure and molasses) in reducing the incidence of tomato bacterial wilt in disease hotspots in farm fields in Tanzania and through in invitro bioassays using soils from the test fields.

Bacterial wilt disease incidence was reduced or eliminated in both on-farm trials and bioassays by ASD treatment of *R. pseudosolanacearum*-infested soils although the efficacy varied by carbon source and by field. Testen and Miller (2018) and Sanabria et al. (2020) emphasized the variation in efficacy of different carbon sources during ASD treatment. Carbon sources vary in composition thus there is variability in type and amount of gases, organic acids and other metabolites that work together to disinfest the soil pathogens and/or the types of pathogens that they affect (Momma et al 2006; Sanabria et al 2020; Hewavithrana et al., 2014; Testen and Miller, 2018). We observed no bacterial wilt in some carbon-source-amended ASD treatments in the field trials and bioassay, indicating low *R. pseudosolanacearum* populations compared to the aerobic controls.

Many factors may have contributed to the observed reductions in disease incidence, including initial *R. solanacearum* population size, soil types and conditions, amount and type of carbon source used, and duration of ASD treatment, as well as surrounding environmental conditions i.e., rainfall and temperature regimes (Momma et al., 2006; Runia et al., 2014; Mazzola and Hewavitharana,2014). Our choice of carbon sources and dosage were guided by results from previous ASD experiments and availability, especially in the subsistence farming community (Momma, 2008; Testen and Miller, 2018; Sanabria et al., 2020), and we hypothesize that carbon source type and amount may need to be site-specific. While all four carbon sources were equally effective in ASD in the on-farm trials, higher rates may have been needed to increase the reduction in bacterial wilt incidence in the tomato crop. The locations selected for this study differ in soil type, altitude, temperature, and rainfall regime, and likely by microbial flora composition. Mlali is a warm area with sandy loam soil and receives less rainfall compared to Image and Misufini that are characterized by cool temperatures, clay loamy soils, and relatively heavy rains. Soil type and temperature regime also affect *R. pseudosolanacearum* population size and distribution (Elphinstone, 2005).

Anaerobic soil disinfestation treatments with wheat and rice bran and fresh grass amendments were reported by others to reduce symptoms and bacterial wilt incidence (Momma et al., 2006; Messiha et al., 2007; Van Overbeek., 2013). Various mechanisms are involved in ASD to reduce soilborne pathogen populations. Anaerobic soil disinfestation enhances the activity of anaerobic microbial populations such as *Klebsiella* (Huang et al., 2016; Testen and Miller, 2018) that degrade carbon source amendments and

increase their population density while releasing metabolites with antimicrobial effects. The anaerobic conditions that develop in ASD-treated soils are generally detrimental to the growth of aerobic microorganisms including most plant pathogens (Katase et al., 2009; Runia et al., 2014). *Ralstonia pseudosolanacearum* is an exception because it can grow anaerobically through nitrogen metabolism and a denitrification pathway (Dalsing et al., 2015; Prior et al 2016), therefore anaerobic conditions during ASD cannot explain its population reduction. Anaerobic soil disinfestation involves other processes that are detrimental to *R. pseudosolanacearum* populations. Increased populations of anaerobes play a direct role in outcompeting soilborne pathogens (Huang et al., 2016) such as *Ralstonia* spp.

The type and amount of organic acids released during ASD determine how acidic the soil and toxic to microbes the soil environment will be (Hewavitharana et al., 2014). Momma et al. (2006) suggested that low pH (5.5) in ASD-wheat bran-treated soil effectively reduced populations of soil pathogens including *R. pseudosolanacearum*. Acetic and butyric acids reduce *R. solanacearum* populations at concentrations of 2000mg/kg and 1500 mg/kg, respectively (Momma, 2008). We did not observe a strong relationship between pH and bacterial wilt incidence in tomato seedlings or later latent infection in our on-farm ASD trials or bioassays. However, we observed consistent significant reductions in wilt incidence indicating that more than one mechanism can be involved in suppressing *R. pseudosolanacearum* populations during ASD.

Increased soil reductive conditions during ASD play a large role in the efficacy of the process (Runia et al., 2014). In previously reported ASD experiments, reductive soil conditions were correlated with IRIS tube iron oxide paint removal (Testen and Miller,

2018; Sanabria et al., 2020). Paint removal signifies low soil oxygen concentration resulting from high metabolic activities of soil anaerobes (Momma et al., 2006; Runia et al., 2014). We did not observe a strong correlation by MANOVA between iron oxide paint removal and reduced bacterial wilt incidence and latent infections in tomatoes grown in soil treated with ASD. However, significant increases in iron oxide paint removal compared to the aerobic control were observed in eight of nine tomato fields, while increased paint removal compared to the anaerobic control was observed in seven of nine fields.

We also suggest that the use of hand hoe in mixing carbon sources into the soil may have contributed to the differences in incidence between the on-farm trial and bioassay. Application of a carbon source requires vigorous mixing; thus, distribution may have been uneven in individual field plots, whereas carbon sources were uniformly distributed in soil samples for the bioassay. *Ralstonia* spp. have been reported to survive in deep (approx. 75cm) layers of soil, especially in environments with favorable growth conditions (Van Elsas et al., 2012). In this case, any soil treatment approach should target a reasonable depth to reduce bacterial populations effectively. This could help explain less than 100% bacterial wilt suppression when conditions favor the multiplication of a few bacterial cells that survived the ASD treatments (Momma, 2008). We observed disease occurrence in the later stages of tomato production in our field experiments. We hypothesize that our treatments were effective, but the carbon sources were not mixed deep enough to disinfect the lower soil layer that could be reached by mature tomato plants. We also encountered unusually heavy and frequent rains throughout the study, with almost no days without rain. The rains

led to flooding and landslides that were likely a source of cross-contamination among our ASD-treated plots, especially along the field borders.

In our ASD experiment, raw cow manure was one of the most efficient carbon sources in reducing bacterial wilt. However, its recommendation to be used as a carbon source for ASD should be investigated in a broader spectrum of food safety especially in tomato and vegetable farming systems where the produce is consumed raw. Cow and other animal manure are a source of foodborne pathogens such as enteropathogenic *Escherichia coli,* and present a high risk for produce contamination. Therefore, although manure is an inexpensive and effective option for ASD, farmers should follow best practices to minimize the risk of cross-contamination of produce. In addition, studies to determine the effectiveness of composited cow manure as a carbon source may be warranted as composited manure presents a much lower safety.

Integrated disease management approaches should be employed to reduce the risk of unexpected bacterial wilt occurrence after ASD. These approaches may include cultural practices such as delayed planting time (cool months), rotation with non-host crops, and the use of resistant tomato varieties or seedlings grafted onto wilt-resistant rootstocks.

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Table 4. 1: Mean soil pH, Indicator of Reduction in Soils (IRIS) tube paint removal and soil temperature after 3 weeks of anaerobic soil disinfestation (ASD) treatment with different carbon sources in nine on-farm trial sites in Mlali (ML), Image (IMG) and Misufini (MS) villages in Tanzania

ASD indicator factor	Carbon source	Mean [x]								
		IMG1	IMG2	IMG3	MS1	MS2	MS3	ML1	ML2	ML3
pH	Aerobic control	5.6	6.8 a	6.6 a	7.0 a	6.7	7.1 a	6.5 a	6.8 a	7.1 a
	Anaerobic control	5.5	6.8 a	6.2 c	6.7 ab	6.3	6.0 c	6.1 d	6.3 c	7.1 a
	Wheat bran	5.5	6.6 b	5.8 e	6.4 bc	6.3	6.5 b	6.5 a	6.5 b	7.1 a
	Rice Bran	5.5	6.8 a	6.5 b	6.5 b	6.2	5.1 d	6.2 c	6.5 b	7.1 a
	Molasses	5.5	6.2 c	6.5 b	6.1 c	6.1	6.4 b	6.4 ab	6.2 c	7.1 b
	Cow manure	5.5	6.1 d	6.1 d	6.6 ab	6.3	6.2 c	6.4 ab	5.3 d	5.7
	P value	0.3071	<0.0001	<0.0001	0.0021	0.1557	<0.0001	<0.0001	<0.0001	<0.0001
Paint removal (%)	Aerobic control	28.5 c	2.3 f	19.0 f	5.9 c	17	6.7 c	8.5 d	17.8 c	9.1 c
	Anaerobic control	36.6 c	13.8 e	24.2 e	18.0 ab	18.3	9.7 c	9.5 d	16.4 c	12.3 c
	Wheat bran	44.6 ab	38.0 a	44.9 c	26.5 a	18.7	20.0 b	32.4 a	29.2 ab	14.8 c
	Rice Bran	53.8 a	17.3 d	75.2 a	18.4 ab	21.6	39.1 a	25.1 b	25.7 bc	40.5 a
	Molasses	41.2 abc	36.7 b	36.1 d	15.4 bc	25	28.1 b	15.3 c	25.0 bc	40.2 a
	Cow manure	47.0 ab	34.3 c	52.8 b	19.3 ab	29.7	26.8 b	19.2 c	37.1 ab	29.1 b
	P value	0.0478	<0.0001	<0.0001	0.0167	0.4565	<0.0001	<0.0001	0.0066	<0.0001
Temperature (°C)	Aerobic control	27	24.7 c	24	26.5 b	34.7	31.0 c	27.9 b	33	34.1 c
	Anaerobic control	27.8	25.7 ab	24.3	30.1 a	38.1	34.9 ab	32.5 a	35.6	37.6 ab
	Wheat bran	27.8	26.2 a	24.8	29.9 a	37.5	35.4 a	33.2 a	35.9	38.4 ab
	Rice Bran	27.6	26.1 a	24.2	30.8 a	38.2	35.5 a	32.4 a	36.4	39.1 a
	Molasses	27	25.7 ab	24.1	30.3 a	35.8	34.3 b	33.1 a	34.9	36.7 b
	Cow manure	27.9	25.3 bc	24.9	30.7 a	37	34.7 b	32.3 a	35.9	36.5 b
	P value	0.5749	0.0025	0.4319	<0.0001	0.1106	<0.0001	0.0078	0.1855	0.0018

[x] Means of four plots representing each of six asd treatments in a field, treatments with the same letters within a row are not significantly different from each other

Table 4. 2: Output of multivariate analysis of variance (MANOVA) summarizing relationship between dependent variable bacterial wilt incidence of tomato plants to anaerobic soil disinfestation toindependent variables soil pH, paint removal (PR), and temperature during on-farm trials

Response factor	**Location**	**r^2 & P^x**	**pH**	**PR**	**Temperature**
Bacterial wilt incidence	Misufini 1	r^2	-0.1371	0.0762	0.2353
		P	0.6126	0.779	0.3795
	Misufini 2	r^2	-0.4162	0.3018	0.0463
		P	0.1088	0.2559	0.0687
	Image 1	r^2	0.0788	0.4078	0.4512
		P	0.7718	0.1169	0.0794
	Image 2	r^2	-0.0461	-	0.1154
		P	0.8653	-	0.6703
	Image 3	r^2	**0.5346**	-	0.295
		P	**0.0329**	-	0.2673
	Mlali 1	r^2	-0.1561	-0.2547	0.1468
		P	0.5638	0.3396	0.5873
	Mlali 2	r^2	-0.295	**0.6719**	0.3815
		P	0.2673	**0.0044**	0.1447
	Mlali 3	r^2	0.3328	0.3399	-0.0898
		P	0.2078	0.2121	0.7407
Yield	Mlali 2	r^2	0.3605	-0.3237	-0.0995
		P	0.1701	0.2212	0.7137
	Image 1	r^2	-0.1971	0.4079	0.3659
		P	0.4644	0.1168	0.1633
	Image 2	r^2	0.1273	-	0.1405
		P	0.6684	-	0.6036

r^2 = Partial correlation coefficient and *P*= 5% alpha value that signifies the relationship between bacterial wilt incidence, or tomato yield and independent variables pH, temperature, IRIs tube paint removal during ASD on-farm trial

Table 4. 3: Mean soil pH and Indicator of Reduction in Soils (IRIS) tube paint removal after 3 weeks of anaerobic soil disinfestation (ASD) treatment with different carbon sources in bioassays of *Ralstonia pseudosolanacearum*-infested soils collected from tomato fields in Mlali (ML), Image (IMG)and Misufini (MS) villages in Tanzania

ASD indicator factor	Carbon source	Mean [x y]								
		IMG1	**IMG2**	**IMG3**	**MS1**	**MS2**	**MS3**	**ML1**	**ML2**	**ML3**
pH	Aerobic control	7.0 a	6.9 a	6.6	7.0 a	6.7 a	6.8 a	6.7 a	6.8	6.8 a
	Anaerobic control	6.6 b	6.5 b	6.2	6.6 bc	6.3 c	6.5 cb	6.5 c	6.6	6.7 ab
	Wheat bran	6.4 cb	6.4 bc	6.4	6.8 abc	6.5 b	6.5 cb	6.6 ab	6.6	6.5 c
	Rice Bran	6.2 cd	6.4 bc	6.4	6.7 bc	6.5 cb	6.6 b	6.5 c	6.7	6.5 c
	Molasses	6.2 cd	6.3 c	6.4	6.9 a	6.4 c	6.3 d	6.5 c	6.6	6.6 bc
	Cow manure	6.0 d	6.6 b	6.5	6.6 c	6.5 b	6.5 cb	6.5 c	6.6	6.6 bc
	P value	<0.0001	<0.0001	0.105	0.0341	<0.0001	<0.0001	0.0005	0.0977	0.0023
Paint removal (%)	Aerobic control	6.8 f	0.9 f	6.5 f	2.2 c	2.8 d	2.9 d	5.4 bc	4.7 d	12.1 dc
	Anaerobic control	12.1 e	4.5 e	8.1 e	3.0 cb	3.3 cd	3.6 cd	4.8 c	5.5 d	9.2 d
	Wheat bran	17.5 b	12.8 a	14.9 c	6.2 a	4.7 cbd	4.6 cb	4.7 c	9.8 b	15.0 c
	Rice Bran	17.8 a	5.8 d	25.1 a	4.3 b	4.9 cb	5.1 b	8.2 a	10.3 b	40.5 a
	Molasses	13.7 d	12.1 b	12.1 d	3.8 cb	5.2 b	5.2 b	4.7 c	7.8 c	40.2 a
	Cow manure	15.7 c	11.3 c	17.5 d	4.6 b	7.2 a	7.2 a	8.2 a	12.2 a	28.9 b
	P value	<0.0001	<0.0001	<0.0001	0.0003	0.0006	<0.0001	0.0165	<0.0001	<0.0001

[x] Means from two combined experiments conducted under similar screenhouse conditions.

[y] Means with the same letters within a row are not significantly different from each other at $P \leq 0.05$.

Table 4. 4: Percentage latent infection by *Ralstonia pseudosolanacearum* in bacterial wilt-susceptible tomato variety Assila seedlings after eight weeks of growth in soil from nine infested tomato fields in Tanzania treated in bioassays with anaerobic soil disinfestation (ASD) using different carbon sources

Carbon source	**Mean percentage seedlings with latent infection** [x, y, z]								
	IMG1	**IMG2**	**IMG3**	**MS1**	**MS2**	**MS3**	**ML1**	**ML2**	**ML3**
Aerobic control	25.0 a	16.5 b	33.0 a	25.0 ab	66.5 a	33.8 a	100.0 a	16.5 ab	50.0 a
Anaerobic control	0.0 b	50.0 a	12.5 b	0.0 c	45.9 b	0.0 c	100.0 a	33.5 a	75.0 a
Wheat bran	0.0 b	0.0 b	0.0 c	29.0 a	0.0 c	12.5 b	58.5 b	12.5 b	75.0 a
Rice Bran	16.5 a	12.5 b	0.0 c	0.0 c	12.5 c	9.4 b	100.0 a	0.0 b	58.5 ab
Molasses	0.0 b	0.0 b	0.0 c	12.5 bc	16.5 c	0.0 c	25.0 c	0.0 b	12.5 c
Cow manure	12.5 ab	0.0 b	12.5 b	0.0 c	0.0 c	29.0 a	58.5 b	0.0 b	50.0 b
P value	0.0029	0.0012	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0016	<0.0001

[x] Means from two combined experiments conducted under similar screenhouse conditions.

[y] Means with the same letters within a row are not significantly different from each other at $P \leq 0.05$.

[z] IMG=Image MS=Misufini ML=Mlali.

Table 4. 5: Output from multivariate analysis of variance (MANOVA) summarizing the relationship between dependent variables bacterial wilt incidence, area under the disease progress curve (AUDPC), and latent infection by *Ralstonia pseudosolanaceaum* in tomato plants to anaerobic soil disinfestation to independent variables soil pH and paint removal (PR) (reducing conditions) in bioassays Multivariate analysis of variance (MANOVA)

		Incidence		**AUDPC**		**Latent infection**	
Location	**r^2 & *P* [x]**	**pH**	**PR**	**pH**	**PR**	**pH**	**PR**
Misufini 1	r^2	0.2909	**-0.4288**	**0.4325**	-0.0424	0.0169	**0.3713**
	P	0.7023	**0.0064**	**0.006**	0.7985	0.9183	**0.02**
Misufini 2	r^2	0.0381	**-0.4452**	-0.0666	-0.2499	-0.2796	**-0.5772**
	P	0.8199	**0.0045**	0.6873	0.1249	0.0848	**0.0001**
Misufini 3	r^2	-0.1206	-0.0026	-0.0319	0.0898	**0.3145**	**0.4901**
	P	0.4465	0.9866	0.9521	0.8656	**0.0481**	**0.0013**
Image 1	r^2	-0.2077	-0.1075	-0.2714	-0.1066	**0.4283**	0.0336
	P	0.2045	0.5147	0.0946	0.5181	**0.0065**	0.8388
Image 2	r^2	0.0188	-0.0212	0.0029	-0.0293	0.1407	0.0044
	P	0.9096	0.8937	0.9861	0.8590	0.3751	0.9776
Image 3	r^2	**0.3495**	0.0067	0.0028	-0.2819	-0.1707	0.0394
	P	**0.0292**	0.9674	0.986	0.8599	0.2987	0.8116
Mlali 1	r^2	-0.0734	0.0345	0.1273	0.0018	-0.2061	-0.0545
	P	0.6569	0.8347	0.438	0.9914	0.2081	0.7414
Mlali 2	r^2	0.1014	0.0718	-0.0573	0.0808	0.0839	0.0076
	P	0.3391	0.6641	0.7292	0.6245	0.6137	0.9635
Mlali 3	r^2	0.1337	-0.0086	0.2368	0.0003	0.2937	0.01
	P	0.4168	0.9585	0.154	0.9984	0.0696	0.9509

[x] r^2 = Partial correlation coefficient and *P*= 5% alpha value that signifies the relationship between bacterial wilt incidence, AUDPC /latent infection and independent variables pH and IRIs tube paint removal during greenhouse ASD bioassay experiment.

