SCREENING AFRICAN EGGPLANT *(SOLANUM SP.)* ACCESSIONS FOR RESISTANCE TO BACTERIAL WILT (*RALSTONIA SOLANACEARUM)*

TEFANIA NIKUZE

MASTER OF SCIENCE

(Plant Health Science and Management)

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

2024

Screening African Eggplant *(Solanum Sp.)* Accessions for Resistance to Bacterial Wilt (*Ralstonia Solanacearum*)

Tefania Nikuze

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Plant Health Science and Management of the Jomo Kenyatta University of Agriculture and Technology

2024

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Signature…… Date……

Tefania Nikuze

This thesis has been submitted for examination with our approval as University Supervisors

Signature…… Date……

Prof. Elijah M. Ateka, PhD

JKUAT, Kenya

Signature…… Date……

Prof. Willis O. Owino, PhD

JKUAT, Kenya

Signature…… Date……

Prof. Jane Ambuko, PhD

UoN, Kenya

DEDICATION

I dedicate this work to my beloved parents, siblings, loving husband Christopher Kimeli Magut, and my children; Gladwel Chepchirchir, Brigid Cheptoo, Newton Kipkirui, Emmanuel Cheruiyot and Abedbarak Kipkemboi for their immense support, encouragement both morally, financially and spiritually during the time of my studies.

ACKNOWLEDGEMENT

First and foremost, I wish to thank the Almighty God for His care and good health during my studies. I would like to thank Jomo Kenyatta University of Agriculture and Technology (JKUAT) for this opportunity to undertake my degree studies. I am proud to be one of the alumni of the JKUAT community.

My sincere gratitude goes to my supervisors Prof. Elijah M. Ateka, Prof. Willis Owino, and Prof. Jane Ambuko for their guidance and support during this work. Am also greatly indebted to the technicians in the Department of Horticulture and Food Security who worked hand in hand with me. This journey wouldn't have been successful without you.

To Patrick Kavagi, many thanks, and may you have strength in the Lord and live to count your blessings. To my colleagues Brenda Muga and Samuel Kiarie Mwaura, I salute you. To my classmates, it was fulfilling walking with you throughout my studies, and may God bless you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABBREVIATION AND ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
AUDPC	Area under Disease Progress Curve
BCAs	Biological Control Agents
BW	Bacterial Wilt
CFU	Colony Forming Unit
CPG	Casamino Acid-peptone Glucose
CRBD	Randomized Complete Block Design
CRD	Completely Randomized Design
DAI	Days after Inoculation
DAP	Di-Ammonium Phosphate
DNA	Deoxyribonucleic Acid
DAT	Days after Transplanting
DI	Disease Incidence
FAO	Food and Agriculture Organization
MAS	Marker Assisted Selection
MT	Metric Tons
PCR	Polymerase Chain Reaction

PDI	Percentage Disease Incidence
QTL	Quantitative Trait Loci
R	Resistant
RPM	Revolution per Minute
RS	*Ralstonia solanacearum*
S	Susceptible
SSR	Simple Sequence Repeat
TZC	Triphenyl Tetrazolium Chloride
WAI	Weeks after Inoculation

ABSTRACT

African eggplant (*Solanum aethiopicum, Solanum anguivi, and Solanum sp*) in the Solanaceae family is an economically important crop produced in sub-Saharan Africa. It is an important vegetable used as a source of food and medicine. Eggplant is known for its genetic diversity in terms of agronomic traits and resistance to various diseases. Despite all these qualities, little has been done in Kenya especially its genetic resistance for the control of major devastating diseases (such as bacterial wilt) of solanaceous crops. African eggplant production like other solanaceous plants is limited by various yield and quality-reducing factors which include diseases, insect pests, and climatic conditions. Eggplant is susceptible to numerous soil-borne diseases; fusarium wilt, verticilium wilt, anthracnose fruit rot, and mainly bacterial wilt causing major economic losses. Control of the bacterium using chemicals, cultural methods, and biological control shows limited success due to its genetic nature, host range, and geographic distribution. However, the use of resistant plants has shown a considerable level of success in the management of the bacteria. Therefore, identifying resistant eggplant genotypes is an alternative for bacterial wilt management. The objectives of the present study were; (i) To determine the phenotypic reaction of African eggplant accessions to bacterial wilt infection and (ii) To identify Molecular markers for bacterial wilt resistance in African eggplant accessions. About 47 African eggplant accessions were collected (*Solanum aethiopicum, Solanum aguivi, and Solanum sp.)* from the African Vegetable Research and Development Center- Regional Center for Africa (AVRDC- RCA) in Arusha Tanzania. The accessions were prepared and separately sown in seed trays and at 4 true leaves were transplanted into a field whose BW history was predetermined and others were established in pots filled with sterilized soil in a greenhouse where they were mechanically inoculated. Symptom development was monitored and rated on a 1-5 scale for each accession and data was recorded weekly. Later 15 SSR markers were used to study resistance on eggplant accessions. Results showed that 13 accessions; RV100386, RV100234, RV100201, RV100245, RV100331, RV100250, RV100447, RV100161, RV100247, RV100240, RV100271, RV100458, and RV100342 were highly susceptible recording disease severity of 2.4 to 3.4. Also, resistant accessions, RV100264, RV100332, RV100265, RV100445, RV100453, RV100239, RV100438, RV100246, RV100242, and RV100455 showed disease severity of 1.1 to 1.2 during the study. Eggplant accessions were amplified by 5 markers (ecm009, emk03O04, SOL5036, emiO4P17, and ecm001). At least resistance markers were present in all the accessions tested. Accession, RV100455, Rv100242, RV100246, RV100438, RV100445, RV100453, RV100360 and RV100352 had resistance markers for bacterial wilt. Inconsistent reactions on the eggplant were observed whereby none of the accessions were immune, and even the symptomatic accessions carried resistant markers. In conclusion, this study showed that some eggplant accessions carried bacterial wilt resistance markers and can be utilized in breeding for BW-resistant varieties. The identified resistant genotypes can be adopted to manage BW to increase eggplant production.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

1.1.1 Eggplant (*Solanum spp*.)

Eggplant *(solanum spp*.) belongs to the Solanaceae family. The Solanaceae family comprises a large number of species distributed in over 50 genera (Taher et al., 2017). They include: potato, tomato, eggplant, pepper, petunia, nicotiana, plant with edible leaves (*Solanum aethiopicum, S. macrocarpon*), medicinal plant like Datura, pepper) (Taher et al., 2017). Solanaceae is the third most important taxonomic group and also the most valuable vegetable for revenue used as a source of food, medicinal value, and revenue-earning (Marques et al., 2012). Nutritionally, eggplant is very low in caloric value and is considered among the healthiest vegetables for its high content of vitamins, minerals, and bioactive compounds for human health (Taher et al., 2017). It is most diverse solanaceous crop in terms of agricultural utility and genetic resources thus used in breeding programs (Plazas et al., 2014; Taher et al., 2017).

Eggplant is native to both the New World and Old World. and originated from Africa. Eurasia and Australia (Knapp et al., 2013). It is grouped into aubergine (*Solanum melongena*) originating from Asia and African eggplant (*S. anguivi, S. aethiopicun, S. insanum, S. macrocarpon)* with the origin in Africa (Taher et al., 2017). Two major categories known for cultivation are from Old-world countries including the Gboma eggplant, *S. macrocarpon* L., and the blackberry eggplant *S. aethiopicum* L. (Knapp et al., 2013; Taher et al., 2017). The *S. aethiopicum* L. commonly known as African eggplant is referred to by several names Mock tomato, bitter tomato, Ethiopian eggplant, and scarlet eggplant (Bacchi et al., 2010).

African eggplant is one of many indigenous vegetables that play an important role in both subsistence production and income generation in rural and urban resource-poor communities in Africa (Hayat Bhatti et al., 2013; Manda et al., 2020). It Itis thought

to have originated in West Africa before spreading to the rest of the world's tropical regions and is mainly grown in Africa, while *Solanum melongena* L. is grown worldwide (Knapp et al., 2013; Taher et al., 2017).

Eggplant is commonly produced in warm tropics and sub-tropics of the Far East, in countries such as India, Bangladesh, Pakistan, China, and the Philippines. Based on FAOSTAT, (2020) statistics, the global eggplant production was estimated at over 50 million MT/year with a net value of more than US$12 billion. The world eggplant production varied between years (FOASTAT, 2020). Although eggplant is considered a tropical crop, only three countries produce more than 1 million MT/year tons annually (Table 1.1), while China (32.4 million tones, world share 27%), Egypt (1.3 Mt), Turkey (720,000 tons), Iran 670,000 tons) (Table 1.1) are ranked the leading world eggplant producers Despite eggplant having its roots in Africa, it accounts for 3.8% of the global production (Table 1.1) (FAOSTAT, 2020). The main African-producing countries are Egypt, Algeria, Morocco, Niger, Libya and Mali. The production has been increasing over the years (Source FAOSTAT 2020). According to (FOASTAT, 2020), China produces over half of the world's production, while Egypt is the leading producing country in Africa. According to FAOSTAT (2020), Rwanda is a major eggplant producer in Eastern Africa. In 2015 and 2016, there was a drop in eggplant production which later picked in the following years (FOASTAT, 2020).

Table 1.1: Annual Eggplant Production (In Million MT Tons) of the Top 10 Countries Worldwide

Country	Global production	2014	2015	Year 2016	2017	2018	2019
China	64.4	29.6	31.6	32.2	33.3	34.5	35.6
India	23.0	13.6	12.6	12.5	12.5	12.8	12.7
Egypt	2.1	1.3	1.2	1.3	1.4	1.2	1.2
Turkey	1.5	0.8	0.8	0.9	0.9	0.8	0.8
Iran	1.2	0.5	0.6	0.7	0.7	0.7	0. 7
Indonesia	1.0	0.6	0.5	0.5	0.5	0.6	0.6
Japan	0.6	0.3	0.3	0.3	0.3	0.3	0.3
Italy	0.6	0.3	0.3	0.3	0.	0.3	0.3
Philippines	0.5	0.2	0.2	0.	0.3	0.2	0.3

(Source: FAOSTATS, 2020).