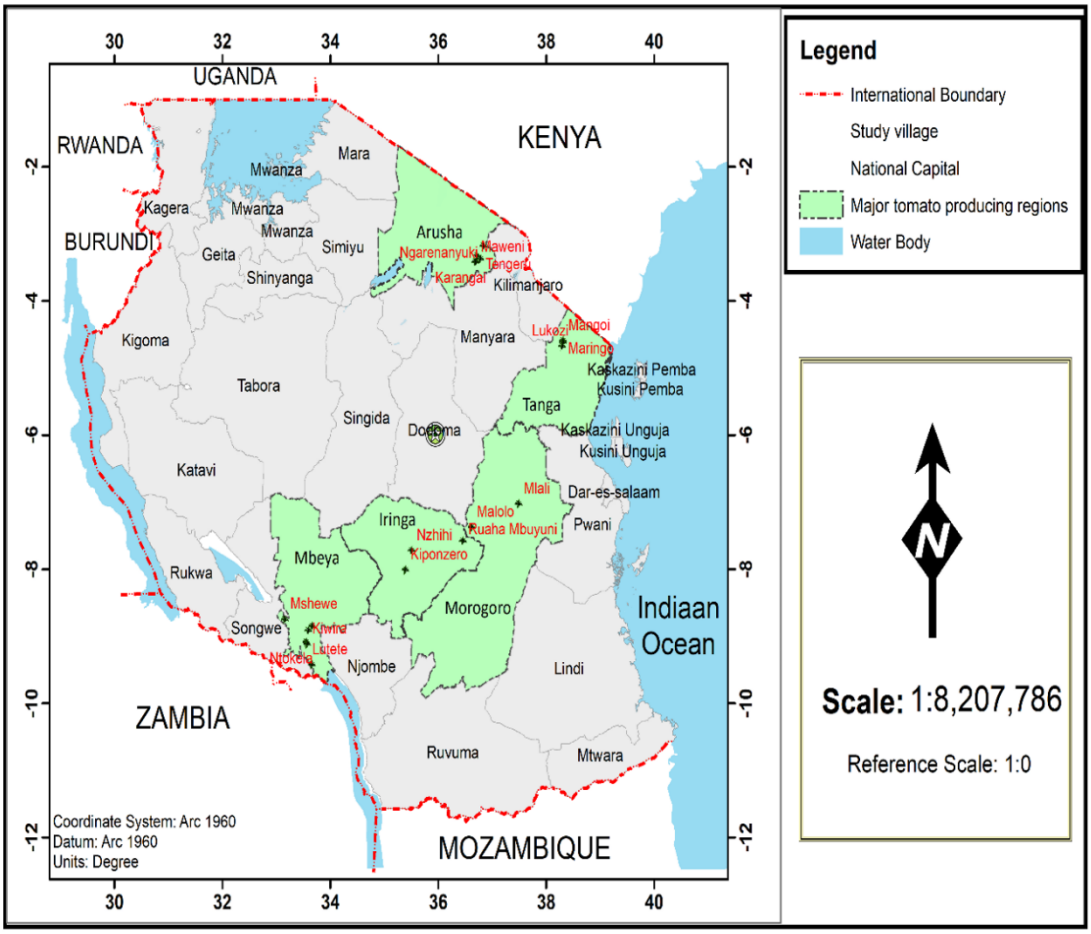

Fig. 4. 1: Map of Tanzania indicating major tomato producing regions highlighted with light green coloration. Dots indicate sites of on-farm anaerobic soil disinfestation experiments and *Ralstonia pseudosolanacearum*-infested soil collection for bioassays.

Fig. 4. 2: A) ASD treated plots after removal of the black plastic sheet showing four blocks each with six treatments in the on-farm trial, B) Eight-week-old tomato plants growing on ASD-treated or control plots in one of the bacterial wilt disease hotspots.

Fig. 4. 3: Bioassay of bacterial wilt in tomato “Assila’ seedlings planted in anaerobic soil disinfestation (ASD)-treated soils collected from nine *Ralstonia pseudosolanacearum*-infested fields in Image, Iringa, Tanzania. The ASD bioassay experiment was conducted at Sokoine University of Agriculture, Tanzania.

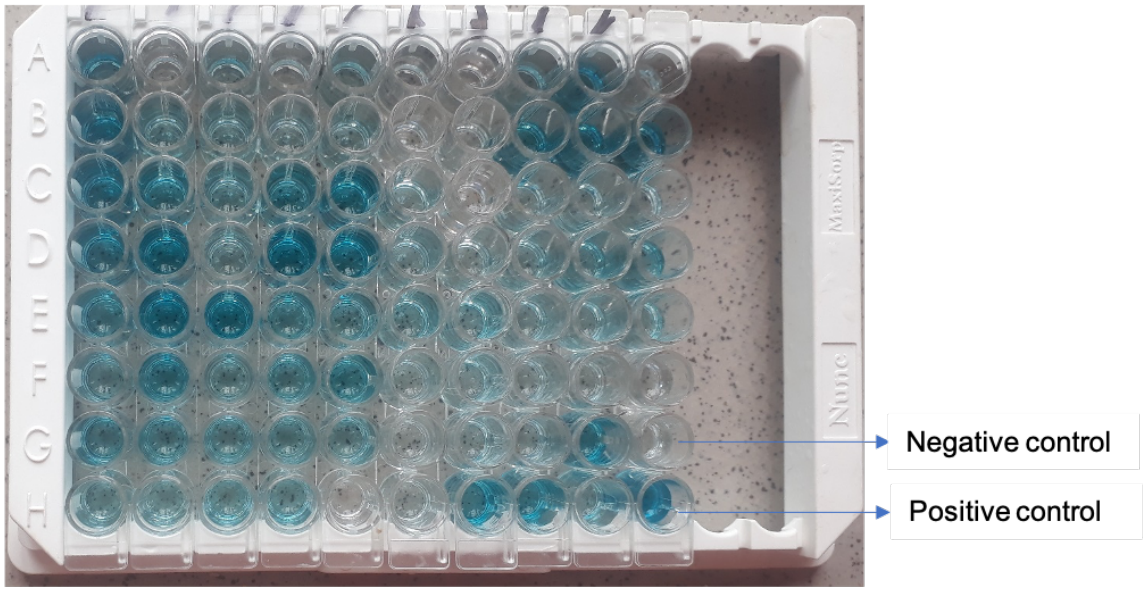

Fig. 4. 4: Agdia Inc. *Ralstonia* double antibody sandwich – enzyme-linked immunosorbent assay (DAS-ELISA) used to determine the presence of latent *R. pseudosolanacearum* infection in asymptomatic tomato seedlings grown in anaerobic soil disinfestation-treated in bioassay experiments. The blue color indicates a positive test result *for R. pseudosolanacearum*, and no color change indicates negative results.

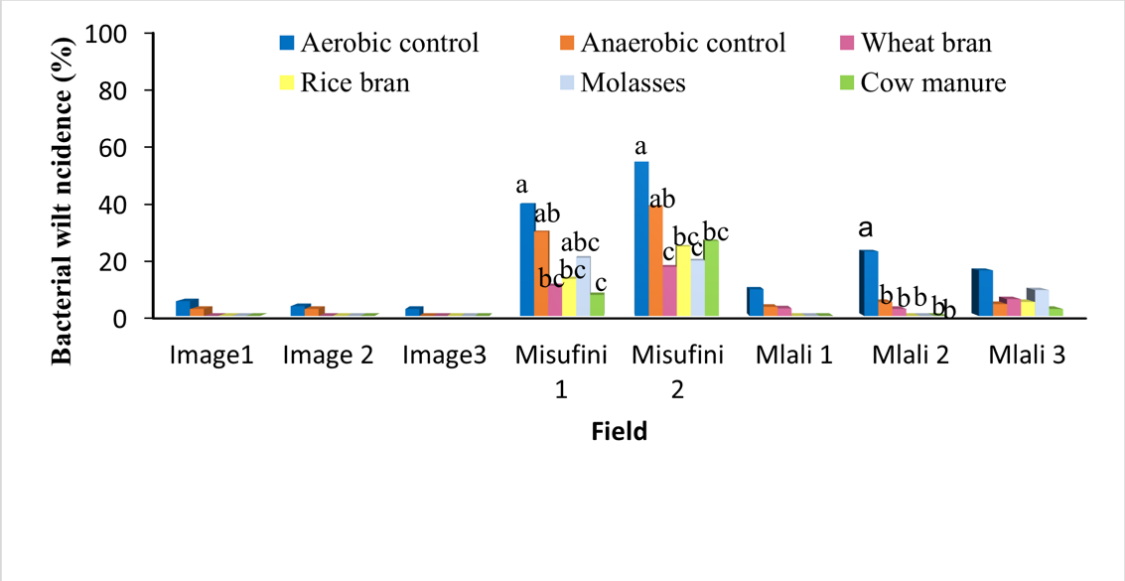

Fig. 4. 5: Bacterial wilt incidence in tomato seedlings twelve weeks after planting in anaerobic soil disinfestation (ASD)-treated soils in fields located at Image (Iringa) and Mlali and Misufini (Morogoro) villages, Tanzania. Values are the mean disease incidence for four blocks of each of six ASD treatments, including aerobic and anaerobic controls and ASD-treated plots amended with wheat bran, rice bran, molasses, or cow manure carbon sources. Values for bars with the same letters in a field are not significantly different from each other at $P \leq 0.05$.

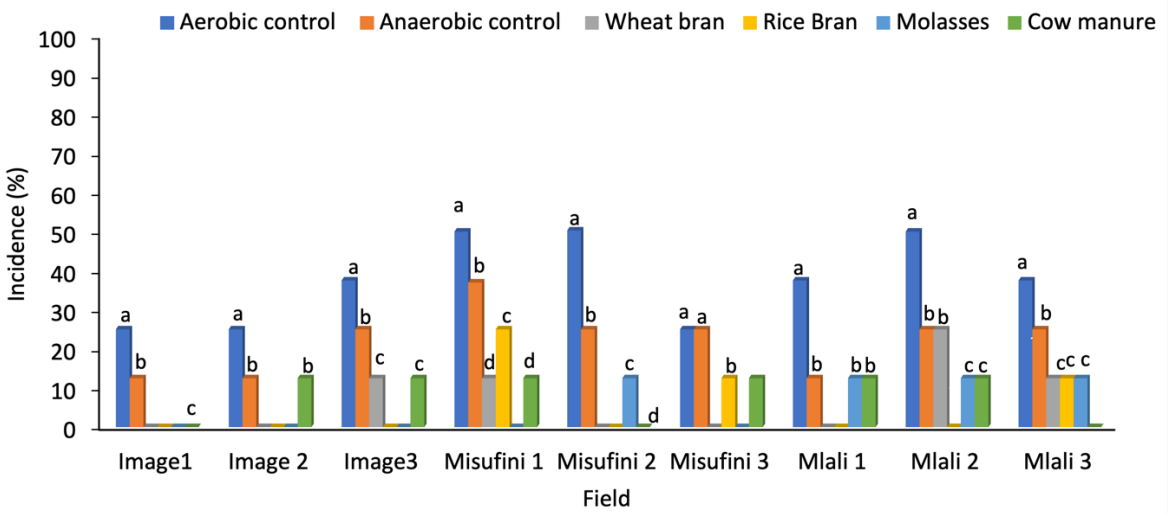

Fig. 4. 6: Bioassay of the effects of anaerobic soil disinfestation (ASD) with different carbon sources on bacterial wilt incidence in tomato variety Assila seedlings planted in soils collected from nine *Ralstonia pseudosolanacearum*-infested fields in Image (Iringa) and Mlali and Misufini (Morogoro), Tanzania. Values for bars with the same letters within a field are not significantly different from each other at a 5% alpha value.

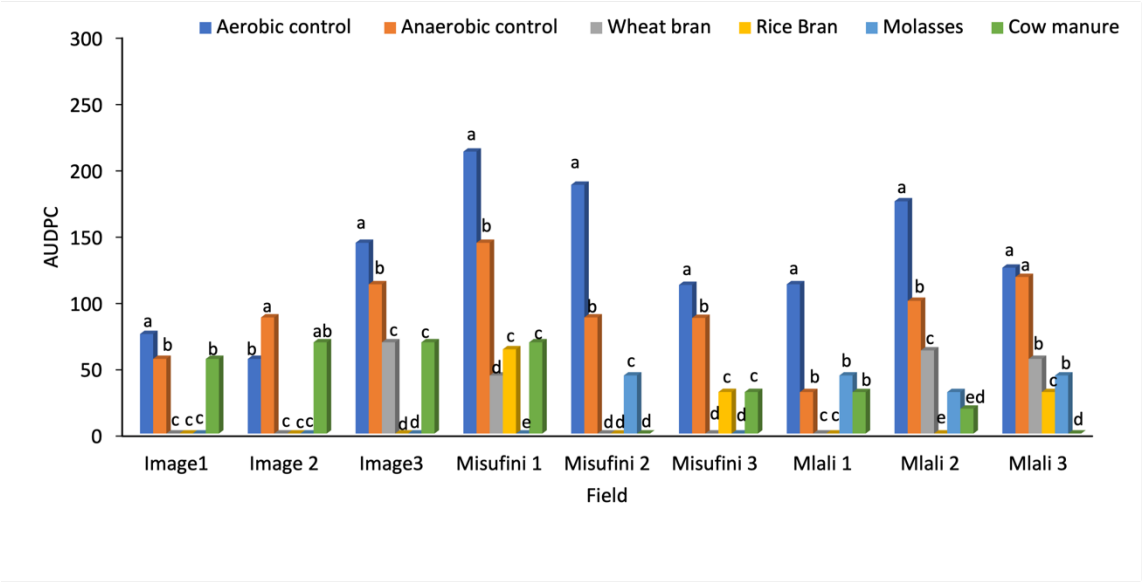

Fig. 4. 7: Bioassay of the effects of anaerobic soil disinfestation (ASD) with different carbon sources on bacterial wilt disease progress (area under the disease progress curve; AUDPC) in tomato variety Asila seedlings planted in soils collected from nine *Ralstonia pseudosolanacearum*-infested fields in Image (Iringa) and Mlali and Misufini (Morogoro), Tanzania. Values for bars with the same letters within a field are not significantly different from each other at a 5% alpha value.

Chapter 5: Bacterial Wilt Disease Prevalence in Key Tomato Producing Regions of Tanzania and Farmer Perceptions of the Disease and its Management.

Abstract

In Tanzania tomato is the most cultivated horticultural crop for local consumption and export to neighboring countries. Bacterial wilt caused by *Ralstonia* spp. has emerged during the past twenty years as one of the most important diseases limiting tomato production. The development of effective and sustainable management practices requires a better understanding of bacterial wilt as well as farmers' knowledge of the disease and their specific management practices. Five key tomato producing regions of the Tanzania mainland, Morogoro, Iringa, Mbeya, Tanga, and Arusha were surveyed to evaluate the prevalence of bacterial wilt disease as well as farmers' awareness of the disease and practices to manage it. On-farm surveys were conducted in July 2017 and November 2019 in which a total of 229 farms producing Solanaceous crops were surveyed, and 103 tomato farmers were interviewed using a questionnaire. During the farmers' survey, 26 tomato varieties emerged as preferred varieties based on fruit shape, size, and yield. Farmers' awareness of bacterial wilt disease and symptoms was high. Some of the farmers surveyed practiced bacterial wilt management through the use of fungicides and/or insecticides (32.9%) or roguing diseased plants (26.2%).

Bacterial wilt disease was present in 61.2% of all Solanaceous crop farms in 2017 and 67.3% in 2019. The prevalence varied significantly between regions (P =0.0026) with Mbeya region having the highest prevalence and Iringa the lowest among five key tomato producing regions. Bacterial wilt prevalence in tomato farms was 38.5% in 2017 and 41% in 2019 with no significant differences between regions (P= 0.9270) and years (P=0.5894). Bacterial wilt incidence and severity were assessed in all tomato fields in which the disease was present. Bacterial wilt incidence did not vary significantly between regions in 2017 (P =0.8657) and 2019 (P =0.1040). Significant variation (P<0.05) was observed within Mbeya region in 2017 and Iringa and Morogoro in 2019. Bacterial wilt severity varied significantly between regions in 2017 (P =0.0106) and non-significantly in 2019 (P =0.1177). Arusha region had the average highest severity (70%) and Iringa the lowest (13.1%). Bacterial wilt incidence was significantly negatively regressed with tomato variety preference and positively with seed source and farmers' awareness of the disease among all farmers agronomical practices and descriptive characteristics tested in this survey. This study demonstrates the potential of using the survey information in testing and improving targeted management practices for bacterial wilt in the key tomato producing regions of Tanzania

Introduction

Tomato is widely cultivated in Tanzania, with an estimated annual production of 314,986 tonnes, contributing 64% of the total vegetable and fruit crop production in the country (De Putter et al., 2011; Luzi-Kihupi et al., 2015, Mutayoba and Ngaruko, 2017). Cultivated tomato is partly grown for local consumption to meet nutritional needs as well as income generation through sales in Tanzania and neighboring countries including Kenya, Rwanda, and Zambia (Minja et al., 2011; Maerere et al. 2006; Mutayoba and Nguruko, 2017). Recently the number of farmers involved in tomato production has increased exponentially due to increased demand and favorable conditions for tomato production (De Putter et al., 2011). In Tanzania tomato is mainly cultivated in the southern highlands (Mbeya, Njombe, and Iringa), northern highlands (Kilimanjaro, Arusha, and Manyara), and coastal and central regions (Morogoro and Tanga (Lushoto)). Smallholder farmers dominate Tanzanian tomato farming systems with an average of 1 Ha/household devoted to tomato production (Maerere et al., 2010). While mean global tomato production amounts to 27.5 t/Ha (FAO, 2005), in Tanzania an average production of 2.2 -16.5 t/Ha has been documented (Maerere et al., 2006). According to Minja et al. (2011), important factors that contribute to reduced tomato production include deteriorating soil fertility, the use of pest- and/or disease-vulnerable and low-yielding varieties, unreliable rainfalls, diseases, pests, and poor farming practices. Pests and diseases account for 56% and 88% of tomato yield losses (UMADEP, 2003; CABI, 2004); up to 100% yield losses have been recorded in areas with high disease and pest pressure (UMADEP, 2003).

Pests and diseases are the leading constraints in the Tanzanian tomato production chain (Opena et al., 1990; Minja et al., 2011; De Putter et al., 2011; Testen et al., 2016). Bacterial spot, bacterial wilt, bacterial canker, and bacterial speck were reported to limit tomato production in the key production regions (Black et al., 1999; Shenge and Mabagala, 2007; Shenge et al., 2010; Mbega et al., 2012; Testen et al., 2018). Among these diseases, bacterial wilt is the most damaging (Black et al., 1999; Muthoni et al., 2012; Asiimwe et al., 2013; Mwankemwa, 2015; Baitani, 2017; Uwamahoro et al., 2018). The disease is caused by members of the *Ralstonia solanacearum* species complex (RSSC), the second most widely recognized bacterial disease-causing crop yield loss globally, especially in Solanaceous crops (Champoseu and Momol, 2009; Mansfield et al., 2012; Meng, 2013). *Ralstonia* spp. are pathogenic soil proteobacteria widely distributed in subtropical, tropical, and temperate areas (Hayward, 1991; Yabuuchi et al., 1995; Elphistone, 2005). Previously only tropical lowland areas with a warm climate were reported to support the perpetuation of members of the RSSC (Hayward, 1991). However, Elphinstone (2005) revealed the presence of RSSC race 3 biovar 2 (R3B2) strains that survive in cooler and high-altitude areas and cause southern wilt, bacterial wilt, and brown rot in geranium, tomato, and potato respectively (Champoseau and Mommol, 2009). The nomenclature of the RSSC has recently been revised (Safni et al., 2014); R3B2 strains are in phylotype 2 and have retained the name *R. solanacearum*, while phylotype I and III strains are now designated *R. pseudosolanacearum*. In Tanzania, phylotype I, II, and III strains have so far been found in association with Solanaceous crops to date (Chapter 2; Mwankemwa et al., 2015; Aloyce, 2020).