Table 1.2: Kenya's Annual Eggplant Production and Value.

Year	Area (Ha)	Tons	Value (M Ksh.)
2017	689	7630	240
2018	996	13381	329
2019	996	11381	329
2020	1586	15286	523

(Source: Agriculture and Food Authority, (2022) – Horticulture Crop Directorate. Kenya's eggplant production from 2017 – 2020.

Although eggplant is not commonly produced in Africa, an annual average of 1.2million MT were harvested from 2014 to 2018 (FOASTAT, 2020). Kenya seems to produce an insignificant amount of eggplant (Aubergins) thus no available statistics (FOASTAT, 2020) but according to Agriculture and Food Authority-Horticulture Crop Directorate (HCD) (table 1.2), a total of 47,678 tons were harvested between 2017 and 2020.

Over 40% of global crop losses are attributed to direct or indirect effects of diseases and possibly short-term or long-term nature. African eggplant yields are well below harvest potential, especially in smallholder production systems. This is due to a variety of yield-reducing factors, including both biotic (viruses, fungi, bacteria, weeds) and abiotic (soil, climatic factors, topography) (Taher et al., 2017; Wicker et al., 2009).

1.1.3 Bacterial Wilt

Eggplant is threatened by various soil-borne diseases such as Fusarium wilt (Phoebe et al., 2016), bacterial wilt (Lebeau et al., 2011, 2013; Mwaniki et al., 2016; Salgon et al., 2017) and Verticillium wilt (*Verticillium dahliae*) (Phoebe et al., 2016). Bacterial wilt (BW) caused by *Ralstonia solanacearum* is a global pathogen. It is one of the most economically important diseases of the solanaceae family causing

significant losses (Manda et al., 2020) in potato, tobacco, tomato, and eggplants (Álvarez et al., 2010).

The disease affects an extensive host range of hundreds of species, in 44 families and over 450 herbaceous and woody plant species (Hayward, 1991; Kumar et al., 2018; Manda et al., 2020). The most economically important crops affected include potato, tomato, eggplant, pepper, tobacco, and banana (Meng, 2013; Xue et al., 2011). (Meng, 2013; Xue et al., 2011). *R. sonacearum* the causal agent for bacterial wilt can colonize non-host plants including a variety of asymptomatic weeds because it can strive in soil for a long time without a host, thus reducing the effectiveness of production sites (Singh et al., 2019; Uwamahoro et al., 2018). Field hygiene and the use of infected seed have significantly increased bacterial wilt disease incidence (Ateka et al., 2001; Bacchi et al., 2010). All these attributes have made it difficult to be effectively controlled (Uwamahoro et al., 2018).

This destructive *R. solanacearum* enters the host through root wounds and latent root emergence points, colonizing xylem cells and causing blockage. The initial wilt symptoms are leaf drooping, followed by full-plant wilting and vascular discoloration. When cut ends of wilted plants are placed in water, milky white exudates can be observed (Singh et al., 2019). In general, irreversible wilting develops rapidly and causes plant death (Hayward, 1991; Mwaniki et al., 2016; Singh et al., 2019). Bacterial wilt dissemination is aggravated by contaminated water sources, farm implements, and latently infected propagation materials (Gildemacher et al., 2009; Mwaniki et al., 2016), and soil through human activities (Swanson et al., 2007).

Bacterial wilt has caused considerable losses in different parts of the world. Uganda, tomato production has been affected by the losses caused by bacterial wilt are 88% (Manda et al., 2020; Uwamahoro et al., 2018). In India, the potato industry losses due to BW were reported. The frequency of bacterial wilt in Ethiopia is practically 100% in pepper, 63% in potato, and 55% in tomato (Manda et al., 2020). Bacterial wilt has remained a threat in Kenya in solanaceous crops producing areas (Mwaniki et al., 2016). Manda et al., (2020) and Mwaniki et al. (2016) observed yield losses of

50% to 100% in potatoes, with prevalence ranging from 35% to 100%, with increasing altitude (Mwaniki et al., 2016).

1.2 Statement of the Problem

Ralstonia solanacearum causal agent of bacterial wilt, is a wide spread soil-borne pathogen present in all continents posing a threat in production of economically important crops (Hasabi, 2014; Mwaniki et al., 2016; Salgon et al., 2017; Tessema et al., 2020). Recently bacterial wilt was ranked the second in the list of the most destructive and economically important bacterial pathogen after blight and induces rapid and fatal symptoms in host plants (Salgon et al., 2017; Yuliar et al., 2015). Bacterial wilt has been reported as a production constraint of cash crops such as tobacco, as well as on major food crops such as potato, tomato and eggplant (Salgon et al., 2017). This disease occurs in wet tropics, sub-tropics and in some temperate regions in different parts of the world (Dheemanth et al., 2018).

Eggplant is susceptible to numerous soil-borne diseases like fusarium wilt (*Fusarium oxysporum* Schlecht. f. sp. melongenae), bacterial wilt (*R. solanacearum),* and verticillium wilt (*Verticillium dahliae*). Eggplant bacterial wilt is caused by race 1 (Singh et al., 2019). It is known to cause significant loss in yield and quality of eggplant. *R. solanacearum* can survive in the soil without a host plant (Singh et al., 2019) and is easily disseminated via latently infected plant tissues, irrigation water, and soil-contaminated equipment and thus infect the subsequent crops (Reddy et al., 2015). Once it is introduced into a cropping system, it is difficult to manage because the host resistance is limited (Uwamahoro et al., 2018).

Direct yield losses by bacterial wilt vary widely according to the host, soil type, cropping systems and strain (Yuliar et al., 2015). Yield losses of up to 91% in tomato, 10% - 30% in tobacco, 33% - 90% in potato, 50% - 100% in banana and up to 20% in groundnuts were reported (Yuliar et al., 2015). Bacterial wilt was reported as a production concern of major crops in Australia causing up to 75% losses. Studies on effects (Bacchi et al., 2010) have been conducted across East Africa. They revealed out that, potato bacterial wilt posed a yield loss ranging from 0-100% (Manda et al., 2020; Mwaniki et al., 2016; Tessema et al., 2020; Uwamahoro et al.,

2018). In Ethiopia, potato bacterial wilt (PBW) was reported at 4% - 53% in all affected areas causing yield losses ranging from 4% - 32% (Manda et al., 2020; Tessema et al., 2020; Yuliar et al., 2015).

In Kenya alone, losses ranging from 50% - 100% were reported in potato production areas (Ateka et al., 2001; Muthoni et al., 2014). African eggplant is one of the most popular Solanaceous vegetable crops cultivated in sub-Saharan Africa. Although farmers have selected many diverse cultivars of African eggplant and germplasm collections, little breeding has been undertaken to explore important characteristics such as resistance to various eggplant diseases, especially bacterial wilt (Osei et al., 2010; Taher et al., 2017).

1.3 Justification

The pathogen (*R. solanacearum*) can survive in soil from season to season for long periods without any host and thus colonizing non-host plants including a vast range of weeds (Singh et al., 2019). This has rendered production sites unsuitable for host plants. Bacterial wilt caused by *R. solanacearum* infection leads to irreversible wilts and eventual death in host plants (Hayward, 1991). Although some plants look healthy, they are feared to carry latent infections. Poor field hygiene and the use of infected seeds have significantly increased disease incidences (Ateka et al., 2001; Bacchi et al., 2010). These features make the disease difficult to control and no single control method was found to be effective (Uwamahoro et al., 2018).

Although several studies have shown bacterial wilt as a serious disease in eggplants, little information is available on eggplant resistance to the bacterium. The high nutritive value of the leaves and the high leaf and fruit yield, as well as the fairly high resistance to pests and diseases, make the crop interesting for development but with little done (Osei et al., 2010). So many characteristics are exhibited by these eggplants, including the level of sweetness, colour, and disease resistance, earliness (Bacchi et al., 2010; Osei et al., 2010). Famers continually select eggplants based on their preferences like large fruits, color, and taste, early maturing as well as high yields (Osei et al., 2010).

Characterization of eggplant based on phenotypic and molecular bacterial wilt resistance has had limited studies. The best way to economically control bacterial wilt is to develop cultivars resistant to the soil-borne pathogen. The management of BW with physical, chemical, biological, and cultural methods has been investigated for decades with little success (Yuliar et al., 2015) but they are not 100% effective method (Manda et al., 2020; Uwamahoro et al., 2018). Identifying resistant cultivars to bacterial wilt is key to its management (Dheemanth et al., 2018).

Progress has been made in mapping and tagging many agriculturally important genes with molecular markers which forms the foundation for marker-assisted selection (MAS) in crop improvement (Chakravarty & Kalita, 2011). Sources of resistance genes have been previously identified in some species such as *S. torvum* and *S. aethipicum* (Lebeau et al., 2013). Although resistant genes exist in these species, little effort has been put up to identify them (Lebeau et al., 2013; Uwamahoro et al., 2018; Xi'ou et al., 2015). . The most successful strategy is to breed resistant cultivars specific to certain locations and strains (Cao et al., 2009; Sanchez Perez et al., 2008). The molecular marker technology has been used to assist breeding of many crops, except eggplant breeding for important traits like disease resistance in eggplant is still unclear (Salgon et al., 2017).

1.4 Objectives

1.4.1 Main Objective

To screen African eggplant accessions for resistance to bacterial wilt (*Ralstonia solanacearum*).

1.4.2 Specific Objectives

1. To determine the phenotypic reaction of African eggplant accessions to *R. solacearum* infection
2. To identify molecular markers for bacterial wilt resistance in African eggplant accessions

1.5 Hypothesis

1. There are no significant differences in reaction to bacterial wilt infection among African eggplant accessions

2. There are no molecular markers linked to bacterial wilt resistance in African eggplant accessions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Distribution of African Eggplant

Solanum sp is the largest known and commercially cultivated genus of plants, including economically important species such as potato, tomato, tobacco, eggplant, and pepper (Caguiat & Hautea, 2014). There are three main cultivated eggplant species. *S. aethiopicum* and *S. macrocarpon* mainly grown in Africa, whereas the better-known *S. melongena* is grown worldwide. *Solanum melongena* eggplant widely distributed in southern Europe, the Middle East, and Asia, is native to Southeast Asia (Knapp et al., 2013). China is considered to be the center of diversity for *S. melongena* (Taher et al., 2017) while African eggplant has its origin in Africa (Caguiat & Hautea, 2014; Knapp et al., 2013).