Bacterial wilt disease has been confirmed to be present in 26 African countries (Elphinstone, 2005; Muthoni et al., 2012). In Tanzania, bacterial wilt was first reported on tomato and eggplant in Zanzibar (Unguja), and in the northern and southern plateaus of the Tanzania Mainland in 1998 (Black et al., 1999). Since that time, bacterial wilt has occurred in other important vegetable producing regions of Tanzania including Mbeya, Arusha, Morogoro, Iringa, and the central, lake and coastal regions, of the Tanzania Mainland and Zanzibar, specifically in Solanaceous crops (tomato, potato, eggplant, and pepper) with increasing disease severity and at an alarming pace (Mwankemwa, 2015; Baitani, 2017; Aloyce, 2020).

As for many soilborne diseases, bacterial wilt management is complex and difficult to accomplish. In East Africa, cultural, chemical, host resistance, and biological control methods have been explored to reduce or eliminate bacterial wilt in Solanaceous crops (Adhikari and Basnyat, 1998; Katafire et al., 2005; Kwambai et al., 2005; Muthoni et al., 2012). However, bacterial wilt persists because farming systems do not ensure the availability of healthy planting materials, and adequate extension services, disease awareness among farmers, and management options are lacking (Gikunda et al., 2015). In Tanzania, the areas used for crop production are chosen based on land availability, favorable environmental conditions, and adequate water supply. Thus, tomato farmers in Tanzania are mainly concentrated in key tomato production regions and rotate tomato with crops such as maize, rice and beans (De Putter et al., 2011; Luzi-Kihupi, 2015). Farmers in these areas practice short crop rotations with few options of crops to rotate. The practice contributes to the long-term saprophytic pathogen survival and perpetuation of the disease

in these areas (Mwankemwa, 2015). Elphinstone (2005) emphasized consideration of factors such as variety selection, soil, cropping systems, farmers' practices, and pathogen characterization in finding sustainable management options. Thus, the use of integrated pest management (IPM) strategies has been suggested for the reduction of bacterial load in *R. solanacearum*-infested soils (Priou et al., 2004; Saddler, 2005; Champoseu and Momol, 2009).

Integrated pest management strategies are popularly known to reduce or eliminate diseases and therefore sometimes are practiced by farmers. However, limited and inadequate information on diseases and their management, especially in African countries due to deficiency of extension services, is the main reason behind the non-adoption of IPM (Uwamahoro et al., 2018; Testen et al., 2018). Accurate disease diagnosis is key to successful and sustainable disease management programs. Farmers must learn how to properly diagnose diseases, decide on management options, and assess the risks associated with the adoption of the strategy (Savary et al., 2011). In addressing this issue scientists are encouraged to involve farmers in various programs that are aimed at mitigating disease effects (Meijer et al., 2015). Current agriculture extension programs emphasize the involvement of farmers in all extension programs (Kiptot et al., 2007). Involvement of farmers or incorporation of farmers' skills has led to the successful adoption of technology. Thus, introducing and improving farmers' practices is of paramount importance as farmers play a key role in disseminating and integrating the findings into their farming systems (Makoya et al., 2008; Testen et al., 2016). Farmer participation in scientific research includes demonstration plots as well as participatory research such as mother and baby

trials (Testen et al., 2016). Studies show that farmers adapt to new or improved technologies by learning and weighing benefits, for instance, costs, risks, and the contribution of technology to enhanced crop quality and yield compared to local practices (Meijer et al., 2015; Oo et al., 2012). Therefore, knowledge of farming practices is important in the development of science-based packages for improving disease management practices.

This study aimed to assess the prevalence of bacterial wilt on smallholder farms in the major tomato producing regions of Tanzania and understand farmers' knowledge of bacterial wilt and practices to manage the disease. We specifically (1) assessed bacterial wilt disease prevalence and extent (incidence and severity) in the key tomato producing regions, (2) determined the depth of farmers' bacterial wilt knowledge and their management practices, and (3) investigated the contribution of farmers' knowledge and practices to bacterial wilt incidence or severity in Tanzania.

Materials and Methods

Study site. The bacterial wilt disease and tomato farmer survey were conducted in Morogoro, Iringa, Tanga, Arusha, and Mbeya, key tomato producing regions of the Tanzania Mainland (Fig. 1). The regions are characterized by bimodal rainfall, a short rainy season (October to December), and a long rainy season (March- June). Additionally, all five regions are characterized by loamy soil types (sandy and clay) with water availability all year round, therefore suitable for horticultural crop production. We selected a total of twenty villages that have a record of high tomato production. The selection included at least three villages from each region: Misufini, Malolo, and Mlali (Morogoro), Ruaha,

Mbuyuni, Nzihi, Image, and Kiponzero (Iringa), Inyala, Mshewe, Ijombe, Kiwira, and Lutete (Mbeya), Mangoi, Maringo, and Lukozi (Tanga) and Maweni, Oldonyo Sapuk, Karangai, Tengeru, and Ngarenanyuki (Arusha).

Morogoro region has an altitude of 300 - 2340 m a.s.l. with a minimum temperature of 14°C during cooler months (June-August) and a maximum of 36°C during hot months (January-February). Average temperatures range from 16°C to 32°C with cooler nights to warm day temperatures; annual rainfall ranges between 890 - 2400 mm. The altitude of the Iringa region ranges between 400 and 1800 m a.s.l. It is characterized by cool temperatures ranging between 12°C and 27°C and annual rainfall of 600-1200 mm. The Mbeya region is medium to high in altitude (500 - 2980 m a.s.l.) and receives an annual rainfall of 650 - 2600 mm, with cooler temperatures than other regions. The average temperatures range from 16°C to 25°C, in cooler months dropping below 10°C. Tanga region is characterized by a coastal climate to hilly cool areas with altitude ranging from 300 - 2100 m a.s.l. Temperatures range from 16°C to 28°C. In hilly areas, temperatures can drop below 10°C during cooler months. The average precipitation is 800 mm annually. Arusha is a high-altitude region, from 900 - 4655 m a.s.l. The average altitude is 1400 m a.s.l. Temperatures range between 14°C and 22°C with an average annual rainfall of 1100 mm.

Bacterial wilt survey and farmer survey instrument. On-farm surveys for bacterial wilt disease were conducted by three researchers with plant pathology and extension expertise. The surveys took place between May and July 2017 and November to December 2019. The villages that were surveyed in 2017 were also targeted for 2019 but not necessarily the same fields or farmers because most farmers rent farms seasonally. The

bacterial wilt disease survey included at least five fields in each village making a total of 128 Solanaceous crop fields in 2017 and 100 in 2019 as summarized in Table 5.1. The exceptions are Malolo, Mshewe, and Karangai villages surveyed in 2017 but not 2019. Participating farmers were selected in village meetings organized by extension officers and village leaders. The selection was based on farms with a history of bacterial wilt disease symptoms and representation of male and female farmers. Up to twelve farmers from each village were selected randomly for bacterial wilt surveys on their farms and up to six among them were who were willing to participate in the interview. A total of 52 farmers in 2017 and 51 in 2019 were interviewed in or near their fields. A semi-structured questionnaire with guided and open questions was administered in Kiswahili by a researcher fluent in both English and Kiswahili and the responses were recorded in English (File 5.1). The same questionnaire was used in the 2017 and 2019 surveys. The questionnaire was divided into three sections (Table 5.2). For questions in section 3, farmers were asked to mention the names and symptoms of diseases or pests, and they were provided with a compendium with photos of symptoms and damage (Anonymous, 2017) to confirm the identity of the pest or disease. Research permit and clearance (RPGS/R/ETHICS) was issued by the Sokoine University of Agriculture research board to ensure adherence to high ethical and scientific standards.

Bacterial wilt disease assessment. Bacterial wilt incidence and severity were assessed in fields owned or leased by farmers who participated in interviews. Bacterial wilt was diagnosed based on symptoms including wilting and stem browning, as well as bacterial streaming from cut basal tomato stems (Champoseu and Momol, 2009). A

serological assay specific for the pathogen (Immunostrip® for *Ralstonia*; Agdia, Inc., Elkhart, IN, USA) was used to confirm bacterial wilt disease before assessment procedures. A lower stem section of plants with bacterial wilt symptoms was sampled and macerated in a buffer that was provided in a sample bag. The immunostrip was immersed in the extract as per manufacturer instructions and samples that produced double lines were recognized as positive for bacterial wilt disease. After confirmation of bacterial wilt in each field, five 2 m^2 plots at least 5 m apart were selected using a zigzag pattern for the assessment of bacterial wilt disease incidence and severity. Disease incidence was assessed by counting the total number of plants (N) and the number of plants with bacterial wilt symptoms (n) in the five plots. Incidence was calculated using the formula (n/N) X 100. Disease severity ratings were obtained by scoring 15 random plants in each of the 2 m^2plots using a 1-5 scale (Horita and Tsuchiya, 2001) i.e., asymptomatic (1), two leaves withered (2), three leaves (3), at least four leaves wilted (4), and dead plant (5) (Horita and Tsuchiya, 2001). Each severity score number was converted to a leaf damage scale (0-100 %), where 0 represented asymptomatic leaves and 1, 2, 3, 4, and 5 represented 20, 40, 60, 80, and 100% severity, respectively. Mean disease severity was expressed as the mean of all leaf damage percentages in the five plots.

Statistical analysis

Data from farmer's survey: All categorical survey data were summarized and coded using specific assigned numerical values in the Statistical Package for the Social Sciences (SPSS) (IBM SPSS statistics 2016, IBM corporation). Chi-square test (χ^2) was used to compare farmers' practices and/or management practices between key tomato

producing regions and survey years. In this case, χ^2 was used to estimate the probability of the compared factors varying from each other. The contribution of farmers' stated practices/management to bacterial wilt disease incidence was assessed using binary multiple regression analysis, in which bacterial wilt disease incidence was treated as a dependent response variable and farmers' agronomic practices and information (variety preference, nursery preparations, rotational practices, seed source, irrigation water sources and management tactics) as independent variables (McCullagh and Nelder, 1989). Binary regression analysis estimated the probability of independent variables to influence the response variable at a given alpha (*P*-value).

Data from disease assessment. Data collected from disease assessments were checked for normality, no transformation was made for the data. Checked data were subjected to one-way analysis of variance (ANOVA) to compare factors among and within the five key producing regions for disease prevalence, incidence, and severity. Means were separated using the Least Significant Difference (LSD) test with Proc GLM (SAS software 9.4, SAS Institute).

Results

Farmers' survey

Demographics. Farmers' demographic responses to the survey are summarized in Table 5.3. The gender of respondents did not differ significantly between survey years (P =0.072) or) among regions (P=0.721). In the 2017 survey 82.9% of respondents were male and 17.1% female while in 2019 63.2% were male and 36.8% female. Among the 103 respondents across both survey years, 73.1% were male and 26.9% were female. Farmers'

experience in tomato production did not differ significantly between years (P = 0.173) and regions (P = 0.176).

The amount of land allocated for tomato production did not differ significantly between years and regions *(P* = 0.519*)*. Mean land allocation by farmers was 0.9 Ha and 1.0 Ha for 2017 and 2019, respectively (Table 3.5, Table D.2). Land allocation for tomato across all regions ranged from 0.4 to 1.8 Ha in 2017 and 0.4-1.4 Ha in 2019. An average of 0.45 Ha was allocated by farmers in Morogoro, while for Iringa and Mbeya farmers the mean land allocation was 1.2 and 1.1 Ha, respectively. Tanga farmers devoted an average of 0.7 ha while Arusha farmers devoted 1.2 Ha of land for tomato production.

Overall, across the regions, surveyed farmers were experienced in tomato production in 2017 (mean = 60.5% with less than five years' experience than in 2019 (mean = 35.7%). In Morogoro region, 46% of farmers indicated having less than five years of experience producing tomatoes in 2017, while in 2019 10% of farmers surveyed had less than five years of experience and 50% indicated 6-10 years of experience in tomato production. The remaining categories were indicated by <20% of farmers (Table 5.3). The majority (58.8% and 75%) of tomato farmers in Iringa indicated experience of few than 5 years in 2017 and 2019, respectively, with the remaining experience categories indicated by <20% of respondents in both years. Tomato farming experience of more than 5 years was indicated by 64.3% of Mbeya farmers in 2017 and 33.3% of farmers in 2019. The majority (55.6%) of Mbeya farmers indicated experience of 6-10 years in 2019. Half of tomato farmers in Tanga indicated 6-10 years of experience in both 2017 and 2019. In the Arusha region, all of the farmers surveyed had less than 5 years of experience in 2017 while

in 2019, 30% were in this category and 70% indicated between six and 15 years of experience producing tomatoes.

Farmer-reported tomato yield varied significantly between years (P = 0.0001) but did not differ (P = 0.111) between regions (Table 5.3). Mean tomato yield across the regions was 11.8 tonnes/Ha in 2017 and 5.9 tonnes/Ha in 2019. The average tomato yield for Morogoro was 8.2 tonnes/Ha, Iringa 10.8 tonnes/Ha, Mbeya 10.6 tonnes/Ha, Tanga 4.3 tonnes/Ha, and Arusha 8.4 tonnes/Ha (Table D.3). The range was 3.9 to 15.5 tonnes/Ha in 2017 and 4.5 to 7.1 tonnes/Ha in 2019. Farmers' perspectives on trends in tomato production varied significantly between regions (P =0.030) but marginally significant (P =0.6) differences between regions (Table D.4). While 33% of respondents indicated increased production in 2017, 26% indicated decreased production, and 17% no change in production. In 2019, 35% of surveyed farmers indicated increased production while 63% indicated a decrease and 2% reported no change. Reasons for increased or decreased production trends differed significantly between years (P =0.045) and regions (P =0.013) (Table D.5). Farmers who reported increased production indicated good weather (13.1%), the advice provided by an extension (8%), high quality seeds (8%), good management (13.1%) as major factors for increased tomato yields. Other factors that were reported by fewer than 5% of respondents to contribute to increased production included access to improved farming technology, variety changes and access to farm implements (Table D.5). On the other hand, respondents indicated that unpredictable weather (22.9%), pests and diseases (16.1%) and poor seeds (12.5%) as major factors that contributed to decreased tomato production. Other factors contributing to reduced tomato production mentioned by

fewer than 5% of respondents included lack of access to Extension, low market prices, expensive and unaffordable farm implements and drought while 7% of respondents reported to not know the reason for increased, decreased or no change of production. (Table D.5)

Tomato variety preference. Tomato variety preference by the farmers surveyed varied significantly between years (P =0.010) and (P = 0.0001) regions (Table D.6). Farmers across all five key regions named a total of 26 tomato varieties produced on their farms, namely 'Assila', 'Mwanga', 'Onyx', ''RioGrande'', 'Cal J', 'Roma vfn', 'Tengeru 97', ''Tanya'', 'Dumudumu', 'Eden', 'Anna', 'Safari', 'Mkulima', 'H070', 'HO82', ''MT56'', 'Tamia', 'Simlo', 'Moyo', 'Kilele', 'Holland', 'Mshumaa', 'Devine', 'Monica', 'Komboa' and 'Pana'. Morogoro respondents cited six varieties of which 'Asilla' was most preferred (38.5%) in 2017 and 'Cal J' and 'Onyx' (both 30%) in 2019. Iringa farmers indicated seven varieties in 2017 but only two varieties in 2019. Mbeya farmers reported growing nine varieties in 2017 but only three in 2019. ''Tanya'' (28.6%) was cited most in 2017 and ''RioGrande'' (50%) in 2019. Tanga farmers preferred seven varieties of which 'Tengeru 97' (33.3%) and 'Anna' (33.3%) were grown by the most respondents in 2017 and Tengeru 97' (30%) and ''Tanya'' (30%) were preferred in 2019. Only two farmers were interviewed in Arusha in 2017 and they both cited 'Tengeru 97' as preferred. In 2019 ten farmers were interviewed and 80% reported that they grew 'Tengeru 97', while 20% of the respondents grew ''Tanya''.

Stated reasons for tomato variety preference varied significantly (P = 0.003) between regions but not (P =0.162) between years (Table D.7). Overall, varieties were preferred

mainly based on yield, shape, color, and marketability. In 2019, 20.8% of Morogoro farmers cited shelf life as an important factor in variety selection, but less than 7% of farmers in the other regions considered shelf life when choosing varieties. Disease resistance was rarely a factor in variety selection in either year throughout the five regions.

Crop intercropped or rotated with tomato. Choice of crops to be intercropped or rotated with tomato varied significantly between regions (P =0.001) and not between years (P =0.202). (Table D.8). On average maize was mentioned by 42.3 of respondents across all regions and both years, followed by beans (17.5%). Averaged over both years, maize was intercropped or rotated the most in Morogoro (75.6%), Tanga (46.3%), and Arusha (70%) while in Iringa maize (33.3%) and beans (20.8%) were the main rotational crops in 2017 and maize (41.7%) and sunflowers (41.7%) predominated in 2019. In Mbeya beans (28.6%) and sweet pepper (23.8%) were commonly rotated with tomato in 2017, while in 2019 beans (33.3%) and potatoes (22.2%) were most common. Other crops including soybean, banana, butternut squash, pigeon pea, watermelon, cabbage, onion, Chinese cabbage, wheat, coffee, potato, carrot, and eggplant were mentioned as rotational/intercropping crops by less than 10% of respondents.

Factors limiting tomato production. Respondents indicated 11 factors that limit tomato production in the key tomato producing regions of Tanzania. Differences in these factors were significant *(*P =0.0001) between years but not between regions (P =0.08) (Table D.9). In 2017, respondents cited insect pests (34.4%), limited information (16.6%), diseases (17.8%), and markets (7.2%) as the most limiting factors for tomato production across all five tomato producing regions. In 2019, the vast majority of respondents cited

insect pests (84.4%) as limiting factors. Other factors including weeds, unpredictable weather, poor soil, post-harvest losses, low-quality pesticides, low-quality seeds, and poor technology were indicated by fewer than 5% of respondents across all regions.

Responses of farmers identifying important insect pests of tomato did not vary significantly between years (P =0.501) or regions (P = 0.368) (Table D.10). While on average 36% of farmers could not name the most damaging insect pests of tomato, of the remaining 64% of respondents who named insect pests, 34.9 indicated *Tuta absoluta*, 21.4% named aphids (*Myzus* spp. and other aphids), and 21.8% cited whiteflies (*Bemisia tabaci*) as the most damaging pests. Fewer than 10% of farmers cited bollworms, thrips (*Frankiliniella* spp.), spider mites, leaf miners, and leafhoppers across all regions.