Based on hybridization and biosystematics data, wild eggplant relatives are generally recognized under two widespread species, *S. incanum* and *S. torvum*. A recent taxonomic revision based on morphological and molecular data recognized 10 different species among these two broadly classified eggplant relatives (Caguiat & Hautea, 2014). African eggplant *S. aethiopicum* and *S. Macrocarpon* is a traditional native vegetable of western and central Africa with relatively low production (Sękara et al., 2007). In West Africa, the scarlet eggplant, African eggplant, also known as garden eggplant (*Solanum ethiopicum* L.), is one of the most popular edible non-nodular cultivated eggplant species. African eggplants are mainly grown in gardens and small fields near villages (Sękara et al., 2007). Eggplant is grown as a leafy and fruit-bearing vegetable. In addition, red eggplant may be used for genetic improvement of eggplant (Aslam et al., 2019).

2.2 Eggplant Production

Global eggplant production in 2019 was estimated at over 322 million tons (FOASTAT, 2020). In Africa, eggplant is majorly produced in West and Central African countries. African eggplant production increased from 300 tons in the 1970s

to 1.85 million tons in 2019, at an average increase rate of 3.97% per annum (FOASTAT, 2020). The top eggplant-producing countries in Africa include Egypt (1.4 MT), Algeria (0.18 MT), and Côte d'Ivoire (0.1 MT), while others produce less than 0.1 MT annually (FOASTAT, 2020). In 2019, the African eggplant area was 114,579 ha, which is an estimate of 161,759Hg/ha.

According to FAOSTAT (2020), Egypt's eggplant production was estimated at 1.4 MT, making it the third-highest eggplant producer in the world. Eggplant production is minimal in East Africa, with Rwanda being the largest producer with an estimated 27,978 tons/year (FOASTAT, 2020). Kenyan eggplant production is carried out on a small scale and is mostly for export markets, estimated at $1,500 by FAOSTAT (2020). However, due to the lack of statistical records, it is not possible to accurately estimate the total production per unit area and yield of eggplant production in Kenya (FOASTAT, 2020).

2.2.1 Eggplant Production Constraints

Crop productivity and value have greatly been reduced by a wide range of biotic and abiotic constraints notwithstanding farmer's practices (Reynolds et al., 2015). These biotic factors include different micro-organisms; fungi, bacteria viruses, and insect-transmitted diseases. The abiotic factors are exacerbated by climatic factors and human activities (Ubisi et al., 2017). Climate change favors the introduction and spread of new and existing pests and diseases. Unpredictable weather with extreme temperatures, droughts, and/or floods affect yield and quality of eggplants (Taher et al., 2017; Ubisi et al., 2017) by influencing multiple microorganisms interactions.Direct yield losses due to pathogens, animals, and weeds account for approximately 20-40% of global agricultural productivity (Savary et al., 2012).

The most common diseases in eggplant include Bacterial wilt, Verticilum wilt, Fusarium wilt, Anthracnose fruit rot, Alternaria fruit rot, damping off, Phytophthora blight, Phomopsis blight and fruit rot, leaf spot, little leaf spot of brinjal and mosaic (Manda et al., 2020; Taher et al., 2017).The occurrence of bacterial wilt, is one of the most important limiting factors for the production of solanaceous plants especially eggplant in warm and humid climates (Manda et al., 2020). According to

Muradashvili (2015), bacterial wilt is estimated to cause significant economic losses worldwide of over US$1 billion annually.

However, yield losses varied from one crop species to the other (Karim et al., 2018; Mwaniki et al., 2016). On the other hand, Yulia (2015), cited direct yield loses associated with bacterial wilt vary widely according to the host, cultivar, climatic conditions, soil type, cropping pattern and bacterial strain. Bacterial wilt ultimately causes significant losses in crops such as tomatoes, eggplants, potatoes, tobacco, and bananas (Manda et al., 2020). Eggplants are also affected by numerous pests, including nematodes, whiteflies, aphids, eggplant nuts, stalk borers, and blister beetles (Taher et al., 2017).

2.3 Ralstonia solanacearum

2.3.1 Origin and Taxonomy

Ralstonia solanacaerum the causal agent of bacterial wilt is a complex species organism (Hayward, 1991; Lebeau et al., 2013; Paudel et al., 2020; Salgon et al., 2017). The causative agent of bacterial wilt *R. solanacearum* was first isolated and described from potatoes, tomatoes, and eggplants in the 1880s and classified into the genus - *Bacillus*. Subsequently, several scientific studies based on phenotypic, chemical behavior, and molecular studies were conducted and multiple taxonomic nomenclatures were identified (Castillo & Greenberg, 2007; Hayward, 1991; Lebeau et al., 2013; Marques et al., 2012; Paudel et al., 2020; Salgon et al., 2017; Xue et al., 2011).

According to Paudel (2020) and Hayward (1991), the wilt-causing pathogen *R. solanacearum*, in 1885 was placed into the genus *Bacillus.* This was followed by several classifications into other genera. 1898 - *Bacterium*, 1914 - *Pseudomonas*, 1923 - *Phytomonas*, 1939 - *Xanthomonas* and in 1948 was reclassified to *Pseudomonas* and in 1945 it was placed into its current genus *Ralstonia* (Salgon et al., 2017).

2.3.2 Aetiology

Raslstonia solanacearum bacterial wilt-causing agent strains exhibit a wide range of genetic diversity. The taxonomy of *R. solanacearum* has been difficult due to the complexity of the species. In the recent past, molecular tools have been used to better understand *R. solancearum* (Marques et al., 2012; Paudel et al., 2020; Salgon et al., 2017). These led to the classifying of *R. solanacearum* into three structured ways including; (i) phylotypes – related to the geographical origin of the strain, (ii) race – based on host type, and (iii) biovars by biochemical reactions (Manda et al., 2020; Muthoni et al., 2014; She et al., 2017).

R. solanacearum was distinctively divided into races which were loosely based on host range and organised in diverse genetic groups (Hayward, 1991; Lebeau et al., 2013; Mamphogoro et al., 2020; Paudel et al., 2020; Salgon et al., 2017). It was organized into five races. Race 1 strains primarily affect tobacco and many other solanaceous crops and hosts of other botanical families. Race 2 strains were limited to Musaceae species, including *Heliconia sp.* and triploid bananas. Race 3 strains are a major threat to potatoes. Race 4 strains are particularly virulent to ginger and Race 5 strains affect and cause disease in mulberry and are present in China (Hayward, 1991).

At phylotype classification, *R. solanacearum* was strongly associated with geographic distribution. Phylotype I is believed to have its origin mainly in Asia and Australia and it included biovars 3, 4, and 5. Phylotype II are strains from the Americas, while phylotype III is widely distributed in Africa and phylotype IV is from Indonesia (Hayward, 1991; Lebeau et al., 2013). Furthermore, phylotype II was divided into two subgroups. That is, IIA and IIB. Phylotypes IIA and B have been reported to cause bacterial wilt disease in potatoes in cold and temperate regions.

Due to their ability to utilize and oxidize various sugars alcohols and carbohydrates, *R. solanacearum* was classified into biovars, and five biovars were identified. Biovar 1 strains do not metabolize sugars and carbohydrates; biovar 2 strains were reported to metabolize disaccharides. Biovar 3 strains metabolize all the sugars and carbohydrates and biovar 4 strains metabolize only hexose alcohols while biovar 5

strains metabolize disaccharides and hexose alcohols except dulcitol and sorbitol (Castillo & Greenberg, 2007; Lebeau et al., 2011, 2013; Meng, 2013; Siljak-yakovlev & Paris-sud, 2001).

Ralstonia solanacearum is a highly heterogeneous bacterial pathogen that causes severe wilting on important crop plants (Álvarez et al., 2010; Meng, 2013). According to Álvarez et al. (2010), *R. solanacearum* is second to *Pseudomonas syringae* in economic importance followed by *Agrobacterium tumifaciens*. Due to its nature, *Ralstonia* can maintain rapid dissemination and adapt to different ecological niches such as soil, water, and plants for a long period (Lebeau et al., 2011).

2.3.3 Distribution and Spread

Bacterial wilt caused by *R. solanacearum* occurs in many parts of the world in tropical, subtropical, and temperate regions Hayward (1991) and Swanson et al., (2007). The spread has been aided by environmental factors that accelerate development, and dissemination of bacterial wilt (Gbonamou et al., 2021; Manda et al., 2020). , *R. solanacearum* can spread over considerable distances by vegetative propagative materials which undoubtedly contributes to the longer survival period forming a reliable source of the inoculum (Karim et al., 2018; Mwaniki et al., 2016)

Weeds and infested moist soil, contaminated water and agricultural equipment, wastes from the crop processing industries, and latently infected plants, e.g., potato tubers, and tomato seeds are all at high risk of harbouring and spreading *R. Solanacearum* (Champoiseau et al., 2009; Gbonamou et al., 2021; Manda et al., 2020; Salgon et al., 2017; Sanchez Perez et al., 2008). Crop residues infected with bacterial wilt, and infected fields, serve as a source of inoculum for the nearby fields. In addition, insects have been considered as vectors that normally spread *R. solanacearum* race 3. Therefore, its wide adaptation and long saprophytic persistence in nature make the control measures of the bacterial wilt more difficult (Aslam et al., 2017; Gbonamou et al., 2021).