Responses citing the most damaging tomato diseases varied significantly between regions (P = 0.0015) and years ($P < 0.0001$) (Table D.11). On average 29.2% of respondents could not name the most important diseases that damage tomatoes. Among the remaining 74% of farmers who could name the diseases, 29.2% indicated early blight, 22.5% cited late blight, and13.3% cited bacterial wilt as most important. Less than 5% of respondents mentioned yellow leaf curl, *Fusarium* wilt, bacterial canker, bacterial spot, leaf rust, nematodes, and other viral diseases. Respondents in Morogoro cited early blight (25.7%), late blight (25.5%), and bacterial wilt (14.6%), those in Iringa mentioned early blight (33.9%), late blight (22.4%), and bacterial wilt (12.9%), and those in Mbeya indicated early blight (30.1%), bacterial canker (15.6%), late blight (12.2%) and bacterial wilt (19%) as the most limiting diseases. Tanga respondents indicated early blight (31.5%), late blight (29.6%), and bacterial wilt (5.8%), while Arusha farmers indicated early blight

(24.6%), late blight 22.5%, and bacterial wilt (14.6%) as the most important diseases. Relatively few farmers (2.2%) named bacterial wilt as an important disease in 2017, but in 2019 the percentage of farmers identifying bacterial wilt increased to 24.5%.

Responses citing the most damaging weeds varied significantly (P =0.0001) between years and (P=0.001) regions (Table D.12). Most farmers (74%) could not name the weeds affecting their tomato crops. From the 26% of respondents who could name the weeds, grasses (*Lolium* spp.) were cited by 20.4%, 14.3% nutsedges (*Cyperus* spp.) speargrass (*Heteropogon* spp. (12.2%) while 12.7% Mexican poppy (*Argemone* spp.)., 10.4% late weed (*Trichodesma* spp., and13.2% Dandelion (*Taraxacum*). Other weeds mentioned by fewer than 2% of respondents are wandering Jew (*Commelina* spp.), Tarvine (*Boerhavia* spp.), African spider flower (*Cleome*), *Oxalis*, nightshade (*Solanum* spp.), and carrot weed (*Daucus* spp.).

Tomato seed source. Responses naming tomato seed sources varied significantly between years (P =0.0001) and among regions (P =0.011) (Table D.13). Farmers indicated different seed sources depending on availability, yield potential, and price. The main seed sources cited were mixed, farmers' own saved seeds, agrostores, markets, seed researchers, and neighbors. Respondents in Morogoro cited mixed and agrostore as seed sources (23.1%) in 2017 and mixed sources by 40% in 2019. those in Iringa mentioned Mixed source, neighbors, agrostores, and local markets (23.5%) whereas in 2019 mixed seed source dominated by 30%, and those in Mbeya indicated mixed sources, neighbors, and agrostore as their main sources of seeds (21.4%) in 2017 and 2019 mixed source by 30%. Tanga respondents indicated mixed source by 33.3 and 30% in 2017 and 2019 respectively

as their main seed source.), while Arusha the 2 farmers indicated to get their seeds from agrostore in 2017 and mixed source by 50% in 2019.

Irrigation water sources and methods. Farmers' responses regarding sources of water used to irrigate tomato farms varied significantly (P = 0.001) between regions but not years (P =0.456) (Table D.14). On average 21.5% of all tomato farms across the regions were rainfed; 39% of all respondents indicated that tomato production started as rainfed but was supplemented by irrigation later during the season (mixed sources) and 58.8% relied on rivers. While Morogoro most farmers relied on rivers (76.9%) and used mixed sources (22%). Iringa farmers relied on rivers (58.8%). For Tanga, 50% of farmers indicated that they relied on rivers while 50% used mixed sources. Arusha farmers cited mixed sources and rivers (50%) for irrigation water. Mbeya respondents relied on rivers (50%) and mixed sources (43%) of water. For the remaining sources, fewer than 10% of respondents across the regions indicated ponds, wells, streams, and tap water as sources of irrigation water for tomato farms. Methods of irrigation differed significantly (P =0.0001) between years and regions (Table D.15). While most Morogoro farmers relied heavily on water cans (61.5% in 2017 and 100% in 2019) to irrigate their tomato crops, Iringa farmers used more varied water sources including water cans (35.3% in 2017 and 27.3% in 2019). In Mbeya respondents used watering cans (21.4%), furrow by 21.4% or no irrigation (57.1%) in 2017 while in 2019 water cans were used by 80% of respondents. For Tanga two thirds of farmers reported using furrow (33.3%) or drip (33.3%) irrigation in 2017 but in 2019 farmers indicated that they used watering cans by 40% while 60% used no irrigation. The two Arusha farmers interviewed in 2017 used furrow irrigation but in 2019

responses among ten farmers interviewed were split among those citing watering cans (80%) and pump and laid pipes 20%. (Table D.16).

Tomato seedling nursery preparation. Tomato seedling nursery preparation and management varied significantly between regions and non-significantly between years. Nursery management by soil sterilization varied significantly between regions (P = 0.037) and non-significantly (P =0.921) between years (Table D.17). While in Morogoro and Arusha 50% of farmers did/did not use sterilized nursery soil, in Iringa, Mbeya, and Tanga > 75% of farmers used sterilized soil for nursery preparation (Table D.17). The use of raised beds in the nursery did not differ significantly between (P =0.131) regions and (P =0.443) year. <60% used raised beds except for Arusha that indicated 50% of use or not use raised beds. This trend was also observed with non-significant variation (P =0.343 and P =0.511) for years and region respectively in the use of mulching in nurseries where <60% of all respondents across regions except for Arusha with 50%. (Table D.17)

Bacterial wilt awareness and disease management

Bacterial wilt awareness. Awareness of bacterial wilt among farmers did not differ significantly (P=0.394) between years (Fig 2). In 2017, 84.9% of all respondents were aware of bacterial wilt disease and symptoms, while in 2019, 90.2% were aware of the disease. There was a significant difference in the proportion of farmers aware of bacterial wilt between the regions (P =0.0001). Bacterial wilt awareness among respondents was 96% in Mbeya, 100% in Morogoro Tanga and Arusha, and 58.8% in Iringa in 2017. In 2019 awareness was 100% in Morogoro, Tanga and Arusha, 66.7% in Iringa and 87% in Mbeya. The causes of bacterial wilt varied significantly (P=0.002) between regions and

non-significantly (P=0.773) between years (Table D.18). A majority (66.7%) of respondents in Tanga indicated to knot know the cause of the disease in 2017 while in 2019 it was 90%. This trend was also observed in Mbeya by 92.9% not know the disease causes in 2017 and 80% in 2019. In Morogoro, about 40% indicated not know the cause of the disease in both years. This trend was also observed in Arusha by 50% and 90% in 2017 and 2019, respectively. Iringa farmers indicated poor farm management (69.2) in 2017 and do not know by 45.5 % in 2019. The remaining possible causes of bacterial wilt be weather (excessive) rain 5.9%) by Iringa farmers in 2017, insect pests by Morogoro farmers 15.4 and 20% in 2017 and 2019 respectively and Iringa farmers by 17.6 % in 2017, and pathogens by Arusha farmers (50%) in 2017 and 10% in 2019, Tanga by 16.7% in 2017 and 10% in 2019 and Morogoro farmers by 7.7% in 2017 and 40% in 2019.

Bacterial wilt management. The percentages of farmers surveyed who reported using the two most cited management options or no management for bacterial wilt in the five major Tanzanian tomato-producing regions are presented in (Fig 3 and 4). The responses varied significantly (P= 0.003) between regions and non-significant (P= 0.180) between years. Farmers had limited knowledge of management tactics for bacterial wilt, and the two most cited tactics were the removal of diseased plants (roguing) and applying fungicides and/or insecticides. In 2017, 32,7% of respondents across all five regions declared they used no management options, 40.4% applied fungicides and/or insecticides and 27% rogued diseased plants (Fig 3). In 2019, 49% of respondents did not use any management tactic, 25.5% applied fungicides and/or insecticides and removed infected plants. Farmers in Morogoro cited uprooting and spraying by 46% as their management

practice in 2017 while in 2019 60% cited roguing and 20% pesticide application. For Iringa, 62.9 % sprayed fungicide and only 5.9 rogue diseased plants in 2017 while in 2019 only 36.4% sprayed and no roguing was reported. Roguing and spraying of pesticides were cited by 28.6% of Mbeya farmers in 2017 while in 2019 50% of farmers sprayed and none did roguing of diseased plants. For Tanga farmers, 50.4% rogued diseased plants and 33.3% did spraying of pesticides while in 2019 roguing and spraying was practiced by 20% of farmers. No spraying or roguing was cited by Arusha farmers in 2017 while 60% indicated roguing in 2019 and 20% spraying pesticides (Figures 3 and 4)

Bacterial wilt disease assessment

Bacterial wilt disease prevalence. The prevalence of bacterial wilt did not differ significantly (P =0.053) between years, Bacterial wilt was present in 61.2% and 67.3 of Solanaceae (eggplant, pepper, and potatoes) fields surveyed in 2017 and 2019 respectively. Bacterial wilt prevalence varied significantly (P =0.0026) between regions in both survey years (Table 5.4). In 2017 bacterial wilt prevalence was highest and did not differ significantly in Mbeya (88.3%), Tanga (71.3%) Morogoro (68.4) while Morogoro and Iringa recorded the lowest prevalence of 30 and 11% respectively (Table 5.4).

For tomato farms, no significant difference in prevalence was observed between years (P =0.5894) and between regions (P = 0.9270). bacterial wilt was present in 38.5% of 52 tomato fields surveyed in 2017 and 4.21% of 52 fields surveyed in 2019 (Table 5.5) with an average prevalence of 54.8% In 2017 and 41,1% in 2019 (Table 5.5).

Bacterial wilt incidence and severity. Bacterial wilt incidence did not differ significantly between regions in 2017 (P = 0.8657) and (P = 0.1040) in 2019 (Table 5.6 &

Table D.1). Tanga recorded the highest incidence in 2017 while recorded the lowest (4.23%). In 2019 Morogoro recorded a high incidence (17.5%) and Arusha the lowest (0.2%), with some regions showing a significant difference (P<0.05) in bacterial incidence within region i.e. Mbeya in 2017 and Morogoro and Iringa in 2019 (Table 5.6).

In terms of disease severity, a significant difference was observed (P =0.0106) in 2017 and non-significant (P =0.1177) in 2019 between the regions. Arusha and Tanga had the highest bacterial severity 70 and 58.7% respectively in 2017 while Iringa scored lowest (13.1%). Within the region, significant differences were significant (P =0.05) in Arusha, Mbeya, and Tanga in 2017 and Iringa, Mbeya, and Morogoro in 2019. (Table 5.7)

Assessment of farmers' practices contribution to bacterial wilt disease incidence

Regression analysis indicated variety preference, seed source, and bacterial wilt awareness significantly contributed to bacterial wilt incidence (Table 5.8). Variety preference was significantly (P =0.006) giving a negative regression coefficient (-0.15) while seed source (P =0.014) and bacterial wilt awareness were significantly (P =0.024) regressed with incidence giving a positive regression coefficient of 1.05 and 2.2 respectively. The remaining 13 factors gave nonsignificant regression coefficient with incidence (Table 5.8)

Discussion

A semi-structured, in-person questionnaire was used to assess the awareness of bacterial wilt disease and practice of disease management tactics among 52 and 51 tomato farmers in 2017 and 2019, respectively in the five-key tomato-producing regions of the Tanzanian mainland.

Our research highlighted the dominance of male farmers in tomato farming systems across the five regions surveyed; on average about 70% of tomato farmers interviewed in the surveys were men. This observation is in line with those of other researchers in Tanzania (Testen et al., 2016, Palilo,2019) and neighboring countries (Uwamahoro et al., 2018). Many factors contributed to this unequal distribution of males and females in owning farms, especially in African farming systems. One of the reasons could be land ownership policies as well as economic reasons and manpower. Tomato farming requires intensive management and farming practices considering tomato growing conditions. These conditions do not favor females following their gender roles according to the culture and customs of many Tanzanian tribes. Women's main role is to provide care and services for the family at home. When combined with land ownership policies that favor men, most women are then compelled to take care of their families and with minimum participation in other economic activities (Rehmtullah, 1999; Tsikata, 2003; Mutangadura, 2005).

Tomato yield was significantly low (<15 tonnes/Ha) across all tomato producing regions and far behind the global standards of 27.5 t/Ha (FAO, 2005). In their study, Maerere et al (2016) observed low tomato yields of up to 16 tonnes /Ha. One of the key factors for optimal yield is the use of certified seeds of improved varieties as well as the use of good farming practices. However, our survey indicated that the use of improved seeds by tomato farmers was very limited in the key tomato producing regions of Tanzania. One of the contributing factors was seed availability and high prices of the quality improved seeds. Lack of extension services also contributed to the poor dissemination of seed education to farmers. Farmers opted for stocked seeds which mostly were neither high yielding nor

tolerant of some challenging abiotic and biotic stresses. The use of poor seeds may have contributed to the poor yields and their trend in our research. Low yield is also attributed to the experience of farmers and the size of land allocated for tomato farming. It was noted that most farms are below 1Ha size and most of the farms are not owned permanently by these farmers i.e., they only lease for a season or hardly 1 year. Small farm size allocation is guided by economic status, where farmers regarding how well they can manage the farm in terms of resources and manpower. It is anticipated that farmers should spend enough time studying soil characteristics or at least know important features of these farms to be able to improve variety/crop choice, rotation schedules as well as fertilization and spraying regimes. With short-time ownership, it is almost impossible to take care and get to know these important facts of the field hence the experience of farming will not matter regardless of the time a farmer has been involved in tomato farming.

Choice and preference of tomato varieties are guided by factors that in one way, or another motivate farmers to choose one variety over others. Availability of seeds, affordability of prices, yield, marketability, shape, size as well as postharvest durability is considered by farmers in choosing the variety. Our results indicated the variety preference was mainly based on yield, shape, size, marketability, and disease resistance. Similar results were also observed by Palilo (2019) in Morogoro. Most of the preferred varieties cited by tomato farmers from all five regions in the survey were susceptible to bacterial wilt with bacterial wilt. Palilo (2019) also indicated susceptibility of ‘Rio’, ‘Assila’ and ‘Cal J’ varieties during their research. In a similar context, Asiimwe et al. (2013) reported high susceptibility of farmers' preferred tomato varieties (Roma type) in Uganda compared

to the moderately resistant variety ‘MT56’. Variety preference was negatively regressed with bacterial wilt incidence in tomatoes in this study. The finding reflects that susceptible varieties were less preferred and most preferred varieties were less susceptible to bacterial wilt disease. With this remark’s researchers, extension, and all stakeholders in tomato farming systems should work together on finding appropriate varieties or management practices that will improve the performance of this bacterial wilt susceptible varieties.

From the results of intercropping and crop rotation in this study, it is indicated that tomato is intercropped or rotated with several short-term (vegetables, maize) and long-term crops (banana and coffee). As indicated by De putter et al., 2011 and Luzi Kihupi et al. (2015) farmers in the key tomato producing regions especially Tanga and Mbeya farmers practice intensive agriculture with very short rotational periods between crops as the fields are used all year round. Maize, a nonhost of *R. solanacearum*, was cited by 37% of the farmers interviewed in our survey as a rotational crop with tomato, however, rotating tomato with maize or other crops cited was not correlated with bacterial wilt incidence in tomato fields in this study. The areas are also the main producers of potatoes, which may serve as a source of inoculum for tomato crops following potatoes in the rotation (Mwankemwa,2015). This contrasts with results in Uganda in which rotations of potato with maize, wheat, sorghum, or finger millet delayed wilt incidence in the next potato crop (Katafiire et al., 2005). Maize is the main staple food crop in Tanzania and is cultivated in these fields especially during the rainy season. As *R. solanacearum* can survive for many years in bare soil and maintain virulence (Prior et al., 2016), the typical one-year rotation program may be ineffective in reducing pathogen populations sufficiently. Other sources

of inoculum, especially surface water used for irrigation, may negate the effects of rotation with a non-host crop.

In this study, farmers highlighted disease and pests as the main constraints for tomato production in the five tomato-producing regions of Tanzania. Testen et al. (2016) and Minja et al. (2011) also highlighted the limitations to tomato production by these factors. Tanzania has a tropical environment that allows a variety of annual and perennial crops to grow all year round, hence perpetuation of disease and pests due to host availability. Besides, poor management programs and fake and/or low-quality pesticides limit effective disease control. Ngowi et al. (2007) and Maerere et al. (2010) reported the overuse of industrial fungicides or insecticides in the control of tomato diseases and pests in Tanzania, especially in Morogoro, Arusha, and Manyara regions. Approximately one-third of farmers in our survey cited the application of fungicides or insecticides to manage bacterial wilt, a completely ineffective tactic against this disease. This reflects both a lack of knowledge of the biology of the disease and its management and a lack of available management tactics.

Although seedborne *R. solanacearum* is not generally considered a major source of inoculum, seedborne transmission of bacterial wilt has been demonstrated in Solanaceous plants (Umesha et al., 2005). At least one-third of the tomato farmers surveyed in this study saved their tomato seeds or bought them from neighbors, in which case quality control is likely lacking and pathogen contamination of seeds is possible. In our results, regression analysis indicated that the seed source was positively regressed with bacterial wilt incidence in Tanzanian tomato fields. This indicates the possibility of seeds source to

influence the incidence levels. Our results are just indicative, however intensive studies are needed to study the possibility of bacterial wilt through seeds and the role of different tomato seed sources in disseminating bacterial wilt pathogens.

Open water sources can be a contributing factor in spreading bacterial wilt disease. Our survey of tomato farmers indicated that among the 46% who relied on irrigation during the growing season, the vast majority 37% used rivers and streams as water sources. Baitani (2017) detected *R. pseudosolanacearum* in river water used by farmers for irrigating their fields in Tanzania. The regions where this study was conducted are subjected to high rainfall regimes associated with floods especially during the long rainy season (March to June). Thus, high possibility of dissemination of bacterial wilt pathogens in new areas or a nearby field. Though farmers in our study area used different Irrigation methods we could not establish the contribution of these methods in bacterial wilt incidences. Palilo (2019) compared bacterial wilt disease prevalence in farms that used drip and furrow irrigation and established a non-significant difference in prevalence between the two methods.

Management Production of healthy tomato seedlings is a key factor towards improved plant health, vigor, and yield. More than half of the farmers surveyed indicated that they used soil sterilization, mulching, and raised beds in their tomato seedling nurseries. Farmers in Tanzania heat sterilize their nursery soil to reduce pathogen load by temperature and limit food availability (Saddler, 2005). Mulching can help to reduce soil temperature and seedling infection (Mwankemwa, 2015). However, none of these tactics as reported by surveyed farmers were significantly contributing to observed bacterial wilt incidence in tomato fields across Tanzania. In comparison between types of nursery and their

contribution to bacterial wilt prevalence Palilo (2019) could not establish bacterial wilt differences in prevalence between raised bed and tray nurseries

On average, more than 85% of tomato farmers surveyed indicated that they were aware of bacterial wilt. Palilo (2019) had 73% of tomato farmers in Morogoro were aware of the bacterial wilt disease. Being aware of the disease is important but most important is to know how to manage the disease. A good proportion of farmers in our study indicated to have limited options of bacterial wilt management being pesticide application and roguing the diseased plants. Farmers indicated the use of roguing to control bacterial wilt in tomato production fields. The use of roguing by 73% of tomato farmers in Morogoro as a management tactic was also reported by Palilo (2019). However, this tactic was observed to not significantly influence bacterial wilt incidence. It is possible that poor handling of uprooted diseased plants including disposing of them in the production field, which we observed during bacterial wilt field assessments, contributed to disease spread. Palilo (2019) established that almost equal proportions of farmers do burn, bury, or dispose of the rogued plants in production fields. We also observed poor weed control on most of the tomato farms. Roguing weeds, especially from the Solanaceae family, could help in reducing *R. solanacearum* inoculum reservoirs (Pradhanang et al., 2005). Palilo (2019)

Bacterial wilt prevalence was also assessed in 128 and 100 Solanaceous crop fields, including 52 and 51 tomato fields, in the regions in 2017 and 2019, respectively. The disease was present in all five regions of Tanzania, but prevalence varied among them. Bacterial wit disease prevalence was high in Solanaceous crops other than tomato in both survey years. Prevalence of bacterial wilt of tomato was 54% in 2017 and 41% in 2019

with Mbeya and Tanga having the highest (>70%) and lowest in Iringa in 2017, while in 2019 prevalence was highest in Morogoro and lowest in Arusha. Aloyce (2020) established the prevalence of bacterial wilt in 55.7% of tomato farms in the main ecological zones of Tanzania. This study observed bacterial wilt incidence of 0.2-17.5% and severity of 3.5 to 17.5% across the regions with very little to no significant variation among the regions. Aloyce (2020) observed a severity of 10.7 to 59.3 and incidence of 5 to 44.6% in tomato fields of the surveyed agroecological zones of Tanzania. Many factors may have contributed to the observed differences in the prevalence of bacterial wilt disease. Warm temperatures and moist soils allow rapid multiplication and spread of the pathogen (Hyaward, 1991). Tanga and Mbeya environments are characterized by acidic loamy clay soils and heavy rainfalls throughout the year (above 1000mm) while Morogoro region is characterized by sandy clay soils, warm climate, and heavy rain throughout the year. These conditions were highly conducive for the growth and perpetuation of *R. pseudosolanacearum* (Lin et al., 2014). These conditions were also highlighted by Minja et al. (2011) and Testen et al. (2016). However, the non-significant difference in the region could be explained by the preference of tomato varieties and similar agronomic practices that were indicated by farmers in this study. As discussed earlier most farmers preferred varieties are susceptible to bacterial wilt, seed sources, and awareness with limited management options across the regions could contribute to the similarities. Seeds and pesticides used were from similar seed companies in addition to having roguing diseased plants and pesticide application as only management options.

In this research, we surveyed the five key tomato producing regions of Tanzania where bacterial wilt is now extensively spread with no differences in prevalence among the regions but variation in incidence in tomato fields within most regions. The disease rate increased between the two surveys and farmers' practices had little contribution to the prevalence of bacterial wilt disease in the key tomato producing areas. This information is important in designing and developing or improving available management strategies such as the deployment of wilt-resistant varieties, preferred tomato varieties grafted on wilt-resistant rootstocks, or soil remediation tactics such as anaerobic soil disinfestation.

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Table 5. 1: Summary of Tanzanian regions, villages and fields surveyed in 2017 and 2019 for bacterial wilt disease and number of tomato farmers interviewed.

Region	District	Village	No. fields surveyed 2017	No. farmers interviewed 2017	No. fields surveyed 2019	No. farmers interviewed 2019
Morogoro	Mvomero	Mlali Peco,	6	5	10	6
		Misufini	5	5	10	5
	Kilosa	Malolo	5	3	0	0
Iringa	Kilolo	Ruaha Mbuyuni	5	5	5	5
		Image	5	5	5	0
	Iringa rural	Nzihi	5	5	5	5
		Kiponzero	5	2	5	0
Mbeya	Mbeya rural	Inyala	6	4	5	5
		Ijombe	5	2	5	0
		Mshewe	5	4	0	0
	Rungwe	Lutete	5	3	5	0
		Kiwira	10	1	5	5
Tanga	Lushoto	Lukozi	12	2	10	5
		Maringo	10	0	5	5
		Mangoi	5	4	5	0
Arusha	Arumeru	Maweni	9	1	5	5
		Karangai	5	1	0	0
		Ngarenanyuki	5	0	5	5
		Oldonyo Sapuk	10	0	5	0
		Hort Tengeru	5	0	5	0
Total			128	52	100	51

Table 5. 2: Types of survey questions and content as administered during tomato farmers' surveys in Tanzania in 2017 and 2019.

Section	Category of questions	Question content
1	Personal data and land use	Farm identification, sex, area used in cultivation, area used in tomato production, other crops cultivation
2	Tomato production and practices	Years involved in tomato production, tomato varieties and preference, irrigation methods, nursery practices, tomato yield, production trend
3	Disease/pest knowledge and management practices	Factors limiting tomato production, important tomato pests/diseases, farmers' knowledge of bacterial wilt, farmers' methods of bacterial wilt management, pesticides and application regimes, success of management practices

Table 5. 3: Demographic responses from 2017 and 2019 tomato farmers' survey in Tanzania by region, and average across regions

		2017						2019					
		Morogoro	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**
Gender	Male	69.2	76.5	85.7	83.3	100	82.9	60	58.3	77.8	60	60	63.2
	Female	30.8	23.5	14.3	16.7	0	17.1	40	41.7	22.2	40	40	36.8
		P=0.721 (Between regions)						*P*=0.072 (Between years)					
Farm size	Up to 0.25	38.5	5.9	21.4	50	0	23.2	50	0	0	0	0	10.0
(Ha)	0.251 - 0.50	38.5	29.4	42.9	33.3	0	28.8	50	25	0	50	20	29.0
	0.51 - 0.75	7.7	11.8	0	16.7	0	7.2	0	8.3	11.1	0	10	5.9
	0.751 - 1.00	7.7	23.5	14.3	0	50	19.1	0	50	44.4	40	40	34.9
	> 1.00	7.7	29.4	21.4	0	50	21.7	0	16.7	44.4	10	30	20.2
		P=0.008 (Between regions)						*P*=0.129 (Between years)					
Year in tomato	< 5	46.2	58.8	64.3	33.3	100	60.5	10	75	33.3	30	30	35.7
production	6 -10	15.4	17.6	21.4	50	0	20.9	50	16.7	55.6	50	40	41.5
	11 - 15	15.4	11.8	14.3	16.7	0	11.6	10	0	0	10	30	10.0
	16 - 20	15.4	5.9	0	0	0	4.3	30	8.3	11.1	0	0	9.9
	> 20	7.7	5.9	0	0	0	2.7	0	0	0	10	0	2.0
		P=0.176 (Between regions)						P=0.173 (Between years)					
Tomato yield	< 5	15.4	0	0	50	0	13.3	8.7	0	0	18.8	16.7	8.8
(tons/Ha)	6 - 10	30.8	5.9	0	0	0	7.3	30.4	24.1	8.7	31.3	16.7	22.2
	11 - 15	0	0	21.4	33.3	0	10.9	8.7	13.8	26.1	37.5	16.7	20.6
	16 - 20	15.4	5.9	14.3	0	0	7.1	17.4	6.9	13	6.3	8.3	10.4
	21 - 30	15.4	29.4	14.3	16.7	50	25.2	17.4	20.7	17.4	6.3	25	17.4
	> 30	23.1	58.8	50	0	50	36.4	17.4	34.5	34.8	0	16.7	20.7
		P=0.111 (Between regions)						*P*=0.0001 (Between years)					

Table 5. 4: Bacterial wilt disease prevalence in Solanaceous crop fields surveyed in key tomato producing regions of Tanzania.

	2017			2019			Region
Region	**No. fields surveyed**	**No. fields w/ bacterial wilt**	**Bacterial wilt prevalence (%)**	**No. fields surveyed**	**No. fields w/ bacterial wilt**	**Bacterial wilt prevalence (%)**	**Mean comparison**
Morogoro	3	0	0	10	6	60	30.0 bc
Iringa	3	0	0	9	2	22.2	11.1 c
Mbeya	17	13	76.5	10	10	100	88.3 a
Tanga	21	15	66.7	10	7	70	68.4 ab
Arusha	32	20	62.5	10	8	80	71.3 a
Total	76	48	61.2	49	33	67.3	-
Mean	15.2	9.6	41.1	9.8	6.6	66.4	-

[a] *P value* =0.0543

[b] *P* value 0.002*6*

LSD value 41.0

Table 5. 5: Bacterial wilt disease prevalence in tomato fields surveyed in key tomato producing regions of Tanzania in 2017 and 2019.

	2017			2019		
Region	**No. tomato fields surveyed**	**No. fields with bacterial wilt**	**Bacterial wilt prevalence (%)**	**No. tomato fields surveyed**	**No. fields with bacterial wilt**	**Bacterial wilt prevalence (%)**
Morogoro	13	3	23	10	6	60
Iringa	17	3	18	11	5	45
Mbeya	14	7	50	10	5	50
Tanga	6	5	83	10	4	40
Arusha	2	2	100	10	1	10
Total	52	20	38.5	51	21	41.2
Mean	10.4	4	54.8	10.2	4.2	41.0
Region $P =$ 0.9270				Year P=0.5894		

Table 5. 6: Mean comparison of bacterial wilt incidence within and among the five key tomato producing regions of Tanzania. Means were calculated from bacterial wilt incidence scores from all tomato fields surveyed in each region.

	2017		2019	
Region	**Mean bacterial wilt incidence**	***P*R > /t/[a]**	**Mean bacterial wilt incidence**	***P*R > /t/ [a]**
Arusha	5.5	0.5682	0.2	0.9683
Iringa	5.9	0.0796	13.7	0.0088
Mbeya	8.2	0.0278	8.7	0.0890
Morogoro	4.31	0.2570	17.5	0.0011
Tanga	11.0	0.5230	3.2	0.5258
P value [b]		0.8657		0.1040

[a] Within region *P*-values.

[b] Among regions *P*-values

Table 5. 7: Mean comparison of bacterial wilt severity among and within the five key producing regions of Tanzania. Means were calculated from bacterial wilt severity scores from all tomato fields surveyed in each region.

	2017		2019	
Region	**Mean bacterial wilt severity**	***P*R > /t/[a]**	**Mean bacterial wilt severity**	***P*R > /t/**
Arusha	70.0	0.0038	3.5	0.6893
Iringa	13.1	0.1040	31.5	0.0007
Mbeya	36.0	0.0001	29.0	0.0017
Morogoro	16.3	0.0766	33.3	0.0004
Tanga	58.7	<0.0001	22.0	0.0150
P value [b]		0.0106		0.1177

[a] Within region *P*-values.

[b] Among regions *P*-values

Table 5. 8: Multiple factor regression analysis of the contribution of farmers' practices to bacterial wilt disease incidence in tomato fields. All farmers' practices were treated as independent variables and disease incidence as the response variable; comparisons were done at 5% probability.

Independent variables	**Regression coefficient**	**Standard error**	***P*** value	**Expected B**
Region	-0.183	0.341	0.591	0.833
Gender	-0.501	0.654	0.443	0.606
Farm size	0.04	0.32	0.908	0.970
Tomato variety preference [a]	**-0.15**	**0.05**	**0.006**	**0.861**
Years of tomato production experience	-0.01	0.04	0.823	0.982
Seed source [a]	**1.05**	**0.43**	**0.014**	**5.607**
Irrigation water source	-0.23	0.27	0.395	0.529
Soil sterilization in nursery	0.003	0.55	0.996	0.971
Use of raised beds in nursery	0.92	1.39	0.509	0.362
Mulching in nursery	-1.39	1.34	0.306	1.522
Irrigation method	0.34	0.22	0.146	1.428
Tomato production trend	0.72	0.48	0.100	5.266
Other tomato disease	0.07	0.08	0.395	1.083
Bacterial wilt awareness [a]	**2.2**	**0.98**	**0.024**	**54.46**
Bacterial wilt causes	0.031	0.04	0.448	1.019
Use of management tactics	0.13	0.37	0.730	1.776
Intercropping/rotational crops	-0.121	0.064	0.059	0.886
Regression constant	-4.93	2.33	0.034	0.001

[a] Independent variables that gave significant *P* value with regression analysis

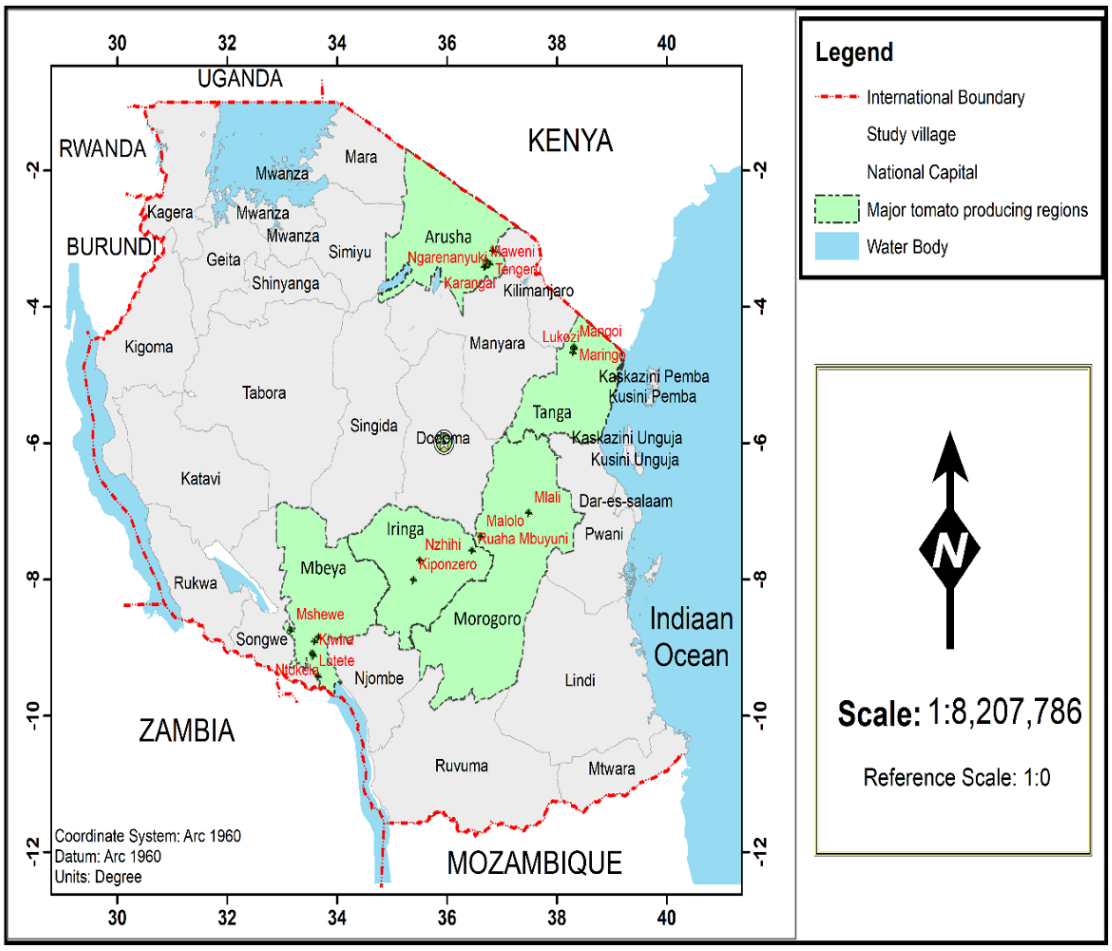

Fig. 5. 1: Map of Tanzania indicating surveyed areas in red in the key tomato producing regions of Tanzania.

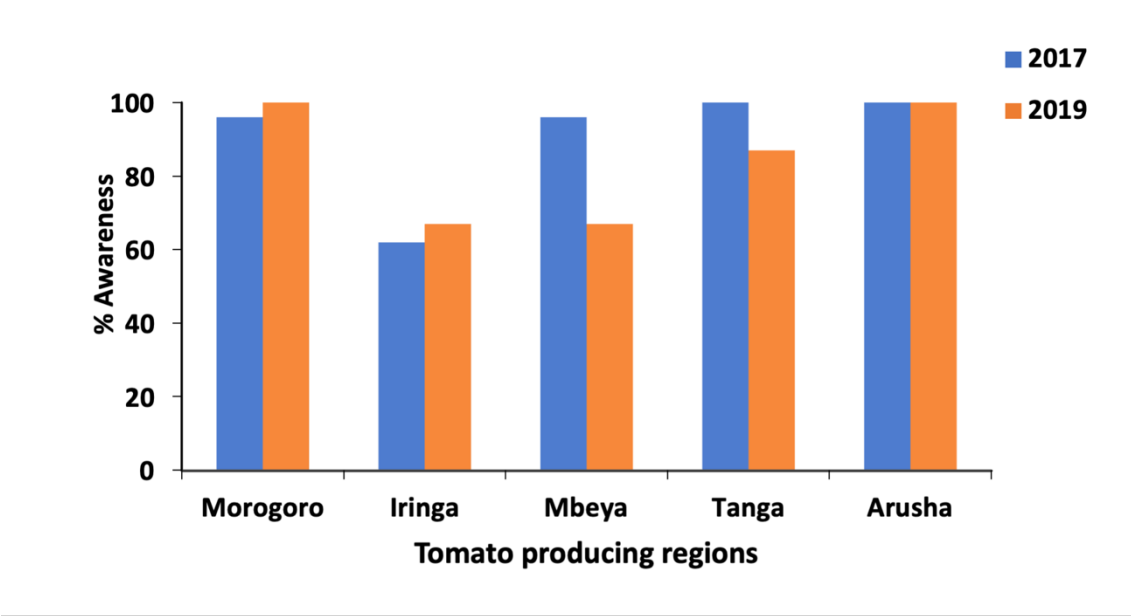

Fig. 5. 2: Proportion of tomato farmers indicating that they were aware of bacterial wilt of tomato during surveys in 2017 (n = 52) and 2019 (n = 51) in the key tomato-producing regions of Tanzania. (P=0.394) between years and P=0.0001 between regions.