2.3.4 Symptomatology in Eggplant

Plants infected with *R. solanacearum* may show typical BW symptoms a few days after infection which is characterized by sudden wilting and drooping of the leaves, followed by underground and eventual death of the plants (Gbonamou et al., 2021; Karim et al., 2018; Manda et al., 2020). During the early stages of the disease development, the initial symptoms are usually seen on the leaves of the plants (Karim et al., 2018; Manda et al., 2020). These symptoms are dominant during the hottest part of the day, showing wilting of the youngest leaves. At this stage, only a few leaves may wilt and at night, when the temperatures are low, the plants regain the turgidity (Muradashvili et al., 2015; Oliveira et al., 2014; Yuliar et al., 2015). Under severe weather conditions, irreversible wilts occur although wilted leaves remain green leading to the eventual death of the plant (Chaudhry & Rashid, 2011; Hayward, 1991).

Another common symptom of bacterial wilt in the field is stunting of crops which may appear at any stage of plant growth. In the young stems of solanaceous plants, vascular bundles are affected, showing visible streaks of long, narrow, dark brown appearance (Manda et al., 2020; Muradashvili et al., 2015; Yuliar et al., 2015).

The favourable temperatures for the expression of symptoms are between 29^{o}C – 35^{o}C, enabling the disease to progress immediately after the infection. In some instances plants that do not show bacterial wilt symptoms may remain unseen for a very long time, thus aiding in inoculum multiplication (Manda et al., 2020; Oliveira et al., 2014). This enables the pathogen to survive in the infected plant and can be spread from the infected plant to healthy plants (Hayward, 1991). At heavily infected plants, sticky, milky-white exudates of bacterial wilt are commonly observed on freshly cut sections of infected eggplants (Muradashvili et al., 2015; Singh et al., 2019). The disease can also be observed on cut stem sections placed under clear water. A viscous whitish spontaneous slime smoky ooze out of the cut end of the stem and the most common sign of bacterial wilt in heavily infected plants (Chaudhry & Rashid, 2011; Muradashvili et al., 2015; Oliveira et al., 2014).

2.3.5 Epidemiology and Disease Cycle

Bacterial wilt caused by *R. solanacearum* is a soil-borne as well as water-borne pathogen, that can survive and spread for a long period in infected or contaminated soil or water (Tessema et al., 2020). It is known to infect the hosts through damaged roots by nematodes and other factors. Cultural practices and damage by insects accelerate the spread and infection of bacterial wilt. The growth and development of the disease is most favored by high temperatures of 29˚C – 35˚C (Gbonamou et al., 2021; Manda et al., 2020; Tessema et al., 2020). Other factors such as soil type, soil structure, soil moisture content pH, and salinity may influence disease survival and development (Manda et al., 2020). .

The survival of *R. solanacearum* ranges from days to years in soils, disease-contaminated irrigation water, and infected weeds. These act as sources of inoculum and are disseminated from the infected to healthy fields by the transfer of soils through machinery and surface runoff. The bacterium can also propagate in infected water sources like ponds or rivers and further spread to non-infected sites after rainfall or using the infested water bodies as irrigation water (Chaudhry & Rashid, 2011; Gbonamou et al., 2021; Manda et al., 2020; Mwaniki et al., 2016; Tessema et al., 2020; Uwamahoro et al., 2018). Infected semi-aquatic weeds can also be considered a source for the spread of the pathogen where the bacteria get released from the roots into the irrigation water (Kumar et al., 2018).

The plant which is infected by bacterial wilt may sometimes not show any kind of symptoms related to the disease even under favorable conditions, (Ateka et al., 2001; Mwaniki et al., 2016; Swanson et al., 2007) but they can survive in their physiological latent state (Manda et al., 2020; Swanson et al., 2007). For instance, *R. solanacearum* race 3 biovar 2 was found to survive during winter in certain semi-aquatic weeds, plant debris, and rhizosphere of alternate and non-host plants, thus acting as reserves for the bacterial inoculum (Gbonamou et al., 2021; Manda et al., 2020). The bacterial wilt of potatoes caused by *R. Solanacearum* is tuber-borne and is disseminated by infected seed tubers or non-certified seed tubers (Gbonamou et al., 2021; Muthoni et al., 2014). Latently infected planting material becomes a source

of bacterial wilt and ensures transmission from place to place in many developing countries. The pathogen stagnates in diseased plant debris, and propagative organs such as tubers, rhizomes, suckers, or seeds of some crops (Swanson et al., 2007; Tessema et al., 2020).

2.3.6 Economic Importance

The extensive economic losses brought about by the pathogen are attributed to its wide host range and its expansive geological dispersal in some warm temperate and tropical regions of the world (Hayward, 1991). Among the plant diseases, soil-borne infections are considered to account for 10 – 20% of yield losses annually (Gbonamou et al., 2021; Manda et al., 2020; Mwaniki et al., 2016; Savary et al., 2012; Yuliar et al., 2015). These include *R. solanacearum* which causes significant yield losses due to pathogen strain, weather conditions, soil type, cropping practices, and the type of plant cultivar (Hayward, 1991; Mamphogoro et al., 2020). It is the most important soil-borne pathogen and is ranked as the second most dangerous among the 10 most fatal bacterial species influencing significant yield loss in solanceous plants (Mamphogoro et al., 2020; Manda et al., 2020).

The pathogen is responsible for serious yield losses in numerous economic plants which include food crops (Bananas, ginger, potato eggplant among many others), trees (Eucalyptus), and shrubs in the family of Zingieberaceae (Gbonamou et al., 2021; Kumar et al., 2018; Manda et al., 2020; Mwaniki et al., 2016). Considerable yield losses of roughly 75% in potatoes have been reported around the globe. The disease has been estimated to affect about 1.7 million hectares of potato and continually expanding due to intensive agricultural practices at the global level, with the damage estimated at over USD 950 million per annum from 2014 to 2018 (Karim et al., 2018; Kumar et al., 2018; Mamphogoro et al., 2020; Muthoni et al., 2014). The bacterium (*R. solanacearum)* has been reported to cause bacterial wilt in commercial Eucalyptus plantations (Kumar et al., 2018). This was first described in Brazil and later China, Taiwan, Australia Venezuela, and South Africa.

In west Georgia, in Chkhorotsku and Kutaisi regions, it was reported to cause up to 100% loss in greenhouse and field-grown tomatoes (Muradashvili et al., 2015). In

Taiwan's fresh tomato market, disease incidences of 15-55% were reported causing losses exceeding 12 million U.S dollars annually (Manda et al., 2020). Tahat and Sijam (2010), cited production of ginger in Hawaii suffered losses of up to 50% during 1998 and 1999 due to the bacterial wilt infection. Research has shown that losses of up to 88% of tomatoes and 70% of potatoes in Uganda and India respectively were recorded and other parts of the world at various severity (Gbonamou et al., 2021; Mamphogoro et al., 2020). In Ethiopia, a yield loss of 100% in pepper, 63% in potato, and 55% in tomato was accounted for due to Bacterial wilt (Champoiseau et al., 2009; Gbonamou et al., 2021; Manda et al., 2020; Muthoni et al., 2014; Mwaniki et al., 2016). In Kenya, bacterial wilt was reported to influence yield loss of potatoes 50% - 100%. On the other hand, losses of up to 40% in eggplant due to bacterial wilt were reported in the field in the state of Amazon (Muradashvili et al., 2015; Oliveira et al., 2014) Despite this, host resistance to *Ralstonia* remains poorly understood (Hayward, 1991).

2.3.7 Management

Diseases are a common occurrence in plants, often having a significant economic impact on yield and quality. Thus, managing diseases in cropping systems is an essential component of crop production (Pradhanang et al., 2000). Conducting an early detection in plant debris and soil or soil-related habitats is essential to the effective management of bacterial wilt in the field, reducing losses and further spread of the inoculums. Understanding the ecology of the pathogen forms the basis for successful disease management and control practices (Pradhanang et al., 2000).

Since *R. Solanacearum* is a soil-borne pathogen with a wide host range, outstanding survival in soil, and wide biological variation, its effective control has not been easy (Cao et al., 2009; Muthoni et al., 2014; Sanchez Perez et al., 2008). Various strategies were developed to control and suppress the disease including phyto sanitation and cultural practices, chemical control, biological control, and host resistance (Muthoni et al., 2014; Yuliar et al., 2015).

2.3.7.1 Cultural Control

The control of *R. solanacearum* is challenging once the pathogen has established in the soil (Kumar et al., 2018; Mwaniki et al., 2016). Although no single control method has proven to be 100% effective, some level of bacterial wilt control has been possible through integrated pest management in areas where the pathogen is established (Muthoni et al., 2014). Among the cultural practices, crop rotation, intercropping or incorporation of green manure, and planting a non-susceptible crop such as mung bean before the cultivation are among the many deployed methods in managing the bacterium inoculum in the soil (Gbonamou et al., 2021; Kumar et al., 2018; Manda et al., 2020; Mwaniki et al., 2016).

Although the use of crop rotation has been shown to reduce disease incidence, it is limited in management as the pathogen population continues to proliferate because it can survive in the soil over a long period but is also complicated by the existence of weeds and volunteer crops of solanaceous family (Kumar et al., 2018; Manda et al., 2020; Mwaniki et al., 2016). Crop rotation with a non-susceptible crop provided some control since this breaks the overlying impact and results in a decrease in disease. In some instances, proper and timely weeding before planting any susceptible crop suppresses the pathogen population density and incidence. Removal of volunteer crops that serve as sources of inoculum significantly reduced bacterial wilt infection (Kumar et al., 2018; Manda et al., 2020; Tessema et al., 2020).

In areas where the pathogen is not present, it is critical to prevent introduction and if accidentally introduced, subsequent spread of the pathogen should be prevented through phytosanitary measures (Manda et al., 2020). Planting certified disease-free seeds/ or seedlings, disinfecting farm equipment, and use of clean irrigation water can reduce the spread of the bacterium. Farmers should ensure frequent scouting to monitor for any infection for early detection because they will be able to destroy any symptomatic plants (Yuliar et al., 2015). However, treatment of seedlings with bioformulations greatly reduced the BW effect (Chakravarty & Kalita, 2011).