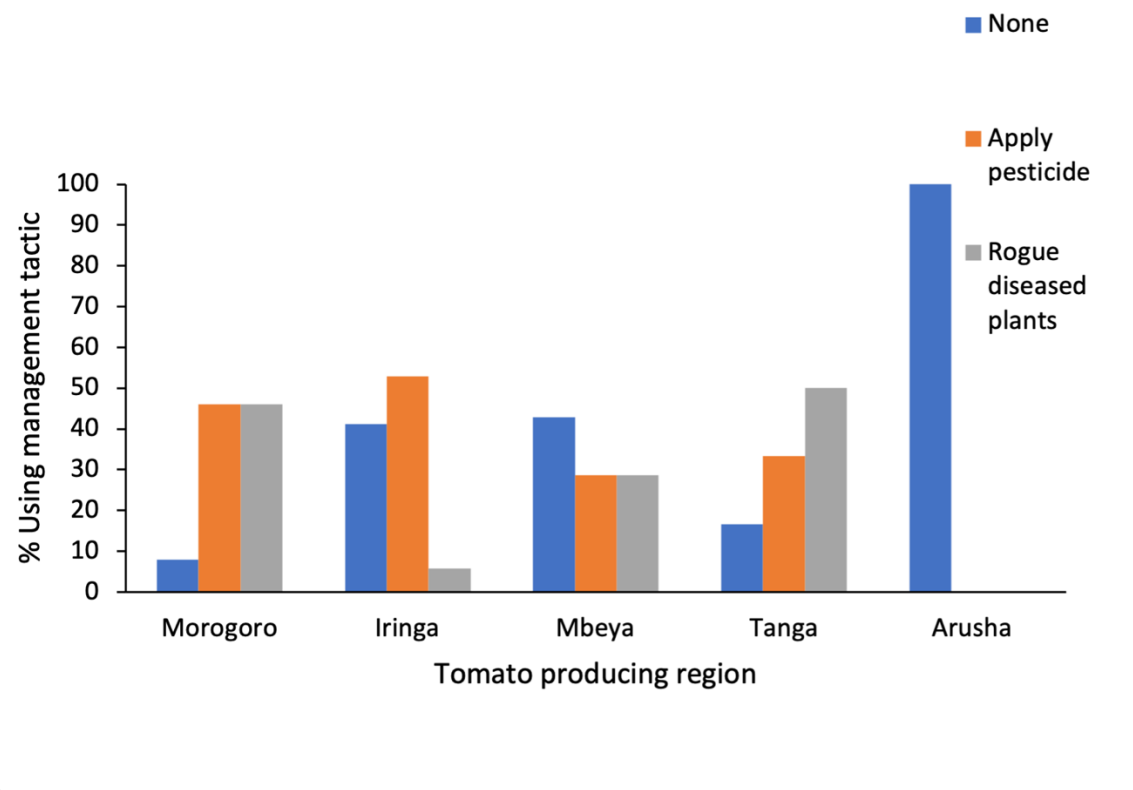

Fig. 5. 3: Proportion of tomato farmers surveyed who reported to use the roguing, pesticide (fungicide and/or insecticide) application or no tactic to manage bacterial wilt of tomato in the key tomato-producing regions of Tanzania. The survey was conducted in 2017 (n = 52 tomato farmers). P=0.003 between regions.

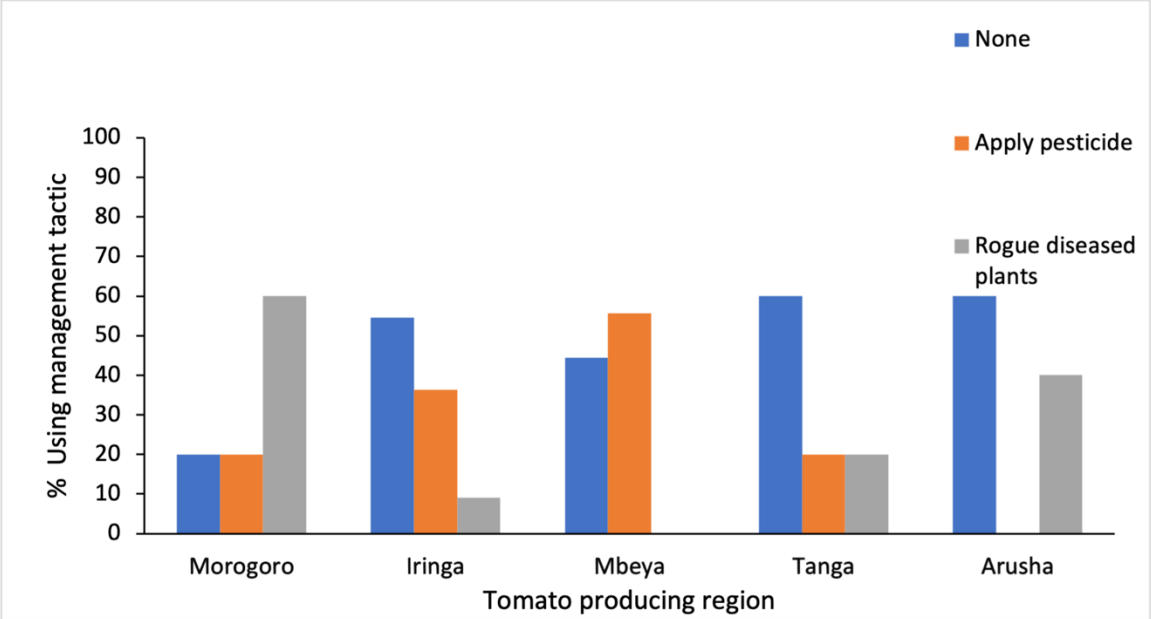

Fig. 5. 4: Proportion of tomato farmers surveyed who reported to use the rouging, pesticide (fungicide and/or insecticide) application or no tactic to manage bacterial wilt of tomato in the key tomato-producing regions of Tanzania. The survey was conducted in 2019 (n = 51) tomato farmers). *P*=0.003 between regions

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Appendix A: Chapter 2 Supporting Materials

Table A. 1: ANOVA table presenting bacterial wilt incidence, latent infection and AUDPC for the varieties, *Ralstonia pseudosolanacearum* strains, and their interaction during pathogenicity evaluation of tomato and sweet pepper carried out in Tanzania.

Response variable	**Source**	**DF**	**Type I SS**	**Mean square**	**F value**	***P*r > F**
Incidence	Rep	1	1192.4	1192.4	1.8	0.1851
	Variety	2	14925.6	7462.8	11.1	<0.0001
	Rep*variety	2	7458.7	3729.4	5.5	0.0046
	Strain	60	102807.5	1685.4	2.5	<0.0001
	Variety*strain	122	133107.1	1091	1.6	0.0016
Latent infection	Rep	1	12939.9	12939.9	34.9	<0.0001
	Variety	2	106067.9	53033.9	143.1	<0.0001
	Rep*variety	2	8556.3	4278.1	11.6	<0.0001
	Strain	60	95105.4	1559.1	4.2	<0.0001
	Variety*strain	122	82332.4	674.9	1.8	0.0001
AUDPC	Rep	1	15430.3	15430.3	1.3	0.2521
	Variety	2	1501135	750567.6	64.7	<0.0001
	Rep*variety	2	109658.7	54829.3	4.7	0.0116
	Strain	60	1005658	14366.5	1.2	0.1800
	Variety*strain	122	995578.1	711.3	0.6	0.9946

Table A. 2: Distribution of *Ralstonia pseudosolanacearum* strains used in the study based on host and region of collection.

Region	**Tomato**	**Sweet pepper**	**Eggplant**	**Total**
Tanga	19	5	0	24
Morogoro	10	6	0	16
Mbeya	7	3	2	12
Iringa	9	0	0	9
Total	45	14	2	61

Table A. 3: *Ralstonia pseudosolanacearum* strains collected from tomato, pepper, and eggplant in five major tomato-producing regions of Tanzania in 2019.

Strain	Host	Origin	Region	Strain	Host	Origin	Region
TZ1	Tomato	Lushoto	Tanga	TZ32	Tomato	Image	Iringa
TZ2	Tomato	Lushoto	Tanga	TZ33	Tomato	Inyala	Mbeya
TZ3	Sweet pepper	Lushoto	Tanga	TZ34	Sweet pepper	Mshewe	Mbeya
TZ4	Tomato	Lushoto	Tanga	TZ35	Sweet pepper	Mshewe	Mbeya
TZ5	Tomato	Mlali	Morogoro	TZ36	Eggplant	Mshewe	Mbeya
TZ6	Sweet pepper	Lushoto	Tanga	TZ37	Tomato	Lushoto	Tanga
TZ7	Tomato	Mlali	Morogoro	TZ38	Tomato	Lushoto	Tanga
TZ8	Sweet pepper	Lushoto	Tanga	TZ39	Sweet pepper	SUA GH	Morogoro
TZ9	Tomato	Lushoto	Tanga	TZ40	Sweet pepper	Lushoto	Tanga
TZ10	Sweet pepper	Lushoto	Tanga	TZ41	Tomato	Image	Iringa
TZ11	Tomato	Lushoto	Tanga	TZ42	Tomato	Mlali	Morogoro
TZ12	Tomato	Lushoto	Tanga	TZ43	Tomato	Lushoto	Tanga
TZ13	Tomato	Lushoto	Tanga	TZ44	Tomato	Mlali	Morogoro
TZ14	Tomato	Lushoto	Tanga	TZ45	Sweet pepper	SUA GH	Morogoro
TZ15	Tomato	Lushoto	Tanga	TZ46	Tomato	Mlali	Morogoro
TZ16	Tomato	Lushoto	Tanga	TZ47	Sweet pepper	SUA GH	Morogoro
TZ17	Tomato	Lushoto	Tanga	TZ48	Sweet pepper	SUA GH	Morogoro
TZ18	Tomato	Lushoto	Tanga	TZ49	Sweet pepper	SUA GH	Morogoro
TZ19	Tomato	Lushoto	Tanga	TZ50	Tomato	Ntokela	Mbeya
TZ20	Sweet pepper	Lushoto	Tanga	TZ51	Sweet pepper	SUA GH	Morogoro
TZ21	Tomato	Image	Iringa	TZ52	Tomato	Mlali	Morogoro
TZ22	Tomato	Image	Iringa	TZ53	Tomato	Mlali	Morogoro
TZ23	Tomato	Image	Iringa	TZ54	Tomato	Image	Iringa
TZ24	Tomato	Misufini	Morogoro	TZ55	Tomato	Inyala	Mbeya
TZ25	Tomato	Misufini	Morogoro	TZ56	Tomato	Inyala	Mbeya
TZ26	Tomato	Inyala	Mbeya	TZ57	Tomato	Inyala	Mbeya
TZ27	Tomato	Image	Iringa	TZ58	Sweet pepper	Mshewe	Mbeya
TZ28	Tomato	Image	Iringa	TZ59	Eggplant	Mshewe	Mbeya
TZ29	Tomato	Lushoto	Tanga	TZ60	Tomato	Lushoto	Tanga
TZ30	Tomato	Kiwira	Mbeya	TZ67	Tomato	Mlali	Morogoro
TZ31	Tomato	Image	Iringa				

Table A. 4: Detailed information of Primers used in the study.

PCR	Primer name	5'-3' Primer sequence	Size	Reference	Remarks
Species-specific and Phylotype multiplex	759R	GTCGCCGTCAACTCACTTTCC	280	Opina et al. 1997	Species specific
	760F	GTCGCCGTCAGCAATGCGGAATCG			
Phylotype Multiplex	Nmult21:1F	F CGTTGATGAGGCGCGCAATTT	144	Fegan and Prior 2005	Phylotype I
	Nmult21:2F	AAGTTATGGACGGTGGAAGTC	372		Phylotype II
	Nmult23:AF	AF ATTACSAGAGCAATCGAAAGATT	91		Phylotype III
	Nmult22: InF	ATTGCCAAGACGAGAGAAGTA	213		Phylotype IV
	Nmult22: RR	TCGCTTGACCCTATAACGAGTA			
Endoglucanase gene (egl)	Endo F	ATGCATGCCGCTGGTCGCCGC	750	Poussier et al. 2000	*egl* gene

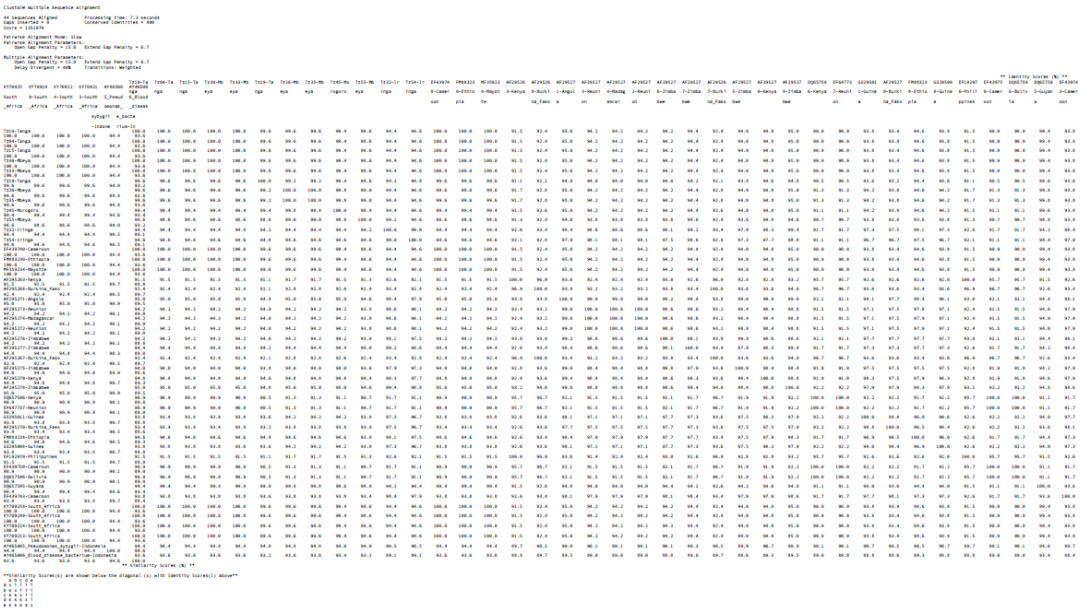

Figure A. 1: Maximum likelihood similarity matrix of twelve Tanzania *Ralstonia pseudosolanacearum* strains and 31 reference strains used in the phylogenetic analysis.

Appendix B: Chapter 3 Supporting Materials

Table B. 1: ANOVA table for incidence of bacterial wilt in eleven Ohio rootstock lines and a wilt-susceptible tomato variety ‘‘Moneymaker’’ inoculated with five strains of *Ralstonia pseudosolanacearum* and their interaction during preliminary rootstock evaluation.

Source	DF	Type I SS	Mean Square	F value	Pr > F
Rep	1	403.3	403.3	1.82	0.1837
Line	11	29050	2640.9	11.91	< 0.0001
Rep*Line	11	1156.7	105.2	0.47	0.91
Strain	4	19146.7	4786.67	21.59	< 0.0001
Line*strain	44	67333.3	1530.3	6.9	< 0.0001

Table B. 2: ANOVA table for severity of bacterial wilt in eleven Ohio rootstock lines and a wilt-susceptible tomato variety ‘‘Moneymaker’’ inoculated with five strains of *Ralstonia pseudosolanacearum* and their interaction during preliminary rootstock evaluation.

Source	DF	Type I SS	Mean Square	F value	Pr > F
Rep	1	114.1	114.1	3.56	0.0654
Line	11	11244.7	1022.2	31.87	< 0.0001
Rep*Line	11	92.8	8.4	0.26	0.9898
Strain	4	8221.4	2055.3	64.08	< 0.0001
Line*strain	44	23537.4	534.9	16.68	< 0.0001

Table B. 3: ANOVA table for incidence of latent infection in eleven Ohio rootstock lines and a wilt-susceptible tomato variety ‘‘Moneymaker’’ inoculated with five strains of *Ralstonia pseudosolanacearum* and their interaction during preliminary rootstock evaluation,

Source	DF	Type I SS	Mean Square	F value	Pr > F
Rep	1	418.1	418.1	0.96	0.3333
Line	11	92978.7	8452.6	19.31	< 0.0001
Rep*Line	11	3891.1	353.7	0.18	0.6318
Strain	4	6840.4	1710.1	3.91	0.008
Line*strain	44	179197.4	4072.7	9.3	< 0.0001

Table B. 4: Bacterial wilt Incidence, latent infection, and severity in 11 Ohio tomato rootstock breeding lines and wilt-susceptible tomato variety ‘‘Moneymaker’’ inoculated with five strains of *Ralstonia pseudosolanacearum* during preliminary rootstock evaluation in Ohio.

		Bacterial wilt		
Line/Variety	**Strains**	**Incidence (%)**	**Latent (%)**	**Severity (%)**
SGH06 220	SM 716	100	0	64
	SM 747	100	0	84
	TZ130	100	0	44
	TZ 48	80	0	60
	TZ 9	10	12.5	70
WG-121	SM 716	70	16.5	50
	SM 747	100	0	70
	TZ130	80	0	48
	TZ 48	20	0	60
	TZ 9	50	12.5	57
SGH06 215	SM 716	100	0	60
	SM 747	100	0	76
	TZ130	40	67	50
	TZ 48	100	0	55
	TZ 9	60	33.5	61
SGH06-211	SM 716	40	67	53
	SM 747	60	100	0
	TZ130	40	67	56
	TZ 48	40	67	60
	TZ 9	20	25	53
SGH06-216	SM 716	60	0	60
	SM 747	20	0	60
	TZ130	80	0	44
	TZ 48	20	25	62
	TZ 9	20	12.5	66
WG12-110	SM 716	80	50	52
	SM 747	40	67	60
	TZ130	20	25	60
	TZ 48	40	67	80
	TZ 9	20	25	60

Continue…

Table B.4: Continued

		Bacterial wilt		
Line/Variety	**Strains**	**Incidence (%)**	**Latent (%)**	**Severity (%)**
WG12-130	SM 716	80	50	52
	SM 747	60	100	40
	TZ130	60	100	60
	TZ 48	20	0	60
	TZ 9	30	29	50
WG12-120	SM 716	80	0	50
	SM 747	80	0	48
	TZ130	0	0	0
	TZ 48	0	0	0
	TZ 9	20	25	60
FGH06- 304	SM 716	20	75	60
	SM 747	80	0	60
	TZ130	20	0	40
	TZ 48	100	0	40
	TZ 9	20	12.5	60
FGH06- 301	SM 716	40	67	60
	SM 747	20	25	60
	TZ130	20	25	40
	TZ 48	40	33	40
	TZ 9	80	30	80
FGH06-302	SM 716	20	0	60
	SM 747	60	100	50
	TZ130	0	67	0
	TZ 48	60	100	40
	TZ 9	40	33	60
‘Moneymaker’	SM 716	70	0	60
	SM 747	80	0	80
	TZ130	60	100	60
	TZ 48	100	0	72
	TZ 9	20	25	62
P value		<0.0001	<0.0001	<0.0001

Table B. 5: ANOVA table for incidence of bacterial wilt in eggplant 'EG190' and tomato 'MT56' rootstock lines and a wilt-susceptible tomato variety ''Moneymaker'' inoculated with 15 strains of *Ralstonia pseudosolanacearum* and their interaction during preliminary rootstock evaluation.

Source	DF	Type 1SS	Mean square	F value	Pr > F
Rep	2	2192.5	1096.2	2.29	0.1073
Line	2	59495.5	29747.8	62.22	<0.0001
Rep*line	4	4450.7	1112.7	2.33	0.0628
Strain	14	15433.8	1102.4	2.31	0.0099
Line*Strain	28	28880	1031.4	2.16	0.0038

Table B. 6: ANOVA table for severity of bacterial wilt in eggplant 'EG190' and tomato 'MT56' rootstock lines and a wilt-susceptible tomato variety ''Moneymaker'' inoculated with 15 strains of *Ralstonia pseudosolanacearum* and their interaction during preliminary rootstock evaluation.

Source	DF	TypeI SS	Mean square	F value	Pr > F
Rep	2	222.6	111.3	0.54	0.5819
Line	2	21534.2	10767.1	52.72	<0.0001
Rep*line	4	1836.4	459.1	2,25	0.0707
Strain	14	18841.1	1345.8	6.59	<0.0001
Line*Strain	28	22522.7	804.4	3.94	<0.0001

Table B. 7: ANOVA table for bacterial wilt latent infection in eggplant 'EG190' and tomato 'MT56' rootstock lines and a wilt-susceptible tomato variety ''Moneymaker'' inoculated with 15 strains of *Ralstonia pseudosolanacearum* and their interaction during preliminary rootstock evaluation.

Source	DF	TypeI SS	Mean square	F value	Pr > F
Rep	2	3720.5	1860.2	2.15	0.1229
Line	2	30564.6	15282.3	17.66	<0.0001
Rep*line	4	16959.6	4239.9	4.9	0.0013
Strain	14	19524.2	1394.8	1.61	0.0927
Line*strain	28	59345.9	2119.5	2.45	0.0009

Table B. 8: Bacterial wilt Incidence, latent infection, and severity in tomato rootstock 'MT56', eggplant rootstock 'EG190' and wilt-susceptible tomato variety ''Moneymaker'' inoculated with 15 strains of *Ralstonia pseudosolanacearum* during preliminary rootstock evaluation in Ohio.

		Bacterial wilt		
Line	**Strain**	**Incidence (%)**	**Latent (%)**	**Severity (%)**
'EG190'	SM 716	22	50	40
'EG190'	SM 727	33	53.3	66.7
'EG190'	SM 732	11	13.3	20
'EG190'	SM 738	0	0	0
'EG190'	SM 747	22	33.3	40
'EG190'	TZ 130	0	33.3	0
'EG190'	TZ 22	0	0	0
'EG190'	TZ 48	0	33	0
'EG190'	TZ 55	0	33	0
'EG190'	TZ 57	0	0	0
'EG190'	TZ 58	0	0	0
'EG190'	TZ 70	0	0	0
'EG190'	TZ 71	0	0	0
'EG190'	TZ 80	0	0	0
'EG190'	TZ 9	0	0	0
MM	SM 716	11	0	20
MM	SM 727	55.6	66.7	40
MM	SM 732	33.3	0	0
MM	SM 738	33.3	0	40
MM	SM 747	78	66.7	60
MM	TZ 130	55.7	53.3	44.7
MM	TZ 22	44.3	66.7	35.7
MM	TZ 48	55.7	83.3	44.3
MM	TZ 55	67	100	47

Continue…

Table B.8: Continued

		Bacterial wilt		
Line	**Strain**	**Incidence (%)**	**Latent (%)**	**Severity (%)**
MM	TZ 57	89	33.3	55.7
MM	TZ 58	67	66.7	55.7
MM	TZ 70	55.7	100	53.3
MM	TZ 71	55.3	33.3	31.3
MM	TZ 80	78	33.3	33.3
MM	TZ 9	67	66.7	40
‘MT56’	SM 716	55.3	33.3	60
‘MT56’	SM 727	66.7	0	26.7
‘MT56’	SM 732	11	66.7	13.3
‘MT56’	SM 738	22.3	0	16.7
‘MT56’	SM 747	55.3	33.3	43.3
‘MT56’	TZ 130	44.7	0	26.7
‘MT56’	TZ 22	0	33.3	0
‘MT56’	TZ 48	0	11	0
‘MT56’	TZ 55	22	11	13.3
‘MT56’	TZ 57	0	33.3	0
‘MT56’	TZ 58	22	33.3	18
‘MT56’	TZ 70	33	16.7	17.7
‘MT56’	TZ 71	0	11	0
‘MT56’	TZ 80	0	33.3	0
‘MT56’	TZ 9	11	33.3	6.7
P value		0.0038	0.0009	<0.0001

Table B. 9: ANOVA table for bacterial wilt incidence in selected tomato and eggplant rootstock lines inoculated with *Ralstonia pseudosolanacearum* strains and their interaction during rootstock evaluation in Tanzania.

Source	DF	Type 1SS	Mean square	F value	Pr< F
Rep	2	938.9	469.4	1.93	0.15
Line	3	59608.3	19869.4	81.68	<0.0001
Rep*line	6	20416.6	3402.8	13.99	<0.0001
Strain	5	13680.6	2736.1	11.25	<0.0001
Line*strain	15	5575	371.7	1.53	0.1071

Table B. 10: ANOVA table for bacterial wilt latent infection in selected tomato and eggplant rootstock lines inoculated with *Ralstonia pseudosolanacearum* strains and their interaction during rootstock evaluation in Tanzania.

Source	DF	Type 1SS	Mean square	F value	Pr< F
Rep	2	115	57.5	0.06	0.9407
Lines	3	5678.2	1892.7	2.01	0.1161
Rep*lines	6	9715.5	1619.3	1.72	0.1221
Strains	5	7942.5	1588.5	1.69	0.1428
Lines*strains	15	15875.2	1058.4	1.13	0.3419

Table B. 11: ANOVA table for bacterial wilt disease progress (AUDPC) in selected tomato and eggplant rootstock lines, *Ralstonia pseudosolanacearum* strains and their interaction during rootstock evaluation in Tanzania

Source	DF	TypeI SS	Mean square	F value	Pr > F
Rep	2	68908.3	344454.2	5.54	0.0075
Line	3	1004277.8	334759.3	53.8	< 0.0001
Rep*line	6	152313.9	25385.7	4.08	0.0028
Strain	5	196450	39290	6.81	0.0002
Line*strain	15	176138.9	11742.6	1.89	0.0553

Table B. 12: Interactions between eggplant 'EG190' and tomato 'MT56' and WG120 rootstock lines, a wilt-susceptible tomato variety ''Tanya'' and six strains of *Ralstonia pseudosolanacearum* in bacterial wilt Incidence, latent infection, and severity during rootstock evaluation in Tanzania

		Bacterial wilt		
Line	**Strain**	**Incidence (%)**	**AUDPC**	**Latent infection (%)**
WG120	TZ 24	46.7	243.3	45.8
WG120	TZ 25	40	173.3	63.3
WG120	TZ 71	26.7	123.3	70.8
WG120	TZ 72	26.7	106.7	80.7
WG120	TZ 95	20	83.3	47.8
WG120	TZ 73	13.3	60	87.5
'Tanya'	TZ 24	86.7	490	50
'Tanya'	TZ 95	73.3	426.7	91.7
'Tanya'	TZ 73	66.7	416.7	66
'Tanya'	TZ 25	66.7	406.7	75
'Tanya'	TZ 71	53.3	286.7	72.2
'Tanya'	TZ 72	40	133.3	56.7
'MT56'	TZ 24	63.3	280	86.1
'MT56'	TZ 25	50	240	91.7
'MT56'	TZ 73	46.7	223.3	83.3
'MT56'	TZ 95	43.3	190	72.1
'MT56'	TZ 72	33.3	126.7	46.7
'MT56'	TZ 71	26.7	103.3	65.3
'EG190'	TZ 24	23.3	90	93.3
'EG190'	TZ 95	10	60	75
'EG190'	TZ 71	6.7	36.7	85.8
'EG190'	TZ 73	6.7	30	75.8
'EG190'	TZ 25	6.7	30	78.3
'EG190'	TZ 72	0	0	73.3
P value		0.1071	0.3419	0.0553

Appendix C: Chapter 4 Supporting Materials

Table C. 1: ANOVA table presenting yield of tomatoes grown in anaerobic soil disinfestation. (ASD)-treated field plots during on-farm evaluation of ASD efficacy in on-farm trials in Tanzania

Farm	Source	DF	Type 1SS	Mean Square	F value	*Pr* > F
Mlali 1	Rep	3	0.7	0.25	0.4	0.752
	Treatment	5	1.1	0.23	0.37	0.8589
Image 1	Rep	3	2.2	0.75	0.52	0.6767
	Treatment	5	3.5	0.71	0.49	0.7798
Image 2	Rep	3	4.2	1.39	2.84	0.0734
	Treatment	5	0.9	0.18	0.37	0.8607

Appendix D: Chapter 5 Supporting Materials

Table D. 1: ANOVA table presenting bacterial wilt incidence and severity on tomato by year and region during on-farm survey carried out in Tanzania in 2017 and 2019.

	Source	**DF**	**Type Iss**	**Mean squares**	**F**	**PR > F**
Incidence	Year	1	98.69	98.69	0.45	0.5013
	Region	4	974.08	243.52	1.1	0.3595
Severity	Year	1	337.73	337.73	0.32	0.5701
	Region	4	5329.39	1332.35	1.28	0.2829

Table D. 2: Distribution of survey responses on size of farms allocated for tomato production during 2017 and 2019 in tomato farmers surveys in the key tomato producing regions of Tanzania.

Survey year	Morogoro		Iringa		Mbeya		Tanga		Arusha		Total	
	n	Mean	n	Mean	n	Mean	n	Mean	n	Mean	N	Mean
2017	13	0.5	17	1.2	14	0.8	6	0.4	2	1.8	52	0.9
2019	10	0.4	12	1.1	9	1.4	10	0.9	10	1.1	51	1.0
Overall	**23**	**0.5**	**29**	**1.15**	**23**	**1.1**	**16**	**0.7**	**12**	**1.2**	**103**	**0.91**

Table D. 3: Distribution of responses for tomato yield during 2017 and 2019 tomato farmers survey in the key tomato producing regions of Tanzania.

Survey year	Morogoro		Iringa		Mbeya		Tanga		Arusha		Overall	
	n [a]	Mean	n	Mean	n	Mean	n	Mean	n	Mean	N[b]	Mean
2017	13	9.1	17	15.2	14	13.1	6	3.9	2	15.5	52	11.8
2019	10	7.1	12	4.6	9	6.8	10	4.5	10	6.9	51	5.9
Overall	23	8.2	29	10.82	23	10.6	16	4.3	12	8.4	103	8.9

Table D. 4: Distribution of responses on trend of tomato production during 2017 and 2019 tomato farmers surveys in the key tomato producing regions of Tanzania.

Region	2017							2019						
	Increased		Decreased		No change		Total farms	Increased		Decreased		No change		Total farms
	N	%	n	%	n	%		n	%	n	%	n	%	
Morogoro	6	46	6	46	1	8	13	4	40	6	60	0	0	10
Iringa	5	29	7	41	5	29	17	4	33	8	67	0	0	12
Mbeya	4	29	8	57	2	14	14	2	22	7	78	0	0	9
Tanga	1	17	4	67	1	17	6	4	40	5	50	1	10	10
Arusha	1	50	1	50	0	0	2	4	40	6	60	0	0	10
Total	**17**	**33**	**26**	**50**	**9**	**17**	**52**	**18**	**35**	**32**	**63**	**1**	**2**	**51**

P=0.697 (Between regions) *P*=0.030 (Between years) n= number of responses

Table D. 5: Distribution of responses on reasons for decreasing or increasing tomato production during 2017 and 2019 tomato farmers survey in the key tomato producing regions of Tanzania.

		2017						2019					
Status	Reasons for Production Trend	Morogoro	Iringa	Mbeya	Tanga	Arusha	Mean	Morogoro	Iringa	Mbeya	Tanga	Arusha	Mean
Increased	Good advice and skills	23.1	0.0	7.1	0.0	0.0	6.0	10.0	0.0	0.0	30.0	0.0	8.0
	Good seeds	7.7	5.9	0.0	0.0	0.0	2.7	10.0	0.0	0.0	20.0	10.0	8.0
	Good management and weather	15.4	11.8	21.4	0.0	50.0	19.7	10.0	33.3	22.2	0.0	0.0	13.1
	Improved technology	0.0	11.8	0.0	0.0	0.0	3.1	0.0	0.0	0.0	0.0	0.0	0.0
	Changed variety	0.0	0.0	7.1	0.0	50.0	11.4	0.0	0.0	0.0	0.0	0.0	0.0
	Access to farm implements	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	10.0	4.0
	Availability of extension service	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.3	0.0	0.0	20.0	5.7
Decreased	Pests and diseases	30.8	5.9	28.6	50.0	0.0	23.1	20.0	8.3	22.2	20.0	10.0	16.1
	Poor weather	7.7	23.5	7.1	0.0	0.0	7.7	30.0	33.3	11.1	20.0	20.0	22.9
	Flower abortion	15.4	5.9	7.1	0.0	0.0	5.7	10.0	0.0	0.0	0.0	0.0	2.0
	Drought	0.0	0.0	7.1	0.0	0.0	1.4	0.0	8.3	0.0	0.0	10.0	3.7
	Poor seeds	0.0	5.9	14.3	16.7	0.0	7.4	0.0	8.3	33.3	10.0	10.0	12.3
	High price of inputs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.1	0.0	0.0	2.2
	Low market price	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	2.0
No change	I do not know	0.0	29.4	0.0	33.3	0.0	12.5	0.0	0.0	0.0	0.0	0.0	0.0
	Total	100	100	100	100	100	100	100	100	100	100	100	100

P=0.013 (Between regions)
P=0.045 (Between years)

Table D. 6: Distribution of response of tomato variety preference among tomato farmers during 2017 and 2019 tomato farmers survey in the key tomato producing regions of Tanzania.

Region		**2017**		**2019**		**Mean**	
		Responses					
	Tomato Varieties	**n**	**%**	**n**	**%**	**N**	**%**
Morogoro	Assila	5	38.5	2	20	3.5	29.3
	Onyx	3	23.1	3	30	3	26.6
	Mwanga	1	7.7	1	10	1	8.9
	CalJ	2	15.4	3	30	2.5	22.7
	RioGrande	2	15.4	0	0	1	7.7
	Mkulima	0	0	1	10	0.5	5.0
	Total	13	100	10	100	11.5	100
Iringa	Assila	3	17.6	0	0	1.5	8.8
	Onyx	3	17.6	5	45.5	4	31.6
	CalJ	1	5.9	0	0	0.5	3.0
	‘Tanya’	1	5.9	0	0	0.5	3.0
	RioGrande	6	35.3	6	54.5	6	44.9
	Eden	2	11.8	0	0	1	5.9
	Safari	1	5.9	0	0	0.5	3.0
	Total	17	100	11	100	14	100
Mbeya	Assila	1	7.1	0	0	0.5	3.6
	Mwanga	1	7.1	1	10	1	8.6
	CalJ	2	14.3	0	0	1	7.2
	Roma vfn	2	14.3	0	0	1	7.2
	‘Tanya’	4	28.6	4	40	4	34.3
	Dumudumu	1	7.1	0	0	0.5	3.6
	Anna	2	14.3	0	0	1	7.2
	Pana	1	7.1	0	0	0.5	3.6
	RioGrande	0	0	5	50	2.5	25.0
	Total	14	100	10	100	12	100

Continue…

Table D.6: Continued

Region		2017		2019		Mean	
		Responses					
	Tomato Varieties	**n**	**%**	**n**	**%**	**N**	**%**
Tanga	Assila	1	16.7	0	0	0.5	8.4
	Tengeru 97	2	33.3	3	30	2.5	31.7
	RioGrande	0	0	2	20	1	10.0
	'Tanya'	1	16.7	3	30	2	23.4
	Roma vfn	0	0	1	10	0.5	5.0
	Onyx	0	0	1	10	0.5	5.0
	Anna	2	33.3	0	0	1	16.7
	Total	6	100	10	100	8	100
Arusha	Tengeru 97	2	100	8	80	5	90
	'Tanya'	0	0	2	20	1	10
	Total	2	100	10	100	6	100

n= number of farms in a region [b] Total number of farms in a year
$P<0.0001$ (Between regions) $P=0.010$ (Between years)

Table D. 7: Distribution of response on reasons for tomato variety preference among tomato farmers during 2017 and 2019 tomato farmers survey in the key tomato producing regions of Tanzania.

	2017						2019					
Reasons for Preference	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**
Yield	22.9	28.3	32.5	18.5	50.0	30.4	20.8	33.3	33.3	41.7	40.0	33.8
Marketability	5.7	13.2	5.0	22.2	50.0	19.2	4.2	13.3	14.8	8.3	30.0	14.2
Shelf life	14.3	7.5	7.5	18.5	0.0	9.6	20.8	6.7	3.7	4.2	5.0	8.1
Color	17.1	20.8	22.5	11.1	0.0	14.3	20.8	20.0	18.5	20.8	5.0	17.0
Shape	17.1	20.8	22.5	11.1	0.0	14.3	20.8	20.0	18.5	25.0	10.0	18.9
Disease resistance	5.7	1.9	0.0	7.4	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0
Seed availability	5.7	3.8	0.0	11.1	0.0	4.2	8.3	3.3	11.1	0.0	10.0	6.5
No staking	2.9	0.0	0.0	0.0	0.0	0.6	4.2	0.0	0.0	0.0	0.0	0.8
Low postharvest losses	5.7	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0
Inexpensive seeds	0.0	1.9	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0
Fetches high price	2.9	0.0	0.0	0.0	0.0	0.6	0.0	3.3	0.0	0.0	0.0	0.7
Fruit Size	0.0	0.0	2.5	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Drought tolerant	0.0	1.9	2.5	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0
Clean seeds	0.0	0.0	2.5	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0
Resistant to bollworms	0.0	0.0	2.5	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	P=0.003 (Between regions)						*P*=0.162 (Between years)					

Table D. 8: Distribution of tomato farmers response on common crops that are intercropped with tomato during 2017 and 2019 tomato farmers survey in the key tomato producing regions of Tanzania.

	2017						2019					
Crop	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**
Maize	61.1	33.3	0.0	62.5	100.0	51.6	90.0	41.7	33.3	30.0	40.0	47.0
Banana	16.7	0.0	4.8	0.0	0.0	4.3	10.0	0.0	11.1	10.0	0.0	6.2
Beans	5.6	20.8	28.6	25.0	0.0	16.0	0.0	8.3	33.3	30.0	10.0	16.3
Pigeon peas	0.0	0.0	4.8	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0
Watermelon	0.0	8.3	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0
Sweet pepper	5.6	4.2	9.5	0.0	0.0	3.9	0.0	0.0	0.0	10.0	20.0	6.0
Cabbage	5.6	0.0	4.8	0.0	0.0	2.1	0.0	0.0	0.0	10.0	0.0	2.0
Butternut squash	5.6	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0
Sunflower	0.0	16.7	9.5	0.0	0.0	5.2	0.0	41.7	0.0	0.0	0.0	8.3
Onion	0.0	4.2	0.0	0.0	0.0	0.8	0.0	8.3	0.0	0.0	0.0	1.7
Chinese cabbage	0.0	4.2	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0
Carrot	0.0	0.0	4.8	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Pumpkin	0.0	8.3	23.8	12.5	0.0	8.9	0.0	0.0	0.0	0.0	0.0	0.0
Wheat	0.0	0.0	4.8	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Coffee	0.0	0.0	4.8	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Potatoes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.2	10.0	20.0	10.4
Eggplant	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	2.0
Total	100	100	100	100	100	100	100	100	100	100	100	100
	P=0.001 (Between regions)						*P*=0.202 (Between years)					

Table D. 9: Responses of farmers regarding limiting factors for tomato production during 2017 and 2019 tomato farmers' surveys in the key tomato producing regions of Tanzania.