2.3.7.2 Chemical Control

Chemical application is a common technique to manage plant diseases. Different kinds of chemicals have been used to manage bacterial wilt for several years (Mamphogoro et al., 2020). Unfortunately, due to the nature of the bacterium, chemicals have not shown satisfactory control of bacterial wilt. To ensure100% control, most chemicals need to be applied before the disease occurs or at the first appearance of symptoms (Mamphogoro et al., 2020; Yuliar et al., 2015). In some cases, chemicals are applied alongside natural enemies but their compatibility usage is highly variable (Muthoni et al., 2014). In the chronological use of chemicals, pesticides fumigants such as (meta sodium, 1,2-dichlorpropene, and chloropicrin), algicide (3-[3-indolyl butanoic acid), and plant activators such as (Val doxylamine and validamycin A) were applied to manage bacterial wilt incidence (Mamphogoro et al., 2020).

Manda (2020) reported a decrease in the bacterial wilt incidence by 72% – 100% while the tomato yields significantly increased by 1.7 to 2.5-fold when methyl bromide was used to control the pathogen. However, its use is controversial because of the rising concerns involved and its pollution effect on the environment (Muthoni et al., 2014). Additionally, the utilization of synthetic substances like antibiotics to control plant pathogens has been seriously questioned on account of their effect on human well-being and nature, and pathogens are becoming resistant to chemicals(Karim et al., 2018; Manda et al., 2020).

2.3.7.3 Biological Control

Ralstonia solanacearum possesses some special biological features. These features include extensively and worldwide distributed major host crops like groundnuts (*Arachishypogae*), *Capsicum annum*, cotton (*Gossypium hirsutum*), rubber (*Heveabra siliensis*) cassava (*Manihot esculenta*), castor beans (*Ricinus communis*), eggplant (*Solanum spp*.) and ginger (*Zingiber officinalis*) with many weeds as asymptomatic alternate hosts to induce a destructive economic impact (De Morais et al., 2015; Karim et al., 2018). The use of biological control products for soil-borne

pathogens has gained popularity in recent years due to environmental concerns raised on the use of chemical products in disease control (Yuliar et al., 2015).

Biological control has been widely used and advocated for as a key practice in sustainable agriculture with a large potential of biological control being microorganisms. Biological control agents show various characteristics that have increased their usage compared to chemicals (Mamphogoro et al., 2020; Manda et al., 2020). Such highlights include the decreased contribution of non-renewable resources and their ability to be self-sustaining and spread after establishment and the capacity to give long-term ailment protection (Mamphogoro et al., 2020; Manda et al., 2020). Various studies have revealed that biocontrol of bacterial wilt disease may be accompanied by utilizing antagonistic rhizobacteria and epiphytic bacteria such as *Bacillus cencus, B. subtilis, B. pumilus, Paenaibacillus macerans, Serratia marcescens, Psuedomonas fluorescenns* and *P. patida* (Mamphogoro et al., 2020; Manda et al., 2020). In other studies, actinobacteria showed the potential for the management of bacterial wilt when combined with other control methods (Mamphogoro et al., 2020; Manda et al., 2020).

Most biocontrol agents of bacterial wilt disease comprise rhizobacteria, endophytic, and epiphytic bacteria species (Mamphogoro et al., 2020). Among the epiphytes, some are beneficial as the bio-agents such as *Paenibacillus macerans*, *Bacillus pumilus,* and *B. subtilis* have been reported to be effective in inducing resistance to *Xanthomonas vesicatoria* and *Ralstonia solanacearum* (Mamphogoro et al., 2020; Manda et al., 2020).

2.3.7.4 Breeding for Resistance

Host resistance is an important area of plant disease management due to the increasing pressure for healthy food and a healthy human environment (Muthoni et al., 2014). Growing plants that are highly resistant to bacterial wilt is the most effective, economical, and environmentally friendly approach to disease control (Mamphogoro et al., 2020; Yuliar et al., 2015). Many plants such as tomatoes, pepper, and eggplant exhibit some resistance to bacterial wilt (Chakravarty & Kalita, 2011; Lebeau et al., 2011, 2013; Salgon et al., 2017; Truong et al., 2015). Breeding

for plants that are resistant to bacterial diseases has been practiced mostly for crops such as potatoes, eggplant, pepper, and peanut (Mamphogoro et al., 2020).

Studies have shown that eggplants carry resistance genes to bacterial wilt (Bainsla et al., 2016; Gopalakrishnan et al., 2014; Namisy et al., 2019; Salgon et al., 2017). According to Knapp et al. (2013), domesticated plants lose their relationships and qualities making them genetically less superior to their wild relatives. This is because resistance is strongly affected by environmental factors and more importantly, by the strain path profile, which can vary among and within different phylotypes of *R solanacearum* species complex (Lebeau et al., 2013). In addition, the unpredictability of *Ralstonia* has prompted the development of several resistances which are successful in some developing regions and different locales (Lebeau et al., 2013).

The use of markers in resistance mapping has become the most important venture in breeding against disease resistance (Lebeau et al., 2013; Salgon et al., 2017). Markers-assisted selection has been used to construct eggplant genetic maps (Nunome et al., 2009). Traits of resistance against bacterial wilt were identified in different eggplant species, such as *Solanum torvum*, *S. sisymbrifolium,* and *Solanum aethiopicum* (Siljak-yakovlev & Paris-sud, 2001; Singh et al., 2019), tomato (Lebeau et al., 2011). The resistant-BW eggplant material was identified in several countries, including India, Taiwan, and Japan (Lebeau et al., 2013).

According to Xi'ou, (2015), previous studies identified *Solanum torvum* and *Solanum aethiopicum* with some resistance but were unstable due to variation in environmental factors and the races and the diversity of the pathogen thus limiting its adoption in different countries. The resistant genes were found to show partial resistance when subjected to virulent strains (Lebeau et al., 2013). Despite the resistance to bacterial wilt, the variation in *R. solanacearum* phylotypes and strains makes it difficult for multi-locational control of bacterial wilt disease (Huet, 2014).

Thus, with all the challenges associated with conventional management strategies, breeding for resistance to the soil-borne pathogen proves as an essential alternative for control in infested areas (Huet, 2014; Salgon et al., 2017). Identifying markers tightly linked to resistance to plant pathogens through marker-assisted selection has

improved the breeding process (Cao et al., 2009; Huet, 2014; Lebeau et al., 2013; Mutlu et al., 2008; Salgon et al., 2017). In a different study in the identification of resistance to *Fusarium oxysporum* Schelcht. F. sp. *melongenae* (FOM), 16 sets of markers yielded polymorphism. These markers included; 4-SRAPs, 4-RGAs, 6-SRAP-RGAs, and 2-RAPDs, which were subsequently tested on resistant and susceptible plants. Further, these markers were detected in all resistant plants and tightly linked to *Fusarium* wilt resistance (Mutlu et al., 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Phenotypic Reaction of African Eggplant Accessions to Bacterial Wilt Infection

3.1.1 Study Site and Experimental Materials

This study was conducted at Jomo Kenyatta University of Agriculture and Technology (JKUAT) between November 2016 and May 2017. JKUAT is situated in Juja, 36 kilometers northeast of Nairobi, and lies at coordinates 1°5'22" S and 37°0'38" E and an elevation of 1,527 m above sea level. Juja is located in the upper midland zone 4 which is semi-humid to semi-arid at 1520 meters above sea level with a mean annual temperature of 20°C and a mean maximum temperature of 30°C. The area receives low rainfall of 856mm with a bimodal distribution. The area has three types of soils which are shallow clay soil over trachytic tuff, very shallow clay sandy soils over murram, and deep clay soils (vertisols).

3.1.2 Planting

Seeds of the forty-seven (47) African eggplant accessions (S. *aethiopicum*, S*olanum sp,* and *S. anguivi*) were collected from African Vegetable Research and Development Center-Regional Center for Africa (AVRDC- RCA) in Arusha Tanzania (Appendix 1). They were collected from Mali, Uganda, Gabon, Senegal Cameroon Malawi, Bukina Faso, Ivory Coast, Tanzania and others from unknown origin.The seeds were sorted to remain with whole and clean seeds. The African eggplant seeds were sown in germination trays containing washed cocopeat and allowed to germinate. Prior to sowing, the seeds were then soaked in warm water (45°C) for 24 hours in order to have uniform germination by breaking the dormancy. The trays were kept in the greenhouse environment and were adequately watered. The temperature in the greenhouse ranged between 25°C – 32°C.

3.1.3 Experimental Design

The study of African eggplant accessions (*Solanum aethiopicum, S. anguivi, and Solanum sp*) resistance to bacterial wilt was carried out in naturally infected fields and the greenhouse. The study aimed to determine the reaction of African eggplant accessions and identify bacterial wilt-resistant genotypes in African eggplant accessions. The experimental treatments (African eggplant accessions) were laid out in a randomized complete block design (RCBD) in field trials. The three blocks measured 100M x 2.5M and were 1.5M apart. The accessions were assigned to forty-seven plots each measuring 2M x 2M. In each plot, there were planted four (4) plants which represented an accession giving a population of twelve plants per accession.

In the greenhouse experiment, plants were sown in plastic pots filled with sterilized soil and manure in four replicates. The pots were arranged in a Completely Randomised Design (CRD) and only four plants represented an accession in the rows.

3.1.4. Field and Greenhouse Experiments

Before transplanting eggplant seedlings, the bacterial load in the soil was determined. This was done by collecting soil from different points in the field by using a zigzag method of soil sampling. Soil was collected by use of a soil auger at a depth of 30cm. The soil was thoroughly mixed to form composite samples. 1 g of the soil sample from the composite was taken and dissolved in 10 ml of sterile water. The mixture was stirred and left to stand for 30 minutes at room temperature and serially diluted to six (6) folds. Plating test by picking 5ul from 10^{-3}– 10^{-6} from each dilution was carried out by lawn spreading on Casamino acid-Peptone-Glucose (CPG) media and incubated for 72 hours at 28°C.