Limiting factors to production	**2017**						**2019**					
	Morogoro	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**
Limited information	0.0	23.5	42.9	16.7	0.0	16.6	0.0	0.0	0.0	0.0	0.0	0.0
Weeds	15.4	0.0	7.1	0.0	0.0	4.5	20.0	8.3	0.0	10.0	0.0	7.7
Insect-pests	61.5	29.4	14.3	16.7	50.0	34.4	80.0	83.3	88.9	80.0	90.0	84.4
Diseases	0.0	5.9	0.0	33.3	50.0	17.8	0.0	0.0	0.0	0.0	0.0	0.0
Poor seeds	0.0	5.9	0.0	16.7	0.0	4.5	0.0	0.0	0.0	0.0	0.0	0.0
Poor weather	0.0	17.6	21.4	0.0	0.0	7.8	0.0	0.0	11.1	0.0	0.0	2.2
Poor market	23.1	5.9	7.1	0.0	0.0	7.2	0.0	0.0	0.0	0.0	10.0	2.0
Poor soils	0.0	11.8	0.0	16.7	0.0	5.7	0.0	0.0	0.0	0.0	0.0	0.0
Post-harvest losses	0.0	0.0	7.1	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0
High input price	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.3	0.0	0.0	0.0	1.7
Poor farm technology	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	2.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	P=0.086 (Between regions)						*P*=0.0001(Between years)					

Table D. 10: Distribution of tomato farmers' response identifying most damaging tomato pests during 2017 and 2019 tomato farmers' surveys in the key tomato producing regions of Tanzania.

	2017						2019					
Pest Name	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**
*Tuta absolut*a	30.8	33.3	35.1	50.0	28.6	35.6	32.0	44.4	36.4	37.0	45.5	39.1
Whiteflies	20.5	11.1	18.9	33.3	28.6	22.5	36.0	14.8	9.1	18.5	27.3	21.1
Leaf miners	7.7	4.4	0.0	0.0	0.0	2.4	4.0	0.0	0.0	0.0	0.0	0.8
Red/Spider mites	7.7	17.8	2.7	0.0	0.0	5.6	0.0	3.7	9.1	7.4	0.0	4.0
Bollworms	15.4	13.3	10.8	8.3	0.0	9.6	8.0	7.4	13.6	0.0	0.0	5.8
Aphids	12.8	15.6	32.4	8.3	14.3	16.7	8.0	25.9	31.8	37.0	27.3	26.0
Thrips	5.1	4.4	0.0	0.0	28.6	7.6	4.0	0.0	0.0	0.0	0.0	0.8
Leaf hopers	0.0	0.0	0.0	0.0	0.0	0.0	8.0	0.0	0.0	0.0	0.0	1.6
Fruit Files	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0	0.7
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

P=0.368 (Between regions) *P=0.501* (Between years)

Table D. 11: Responses of tomato farmers identifying the most important tomato diseases in the key tomato producing regions of Tanzania during 2017 and 2019 tomato farmers survey.

	2017						2019					
Disease Names	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**
Early blight	24.5	26.2	31.5	33.3	20.0	27.1	26.9	41.7	28.6	29.6	29.2	31.2
late blight	16.3	11.5	14.8	22.2	20.0	17.0	34.6	33.3	9.5	37.0	25.0	27.9
Bacterial wilt	6.1	4.9	0.0	0.0	0.0	2.2	23.1	20.8	38.1	11.1	29.2	24.5
Fusarium wilt	8.2	14.8	3.7	5.6	0.0	6.5	0.0	0.0	0.0	0.0	0.0	0.0
Rust	16.3	11.5	7.4	5.6	0.0	8.2	3.8	0.0	4.8	7.4	0.0	3.2
Bacterial canker	10.2	11.5	31.5	5.6	20.0	15.8	0.0	0.0	0.0	0.0	0.0	0.0
Nematodes	10.2	6.6	1.9	11.1	20.0	10.0	0.0	4.2	19.0	0.0	0.0	4.6
Yello leaf curl	2.0	6.6	7.4	0.0	20.0	5.8	3.8	0.0	0.0	0.0	0.0	0.8
Flower abortion	6.1	6.6	1.9	16.7	0.0	7.2	3.8	0.0	0.0	0.0	0.0	0.8
Softrot	0.0	0.0	0.0	0.0	0.0	0.0	3.8	0.0	0.0	0.0	0.0	0.8
Viral disease	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.1	12.5	4.7
Bacteria spots	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.7	4.2	1.6
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	P=0.015 (Between regions)						*P*=0.0001 (Between years)					

Table D. 12: Responses of tomato farmers identifying the most important weeds on tomato farms in the key tomato producing regions of Tanzania during 2017 and 2019 tomato farmers surveys.

	2017						2019					
Weed names	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**
Grasses	13.0	33.3	33.3	0.0	50.0	25.9	13.3	22.2	14.3	10.0	14.3	14.8
Commellina spp.	17.4	0.0	0.0	0.0	0.0	3.5	0.0	0.0	0.0	0.0	0.0	0.0
Tarvine	8.7	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0
Late weed	13.0	0.0	0.0	0.0	0.0	2.6	46.7	0.0	14.3	30.0	0.0	18.2
Nut grass	17.4	16.7	50.0	33.3	0.0	23.5	0.0	11.1	14.3	0.0	0.0	5.1
Spear grass	8.7	22.2	0.0	0.0	0.0	6.2	13.3	33.3	14.3	30.0	0.0	18.2
Wandering jew	8.7	0.0	0.0	0.0	0.0	1.7	6.7	0.0	0.0	0.0	0.0	1.3
Mexican poppy	4.3	5.6	0.0	0.0	50.0	11.9	13.3	11.1	0.0	0.0	42.9	13.5
African spider flowers	0.0	5.6	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0
Dandelion	8.7	16.7	16.7	33.3	0.0	15.1	6.7	11.1	14.3	10.0	14.3	11.3
Oxallis	0.0	0.0	0.0	33.3	0.0	6.7	0.0	0.0	14.3	10.0	0.0	4.9
American poppy	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.1	0.0	0.0	0.0	2.2
Jew mallow	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.3	0.0	0.0	2.9
Night shade	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	2.0
Carrot weed	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	28.6	5.7
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	P=0.0001 (Between regions)						*P*=0.001 (Between years)					

Table D. 13: Distribution of farmers responses citing tomato seed source during 2017 and 2017 tomato farmers' surveys in the key tomato producing regions of Tanzania.

Region	Source of seeds	2017		2019		Total	
		n	Response (%)	N	Response (%)	N	Response %
Morogoro	Mixed source	3	23.1	4	40	7	31.6
	Farmer saved	1	23.1	1	10	2	8.9
	Neighbor	2	15.4	2	20	4	17.7
	Agrostore	3	23.1	1	10	4	16.6
	Local market	2	15.4	2	20	4	17.7
	Given by company	1	7.7	0	0	1	3.9
	Given by scientist	1	7.7	0	0	1	3.9
	Total	13	100	10	100	23	100
Iringa	Mixed	4	23.5	4	36.4	8	30.0
	Farmer saved	1	17.6	2	18.2	3	12.1
	Neighbor	4	23.5	2	18.2	6	20.9
	Agrostore	4	23.5	2	18.2	6	20.9
	Local market	4	23.5	1	9.1	5	16.3
	Total	17	100	11	100	28	100
Mbeya	Mixed	3	21.4	3	30	6	25.7
	Farmer saved	2	14.3	2	20	4	17.2
	Neighbor	3	21.4	1	10	4	15.7
	Agrostore	3	21.4	1	10	4	15.7
	Local market	3	21.4	1	10	4	15.7
	Given by company	0	0	2	20	2	10.0
	Total	14	100	10	100	24	100

Continue…

Table D.13: Continued

Region	Source of Seeds	2017		2019		Total	Mean
		n	Response (%)	n	Response (%)	N	Response %
Tanga	Mixed	2	33.3	3	30	5	31.7
	Farmer saved	1	16.2	2	20	2	18.4
	Neighbor	1	16.7	2	20	3	18.4
	Agrostore	1	16.7	2	20	3	18.4
	Local market	1	16.7	1	10	2	13.4
	Given by company	0	0	1	10	1	0.0
	Total	6	100	10	100	16	100
Arusha	Mixed source	0	0	5	50	5	25.0
	Farmer saved	0	0	1	10	1	5.0
	Neighbor	0	0	2	20	2	10.0
	Agrostore	2	100	0	0	2	50.0
	Local market	0	0	2	20	2	10.0.
	Total	2	100	10	100	12	100
Means	Mixed source	2.4	23.1	3.8	36.5	6.2	29.9
	Farmer saved	1	9.6	1.6	15.4	2.4	12.5
	Neighbor	2	19.2	1.8	17.3	3.8	18.3
	Agrostore	2.6	25	1.2	11.5	3.8	18.3
	Local market	2	19.2	1.4	13.5	3.4	16.4
	Given by company	0.2	1.9	0.6	5.8	0.8	3.9
	Given by scientist	0.2	1.9	0	0	0.2	1.0
	Total	10.4	100	10.4	100	20.6	100

P=0.011 (Between regions) *P*=0.0001 (Between years). [a] Number of farms in a region [b] Total number of farms in a year

Table D. 14: Distribution of farmers responses citing water sources for tomato farms irrigation during 2017 and 2019 tomato farmers surveys in the key tomato producing regions of Tanzania.

	2017						2019					
	Morogoro	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**	**Morogoro**	**Iringa**	**Mbeya**	**Tanga**	**Arusha**	**Mean**
Borehole/pond	7.7	0.0	7.1	0.0	0.0	3.0	30.0	0.0	11.1	0.0	0.0	8.2
Wells	7.7	11.8	0.0	0.0	0.0	3.9	0.0	8.3	44.4	0.0	0.0	10.5
Rainfed	7.7	29.4	42.9	0.0	0.0	16.0	0.0	75.0	0.0	50.0	10.0	27.0
River	76.9	58.8	50.0	50.0	100.0	67.1	50.0	16.7	44.4	50.0	90.0	50.2
Stream	0.0	0.0	0.0	16.7	0.0	3.3	20.0	0.0	0.0	0.0	0.0	4.0
Tap water	0.0	0.0	0.0	33.3	0.0	6.7	0.0	0.0	0.0	0.0	0.0	0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	P=0.001 Between regions)						*P*=0.456 (Between years)					

Table D. 15: Distribution of responses on irrigation methods used by tomato farmers during 2017 and 2019 tomato farmers' surveys in the key tomato producing regions of Tanzania.

Region	Irrigation Method	2017		2019		Mean
		n	%	n	%	%
Morogoro	Watering can	8	61.5	10	100	80.8
	Furrow irrigation	2	15.4	0	0	7.7
	Rainfed	1	7.7	0	0	3.9
	Pump and laid pipes	1	7.7	0	0	3.9
	Drip irrigation	1	7.7	0	0	3.9
	Total	13	100	10	100	100
Iringa	Watering can	6	35.3	3	30	31.3
	Furrow irrigation	5	29.4	2	20	23.8
	Rainfed	5	29.4	5	50	37.5
	Pump and laid pipes	0	0	1	0	4.6
	Drip irrigation	1	5.9	0	0	3.0
	Total	17	100	11	33	100
Mbeya	Watering can	3	21.4	8	80	50.7
	Furrow irrigation	3	21.4	1	10	15.7
	Rainfed	8	57.1	1	10	33.6
	Pump and laid pipes	0	0	0	0	0
	Drip irrigation	0	0	0	0	0
	Total	14	100	10	100	100

Continue…

Table D.15: Continued

Region	Irrigation Method	2017		2019		Mean
		n	%	n	%	%
Tanga	Watering can	1	16.7	4	40	28.4
	Furrow irrigation	2	33.3	0	0	16.7
	Rainfed	0	0	6	60	30.0
	Pump and laid pipes	1	16.7	0	0	8.4
	Drip irrigation	2	33.3	0	0	16.7
	Total	6	100	10	100	100
Arusha	Watering can	0	0	8	80	40
	Furrow irrigation	2	100	0	0	50
	Rainfed	0	0	0	0	0
	Pump and laid pipes	0	0	2	20	10
	Drip irrigation	0	0	0	0	0
	Total	2	100	10	100	100

Table D. 16: Distribution of responses regarding tomato nursery management tactics during 2017 and 2019 tomato farmers' surveys in the key tomato producing regions of Tanzania.

		Nursery management tactic														
		Raised bed					Soil sterilization					Mulching				
Region	Response	2017		2019			2017		2019			2017		2019		
		n	%	n	%	Mean	n	%	n	%	Mean	n	%	n	%	Mean
Morogoro	No	2	15.4	0	0	7.7	7	53.8	5	50	51.9	2	15.4	0	0	7.7
	Yes	11	84.6	10	100	92.3	6	46.2	5	50	48.2	11	84.6	10	100	92.3
	Total	13	100	10	100	100	13	100	10	100	100	13	100	10	100	100
Iringa	No	3	17.6	1	9.1	13.5	4	23.5	3	27.3	25.4	3	17.6	1	9.1	13.4
	Yes	14	82.4	10	90.9	86.7	13	76.5	8	72.7	74.6	14	82.4	10	90.9	86.7
	Total	17	100	11	100	100	17	100	11	100	100	17	100	11	100	100
Mbeya	No	5	35.7	1	10	22.9	12	85.7	3	30	57.9	5	35.7	1	10	22.9
	Yes	9	64.3	9	90	77.2	2	14.3	7	70	42.1	9	64.3	9	90	77.1
	Total	14	100	10	100	100	14	100	10	100	100	14	100	10	100	100
Tanga	No	1	16.7	3	30	23.4	2	33.3	9	90	61.7	1	16.7	3	30	23.4
	Yes	5	83.3	7	70	76.7	4	66.7	1	10	38.3	5	83.3	7	70	76.6
	Total	6	100	10	100	100	6	100	10	100	100	6	100	10	100	100
Arusha	No	1	50	0	0	25	1	50	5	50	50	1	50	0	0	25
	Yes	1	50	10	100	75	1	50	5	50	50	1	50	10	100	75
	Total	2	100	10	100	100	2	100	10	100	100	2	100	10	100	100
Grand total	No	12	23.1	6	11.7	17.4	26	50	25	49	49.5	12	23.1	5	9.8	16.5
	Yes	40	76.9	45	88.2	82.6	26	50	26	51	50.5	40	76.9	46	90.2	83.5
	Total	52	100	51	100	100	52	100	51	100	100	52	100	51	100	100

P=0.443 (Between regions) *P*=0.037 (Between regions) *P*=0.511 (Between regions)

P=0.131 (Between years) *P*=0.921 (Between years) *P*=0.343 (Between years)

n= number of responses in a region

Table D. 17: Distribution of responses regarding possible causes of bacterial wilt during 2017 and 2019 tomato farmers' surveys in the key tomato producing regions of Tanzania.

Region	Possible causes of bacterial wilt	2017		2019		Mean
		n	%	n	%	%
Morogoro	Bacterial	1	7.7	4	40	23.9
	Weather	4	30.7	3	30	30.4
	Insect pests	2	15.4	2	20	17.7
	Do not know	6	46.2	1	10	28.1
	Total	13	100	10	100	100
Iringa	Insect pests	3	17.6	3	27.2	22.4
	Excessive rain	1	5.9	1	9.1	7.5
	Poor seeds	2	11.8	0	0	5.9
	Infected seeds	0	0	1	9.1	4
	Soil Borne	1	5.9	0	0	3.0
	Poor management	9	69.2	1	9.1	31.0
	Do not know	1	5.9	5	45.5	25.7
	Total	17	100	11	100	100
Mbeya	Poor seeds	0	0	2	20	10.0
	Soil Borne	1	7.1	0	0	3.6
	Do not know	13	92.9	8	80	86.5
	Total	14	100	10	100	100
Tanga	Bacterial	1	16.7	1	10	13.4
	Soil Borne	1	16.7	0	0	8.4
	Do not known	4	66.7	9	90	78.4
	Total	6	100	10	100	100
Arusha	Bacterial	1	50	1	10	30
	Do not know	1	50	9	90	70
	Total	2	100	10	100	100

P=0.002 between regions *P*=0.180 between years

File 5. 1: Survey Instrument

Questionnaire No. **:** ……

TANZANIA SURVEY FOR BACTERIAL WILT DISEASE

Questionnaire for weather, soil characteristics, presence of other pests (diseases, insects), pesticides and fertilization, cultivar, nursery preparation and planting methods

Date .....

Region…….

District……

Ward……

Village……

GPS coordinates ……

Temperature……. Soil type…….....

PART 1: PERSONAL DATA AND LAND USAGE INFORMATION

1. Farm No/ ID.....
2. Sex: 1. M ……. 2. F ……...
3. Total cultivated area …….....
4. Total area used for tomato production ……....

5. Other crops cultivated in the area…….

PART2: INFORMATION ON TOMATO PRODUCTION

6. No of years involved in growing tomato…… (yrs)

7. Name the varieties you are growing

   1. ……

   2. …….

   3. ……

   …

   4. ……...

9. Among these what is the most preferred, Why? (Tick the answer given)?

   A. Color, shape …….

   B. Shelf life ……

   C. Yield …….

   D. Market ……

   E. Availability ……...

10. Have you ever used these varieties below, if not why?

    Assila F1, Anna F1, Onyx

    A. Not aware of it

B. It is not available

C. Too expensive.

D. Does not grow well on my farm

E. Other reasons: State the reason(s)

……

11. Seed/ seedlings source of for the preferred variety (Tick)?

A. Farmers saved …….

B. Borrowed from neighbor…… .

C. Purchased from neighbor, market or store……

D. Given by extension, company, scientists……...

E. Other (please specify) ……

12. What is the source of irrigating water for your farm?.....

(Source of Water: borehole, well, river/stream, pond, lake, collected rainwater, etc.)

13. How do you prepare your nursery?

Media: Type……

Sterilization Yes ……. No……. how? ……..

Irrigation methods: Overhead…….

Other……

14. Tomato yield for last growing season(s)……

15. Over the past 3 years, has your tomato production:

A. Increased …….

B. Decreased ……....

C. Stayed the same …….

PART 3: KNOWLEDGE OF PESTS, WILT DISEASES AND IPM PRACTICES

16. What limits your tomato production?

☐Weeds ☐Insect pests

☐Disease/pathogens

☐Infertile/poor Soils ☐ Poorly producing Varieties ☐poor seeds

☐Drought/weather ☐Postharvest Loss ☐Market Access

☐Other,

Specify……

…….

17. Mention the names of the most important diseases/pathogen, insects/pests, weeds that damage your tomatoes

1. Insects/pests

a……...

b…….

c……

2. Disease/pathogen

a…….

b…….

c……

3. Weeds

a…….

b…….

c……

18. Do you know bacterial wilt disease? What causes

it?.....

19. How do you manage this disease?

a. Spraying insecticide…….

b. Fungicide……

c. Any other method…….

20. Did you use pesticides to control this disease last season? Yes……. No…….

21. Did it control the disease? ……. What was the status? Why?

……. increased……. stayed the same; ……. Decreased

Reasons

a: Fake

b: Don't know the dosage

c: Not meant for this disease

d. Any other reason…….

22: What is the basis of your decision on when to use pesticides for this disease?

A. I use pesticides at regular intervals throughout the season……

B. I use pesticides when we see disease in the field …….

C. I use pesticides after field sampling and finding a certain number

of diseased plants ……

D. When I see my neighbors are spraying……

Other…….

23. How many times did you spray your tomato for this disease?

_____ 0 didn't spray

_____ 1, sprayed once

_____ 2, sprayed twice

_____ 3, sprayed three times

_____ X number of times (write-in number)

24. Please list the name of the pesticides you used to control this disease:

i. _____

ii. _____

iii. _____

iv. _____

25. Are you aware of other ways to control pests/diseases besides using pesticides?

Yes ____ No ____

If yes, please give examples (name as many as possible):

i. ----------

ii. ----------

iii. ----------

iv. ----------