At four (4) fully expanded leaves, the seedlings were transplanted into the field at a spacing of 0.9M X 0.5M and in the greenhouse into poly bags of 21 inches x 14 inches x 14 inches (plate 3.1). Each polybag was planted with one seedling and spaced at 30cm. In each replication (greenhouse) four (4) plants per accession were planted and inoculated (the inoculum prepared as discussed in section 3.1.5).

Irrigation was carried out throughout the experiment to ensure that no water stress was created. Plants were monitored daily for bacterial wilt symptom development.

Figure 3.1: Experimental study: (i): Field experiment (ii) greenhouse experiment.

3.1.5 Evaluation of African Eggplant Accessions for Resistance to Bacterial Wilt

The bacterium, *R. solanacearum* was isolated from diseased eggplants obtained from the field showing typical BW wilt symptoms. The inoculum was prepared by isolating the bacteria from an infected plant by the ooze-out method. A stem section of an infected plant was cut, washed off the soil, and sterilized with 70% ethanol to remove unwanted contaminations. The stem section was placed in 10 ml of distilled water to allow bacteria to ooze out. The bacteria suspension was diluted at the 10^{th} fold. 30µl of the diluted suspension was plated by lawn spreading on a media plate. The Bacterium was isolated on 2, 3, 5- and triphenyl tetrazolium chloride (TTC) and incubated at 28°C (Chaudhry & Rashid, 2011). Actively growing colonies irregular in shape, fluidal, and pink centers with the typical characteristics of *R. solanacearum* were harvested from 48 hours' culture by use of a sterile wire loop. Colonies were then transferred to CPG (Casamino hydrolysate 1g, Peptone 10 g, and Glucose 5 g pH 7) nutrient broth and incubated on an orbital shaker (Orbital Shaker-incubator ES-20 Grant- Bio) for 24 hours at 28°C. After the incubation, the bacterium inoculum was prepared by adjusting the concentration to 10^{-7} CFU/ml (OD=0.0977) at 600 nm optical density (OD) using a spectrophotometer (APEL PD-3000UV

spectrophotometer). Plants were inoculated seven days after transplanting (DAT) upon preliminary puncturing of roots 2 cm away from the stem using a sterile knife. The bacterial suspension was then drenched immediately after the roots were damaged. Each plant received 30 ml of bacterial suspension.

The plants were monitored daily for symptom development. Bacterial wilt severity was determined by visual assessment of the symptom development once a week for six weeks according to a disease severity scale of 1-5 by Lebeau et al. (2013) with slight modifications (Table 3.2). At each scoring date, the disease incidence was calculated for each accession as;

$$\%\ Disease\ incidence = \frac{Number\ of\ wilted\ plants}{Total\ number\ of\ plants\ per\ accession} x100$$

While the area under the disease progress curve (AUDPC) was determined according to Lebeau (2013);

$$\sum_{i=1}^{n-1} \frac{x_i + x_{i+1}}{2} x(t_{i+1} - t_i) x \frac{1}{t_n - t_1}$$

where; x_i is the mean wilting symptoms rating (disease score) at the i^{th} date (i = 1 corresponds to the day of transplanting), t_i is the time at the i^{th} observation, and n is the total number of observations.

Table 3.1: Bacterial Wilt Severity Rating Scale of African Eggplant Accessions

Rating (Scale)	Reaction observed
1	Asymptomatic plant
2	One wilting leaf
3	Less than 50 % wilted leaves
4	More than 50 % wilted leaves
5	Completely wilted leaves (plant dead)

Disease severity scale used during the study (Lebeau et al., 2013) with modifications.

3.1.6 Testing Field Plants for Bacterial Wilt Infection

At the end of the experiment, infection tests for bacterial wilt were carried out by taking asymptomatic and symptomatic plants for bacteria isolation according to (Lebeau et al., 2011, 2013) with slight modifications. All the plants collected were washed off the soil in tap running water. To eliminate other non-targeted organisms' stems were rinsed with distilled water and finally sterilized with 70% ethanol. Eggplant stems of about two centimeters in length picked from the stem base, were placed in clear glass test tubes filled with 10 ml sterile water. Stem sections were allowed to stand for 1–2 hours at room temperature (23°C) for bacteria to stream out of the xylem vessels.

Dilution of the bacterial suspension to (10^{-6}) from the original streaming was done. An aliquot of 50 µL from each sample was drawn and streaked onto Kelman's media triphenyl tetrazolium chloride (TTC) agar and incubated at 28°C for 48 hours on orbital shaker-incubator ES-20 (Grant-bio). Stem sections from which characteristic *R. solanacearum* colonies were isolated by the use of plating method (Sanders, 2012), were scored as positive for the presence of bacterial wilt. Only characteristic colonies were considered.

3.1.7 Data Analysis

Analysis of variance (ANOVA) was carried out using SAS software (JMP 9.0.0 2010). Phenotypic reactions; Disease Incidence (DI), severity, and area under disease progress curve (AUDPC) were computed according to (Lebeau et al., 2013).

3.2 Identification of Molecular Markers for Bacterial Wilt Resistance

3.2.1 DNA Extraction

DNA from each accession was extracted from 0.5 gm of the youngest leaves using modified CTAB procedure (Nunome et al., 2009). DNA extraction was done by grinding 0.4 grams of young dried eggplant leaves in 2ml extraction buffer. The slurry was transferred into a 1.5ml eppendorf tube and incubated at 65^{o}C in a water bath for 30min. The mixture was centrifuged at 1300 revolutions per minute (rpm) at 10^{o}C for 10min then the supernatant was carefully transferred to a new Eppendorf. An equal volume of chloroform:Isoamylacohol (24:1) was added and then centrifuged at 13000rpm for 10min.

After centrifuging, 600ul of an aqueous phase was mixed with an equal volume of Isopropanol and centrifuged for 10min at 13000rpm. This was followed by separating the DNA pellet from the supernatant through decanting. The DNA pellets were then washed with 700ul of 70% ethanol and centrifuged at 11000rpm for 5min at 5^{o}C. the ethanol was discarded and the DNA pellets were airdried and resuspended in 40ul of free Rnase water. The quality of DNA was assessed using 2% (w/v) agarose gel electrophoresis (Lebeau *et al.,* 2013).

3.2.2 SSR-Based Diversity Analysis

A polymorphism check was conducted using 15 SSR primers on all the accessions, with 2 µl of genomic DNA (Nunome et al., 2009). The analysis was carried out by pooling the accessions' DNA. Markers with polymorphic bands were then considered for subsequent screening for BW resistance in eggplant. The polymorphic SSR bands for each accession were individually scored for the presence (1) or absence (0) of the expected band sizes (Lebeau et al., 2013).

All eggplant accessions DNA were amplified in a 25 µl reaction volume with 2 µl of genomic DNA. 0.5 µl of each forward and reverse primer was added to the DNA template.9.5 µl of free nuclease water and 12.5 µl of X2 master mix taq (Biolabs, New England) (Nunome et al., 2009). A touchdown PCR protocol was applied, of one cycle of 94ºC for 3 min of denaturation; 10 cycles of 94ºC for 30 s, 65–55ºC decreasing by 1ºC per cycle for 1 min; one cycle of 72ºC for 1 min; 30 cycles of 94ºC for 30 s; one cycle of 55ºC for 1 min; one cycle of 72ºC for 1 min; and a final cycle of 72ºC for 5 min. Amplification was carried out using the GeneAmp PCR system 2720 thermal cycler (Applied Biosystems, USA). The PCR products (100 base pair molecular-weight ladder) were separated through electrophoresis in 2.0% agarose gel run in 5x Tris-Borate-EDTA (TBE) at 80V after staining with ethidium bromide and then photographed under UV light using benchtop 2UV transilluminator (UVP). The bands were scored (1) for presence and (0) for absence (Lebeau et al., 2013).

Table 3.2: Bacterial Wilt Resistance Markers Used in the Study

Name	Forward primer	Reverse primer	Expected band size
ecm009	CACTAGTACCATCAAGTCTAAGCAGCA	TTAACAACAGCTGAGGCCATGAAA	245
emi04P17	CCCAAGAATACAGCAACTTGAGGA	TCATACCCGGCAAGTGTTTGATAA	226
emk03O04	AAGATTTGGGCAGCCACTTTTGTA	TTGGAACCAACTAAACTTAGGGCA	179
est_ae507f01	TAGGAGCAGATGACATCGTCAAGG	CAAACCTGTGTGTGATCAATGGCT	582
SOL7229	TGACAAAGCCAAATTCAACTGCTG	TCAAGCTTTTCTGCTCTTTTAGCCA	289
SOL8240	ATGAGATTCCCCTTGGTTGGAAGT	CTGTGCAGCTTGAAACCAGTTCAT	228
est_rbw03m09	ACTACAGCCGATTGAACCATCACA	GTCCATTTGGAAAGCAAGCATTTG	198
SOL5036	AGACTGTCACAAAAACGCAAACCA	AAACTCTGCCATTTCACTTGAAGG	134
est_rbw01l06	GTTTGCTCAAGAGAATATTGCCCC	ACTCATGGCTAGAGCCCCCACAT	135
SOL8269	GCCAATCTTTGACCCTTTATGCTG	CCAGTGGTAAGGCTGAGTTCATGG	
SOL7124	TGCTCATCATAAGGAGGTTGCTGA	AGGAGCCTTTGTAGGCAAGGAAAA	412
SOL8253	GGGAATCTCACAGGGAGGAAGAA	CTTGAGCTCCTTCACCATCCTCTC	132
ecm001	CCCTTACGCAATTTACACTTCCCC	ATCAATGGCGTCACCTCTCTCTCT	229
SOL7274	CTCCAGCAATTCCTCAGTCTCAAG	ACATCGGAGGTAGCAAAATAGCCA	185
SOL5085	GCCAATCATGAGAGAAGTGCAGAA	GGAAGTACACACAAGCTCCAAATGA	212

SSR markers used to identify resistance eggplant to bacterial wilt were derived from an SSR-enriched genomic library of eggplant (Solanum melongena L.) (Nunome et al., 2009)**.**

3.2.3 Data Analysis

African eggplants were scored for the presence (1) or absence (0) of the resistant markers.

CHAPTER FOUR

RESULTS

4.1 Reaction of African Eggplant Accessions to Bacterial Wilt Infection

The experimental field was found to be infested with bacterial wilt. This was evident from soil collected from the field showing typical characteristics of bacterial wilt colonies on CPG media. All plants did not germinate at the same time even after treating the seeds with hot water. The germination was poor and irregular across the accessions, thus delaying the transplanting process. However, symptom development on accession majorly depended on the genotype of the accession.

Wilt symptoms development varied across the experimental units, where it ranged from one leaf wilted to a whole plant or all plants in the accessions wilted/dead (Plate 4.1). However, African eggplant accessions were observed to display wilting symptoms.

Plate 4.1: Eggplants Showing Bacterial Wilt Symptoms. A – C Leaf Wilting and (D) Brown Dis-Coloration on a Stem Sections.

All accessions were affected with the bacterium where *S. aguivi* showed resistance to bacterial wilt while *S. aethiopicum* was highly susceptible (table 4.1). Some accessions showed mild to severe symptoms to bacterial wilt, whereas severe wilt was observed during the early hours of the day and recovered in the evening (Figure 4.1). Other symptoms such as leaf yellowing, and stem discoloration were also observed during the experiment.

Table 4.1: African Eggplant Classification. Severity, Disease Incidence and AUDPC of African Eggplant Accessions to Bacterial Wilt

Accessions	Severity	% DI	AUDPC	Classification	Family
RV100386	3.4	66.8	472.5	HS	*S. aethiopicum*
RV100234	3.1	60.5	422.5	HS	*S. aethiopicum*
RV100201	3	59.4	414.5	HS	*S. aethiopicum*
RV100245	3	59.4	415.0	HS	*S. aethiopicum*
RV100331	3	58.4	404.5	HS	Unknown
RV100250	2.7	53.8	372.0	HS	*S. aethiopicum*
RV100447	2.7	53.7	374.5	HS	*Solanum sp.*
RV100161	2.7	53.7	370	HS	*S. aethiopicum*
RV100247	2.6	52.3	361.5	HS	*S. aethiopicum*
RV100240	2.6	51.7	358	HS	*S. aethiopicum*
RV100271	2.4	48.8	334	HS	*S. aethiopicum*
RV100458	2.5	48.4	331	HS	unknown
RV100342	2.4	47	327	HS	*S. aethiopicum*
RV100328	2.3	45	316	S	*S. aethiopicum*
RV100432	2.3	45	314.5	S	*Solanum sp.*
RV100431	2.2	43.1	298	S	*S. aethiopicum*
RV100330	2.1	41.9	293.5	S	*S. aethiopicum*
RV100335	2.2	42.9	311	S	*S. aethiopicum*
RV100259	1.8	35.2	244.5	S	*S. aethiopicum*
RV100327	1.8	42.9	230.5	S	*S. aethiopicum*
RV100169	1.7	30.7	248.5	S	*S. aethiopicum*
RV100270	1.6	30.6	223.5	S	*S. aethiopicum*
RV100511	1.5	29.5	215.5	S	*S. aethiopicum*
RV100217	1.5	27.4	195	S	*S. aethiopicum*
GBK050572	1.5	27.5	192	S	*Solanum sp.*
RV100377	1.3	25.7	191	MS	*S. aethiopicum*
RV100185	1.3	24.5	178	MS	*S. aethiopicum*
RV100452	1.4	24.2	177	MS	*Solanum sp.*
RV100364	1.5	26.2	187	MS	*S. anguivi*
RV100261	1.3	23.5	162.5	MS	*S. aethiopicum*
RV100190	1.3	22.5	168	MS	*S. anguivi*
RV100334	1.3	22.6	157	MS	*S. aethiopicum*
RV100333	1.3	20.2	139	MS	*S. aethiopicum*
RV100268	1.3	20.4	140.5	MS	*S. aethiopicum*
RV100352	1.2	19.5	138	MS	*S. aethiopicum*
RV100263	1.2	17.8	130	MS	*S. aethiopicum*
RV100218	1.2	19.6	148	MS	*S. aethiopicum*
RV100360	1.2	15.1	108.5	MS	*S. anguivi*
RV100264	1.2	16.3	112	MS	*S. aethiopicum*
RV100332	1.1	16.8	118	R	*S. aethiopicum*
RV100265	1.1	12.5	95	R	*S. aethiopicum*
RV100445	1.1	13.5	97	R	*S. aethiopicum*
RV100453	1.1	15.3	112.5	R	*Solanum sp.*
RV100239	1.1	17	119.5	R	*S. aethiopicum*
RV100438	1.1	9.7	73.5	R	*S. aethiopicum*
RV100246	1.1	11.4	88	R	*S. aethiopicum*
RV100242	1.1	11.3	85	R	*S. aethiopicum*
RV100455	1.1	5.6	38.5	R	*Solanum sp.*

The disease severity of 47 African eggplant accessions was recorded weekly at a scale of 1 - 5 where 1 represented healthy and 5 dead plants. Disease incidence was calculated as a percentage of wilted plants per accession. Severity scores and disease incidence yielded four categories of disease reaction; 1. HS - highly susceptible, 2. S - susceptible, MS - moderately susceptible, MR - moderately susceptible, and R – resistant.

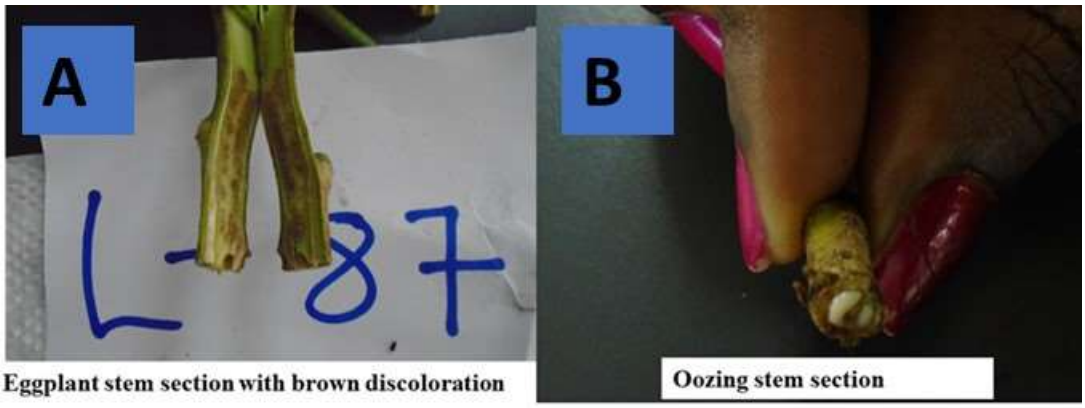

Plate 4.2: Eggplant Stem Parts with Bacterial Signs. Longitudinal Stem Section of Eggplant with Brown Coloration (A) and Bacterial Ooze from a Freshly Cut Stem (B). This Showed how Different Accessions Responded to Bacterial Disease.

Assessment of eggplant accessions to bacterial wilt infection showed that there were no accessions that were highly resistant or immune. The susceptible accessions reacted with bacteria and the BW signs (brown colouration and oozes) were visible on the selected accessions plate 4.2 and 4.3.

A high disease incidence and severity were recorded in *S. aethiopicum* and at least in *S. anguivi* (Table 4.1). Accessions from the *S. aethiopicum* (RV100386, RV100234, RV100201) were highly susceptible to the bacteria and recorded disease severity of 3 to 3.4 (Table 4.1). on the other hand, accessions from the *S. anguivi* RV100364 and RV100190 showed a disease severity of 1.3 and 1.5 respectively hence classified moderately susceptible. RV100335 was susceptible to bacterial wilt recording a severity

of 2.2 and RV100360 resistant with 1.2 severity. The area under the disease progress curve (AUDPC) was also calculated which showed the disease progressed with time (Table 4.1).

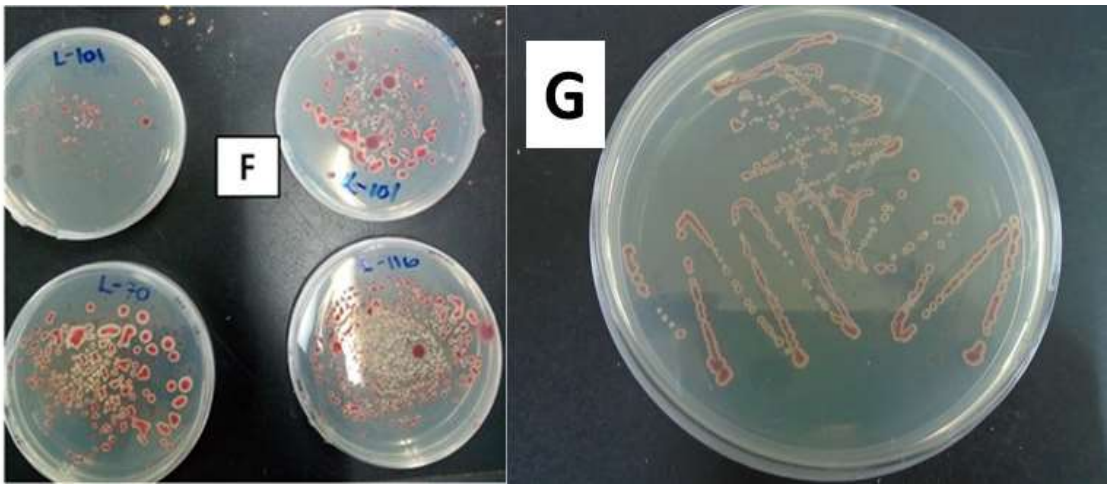

Plate 4.3: *R. solanacearum* colonies on Kelman TZC media.

Thick and thin thread-like milky streams flowed from the stem sections (Plate 4.1 E). Signs of *R. solanacearum* were observed on cut stems of stem browning and exudates oozing out (Plate 4.3A and B). The presence of milky oozing exudates (Plate 4.1 E), and bacterial exudates from the cut stem section was proof that the pathogen was *R. solanacearum.* After the incubation, the culture produced characteristic *R. solanacearum* fluidal colonies with pink centers and whitish margins were observed (Plate 4.3 F and G). The stem sections from which *R. solanacearum* characteristic colonies were observed, were scored as positive for the presence or absence of bacterial wilt. RV100431 had dirty streaming, but no colony was formed even though it showed wilt symptoms both in the field and in green house. On the contrary, some accessions did not show symptoms in the field but produced typical morphological characteristics of *R. solanacearum* on the TZC medium.

Out of the 47 tested African eggplant accessions, thirty-one (31) BW symptoms with disease severity ranging from1.5 – 3.4; RV100386, RV100234, RV100201, RV100245, RV100331, RV100250, RV100447, RV100161, RV100247, RV100240, RV100271, RV100458, RV100342, RV100328, RV100432, RV100431, RV100330, RV100335, RV100259, RV100327, RV100169, RV100270, RV100511, RV100217 and GBK050572 (wilting of younger leaves) 14 days after transplanting (DAT) (Figure 4.1). African eggplants were grouped into four groups depending on the disease progression. These groups included; R-resistant, MR – moderately resistant, S – susceptible, and HS – highly susceptible. Accessions that displayed the lowest disease severity were identified as resistant while those with the highest disease severity as highly susceptible. The disease incidence of 5.6% - 66.8% was recorded during the study and shows no significant (P = 0.05) difference among the accessions. Fifteen of the accessions had the lowest severity of 1.1 – 1.2 with the bacterium with an average DI of 15%, 9 accessions moderately with 18- 25%, and 26 accessions >40% DI respectively (Table 4.2; Figure 4.1). Weather patterns varied in both seasons where medium to lower (<18˚C) temperatures were recorded during the first season.

Accessions RV100245, RV100432, RV100331, RV100201, RV100386, RV100250, RV100240, RV100447, and RV100234 reacted severely of 2.4 – 3.4 and were the first to show the disease symptoms 14 days after transplanting (DAT). Accessions RV100432, RV100217, RV100199, RV100169, RV100352, RV100271, RV100201, RV100386, and RV100200 during the study displayed (BW) symptoms 14 DAT (Table 4.1; Figure 4.4). An average disease incidence of 52% – 67.5% was recorded even though it was highest in some accessions. However, none of the accessions showed complete resistance to the bacterium. Depending on the disease severity, African eggplant accessions were grouped into four categories R- resistant (1.1 disease severity), MS- moderately susceptible (1.2 – 1.3 Disease severity), S- susceptible (1.5 – 2.3 Disease severity), and HS- highly susceptible (>2.5 Disease severity).

4.2 Identification of Molecular Markers for Bacterial Wilt (*R. Solanacearum*) Resistance in African Eggplant Accessions

4.2.1 Bacterial Wilt-Resistance Markers in Eggplant

Young eggplant leaves were harvested for the extraction of genomic DNA. The DNA extraction was done by CTAB method with minor modifications with the aim of identification of bacterial wilt-resistance markers in eggplants. A total of fifteen (15) markers were used and markers that produced bands were scored one (1- resistant) and absence of a band were scored zero (0- susceptible) (Plate 4.1).

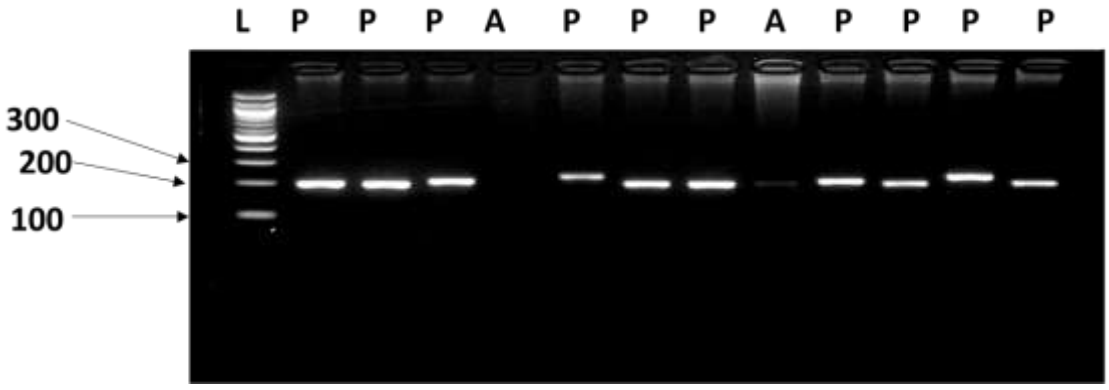

Plate 4.4: The PCR amplicon of African eggplant accessions. Lanes M 100 bp ladder. P is the amplified accession indicating the presence of the marker hence resistant and A standing for the absence of the marker in accessions hence scored Zero (0) for susceptibility. One (1) presence of resistance markers.

A total of fifteen eggplant bacterial wilt-resistance SSR markers were screened. Only 5 markers produced quality bands and were selected for identifying bacterial wilt resistant

accessions (Plate 4.1). The markers produced single bands (Plate 4.1). The observed amplicons were within the expected band sizes (177bp – 229bp) except for a few accessions which produced bands with slight difference from the expected band. Among the identified primers, two (2) ecm009 and emiO4P17 had been previously reported to carry bacterial wilt-resistant markers and they were found in ten (10) accessions. The other primers that amplified the eggplant accessions included; ecm001, emk03O04, and SOL5036 (Appendix 2).

Bacterial wilt resistsnce markers were present in various accessions. It was observed that seven (7) accessions RV100453, RV100246, RV100360, RV100445, RV100268, RV100352, and RV100330 had the resistant gene. Markers ecm009 was found in fourteen (14) and only fifteen (15) accessions were amplified by emi04P17 out of the 47 accessions screened respectively. On the other hand, emk03O04 and ecm001 were present in fifteen (15) and twenty-one (21) accessions respectively, and another twenty-one accessions with SOL5036. The study also revealed fifteen (15) eggplant accessions lacking any of the identified bacterial wilt resistance markers.

Symptomatic accessions did not carry resistant markers except for RV100330 which was grouped as susceptible and was observed to carry resistant gene (Table 4.1 and Appendix 2). African eggplant accessions RV100217 and RV100185 in the susceptible and moderately susceptible category also had the resistant markers, respectively. However, all accessions that had the low disease severity and incidence were amplified by the resistance marker. It was also observed that some accessions with moderate severity had resistance markers (Table 4.1 and Appendix 2).

African eggplant accessions *S. aethiopicum* and *S. anguivi* RV100334, RV100330, RV100242, RV100268, RV100265, RV100261, RV100352, RV100360 and RV100190 carried resistance gene (appendix 4.2). The same accessions had low severity with bacterial wilt in the field. On the other hand, accessions with unknown origins carried the tested markers for resistance and low severity with bacterial wilt (Table 4.1;

Appendix 2). Other markers that expressed in eggplant accessions that did not react with bacterial wilt included emk03O04, ecm001, emi04P17, and SOL5036 (appendix 2)

CHAPTER FIVE

DISCUSSION

5.1 Reaction of African Eggplant Accessions to Bacterial Wilt Infection

African eggplant accessions were tested for their reaction to bacterial wilt infection. The experiment was carried out under natural and artificial inoculation conditions. disease severity and incidence were recorded every week. Bacterial wilt severity varied between the tested accession showing that none was immune with the highest scoring a disease severity of 3.4. The current study revealed that all susceptible accessions showed wilt symptoms 14 -21 days after transplanting (DAT). At this time, majority of the plants had wilted. In studies by Oliveira, (2014) and Siljak, (2001), commercial eggplant species *S. melongena* was found to succumb to the disease 2 weeks after inoculation (WAI) with bacterial wilt, unlike their relatives which exhibited up to 50% resistance and were in agreement with the current findings. On the other hand, Aslam et al. (2017), reported that it only took 4 days for susceptible tomato to show wilt symptoms and complete wilting 14 days later. This study showed similar findings although with a little bit of delay in symptom development which could be due to the physiological difference between tomato and eggplant.

In the current study, a delay in symptom development was observed in susceptible accessions at 14 days after inoculation with 50% of leaves wilted which could be due to differences in the emergence of seedlings during germination. According to Swanson et al. (2007), there was variation in the death rate of African eggplants due to the effect of the BW, and all the accessions were affected, while citing that, the delayed effect could be due to root cortical cells and a high level of phenolics content that prevent the entry and continued multiplication of bacterium (Cao et al., 2009). The same phenomenon was also reported by Salgon et al. (2017) where there was a difference in phenotyped RILs.

The disease incidence varied between the seasons where the second season was high (January – April 2017) compared to season 1 (July – November 2016), which was characterized by low temperatures < 17°C (data not shown). This difference could be due to favourable weather conditions, especially temperature (22°C to 27°C) in season 2 and (Mew & Ho, 1977) reported such findings. The time difference in wilt symptoms development could have been due to weather conditions at the time of inoculation, in which, the first season fell in the month of July to November which are typically colder than the rest of the year. A study by Bainsla et al. (2016) also reported that there was a variation in symptom development as a result of changing weather patterns. These conditions may have affected the survival of the inoculum after being administered to plants.

The variation in symptom development between the 2 seasons was consistent with Bainsla et al. (2016) who in their findings reported that soil-borne diseases such as *R. solanacearum* survival in low temperatures is hindered. The results revealed that in some accessions, symptoms were observed at the first days of inoculation/transplanting and later plant recovered which could be attributed to other factors such as the development of immunity/ or escape mechanism. Such findings were similar to those reported by Bainsla et al. (2016).

The extent of reaction was among the accessions whereby the *S. aethiopicum* showed relatively higher levels of severity. However, the results showed that none of the accessions was resistant to bacterial wilt. In some instances, asymptomatic plants turned positive on culture media which showed that they were latently infected by the bacterium. This shows that the asymptomatic plants were latently infected by the bacterium. This not only confirmed the causal organism as was reported by (Chaudhry & Rashid, 2011; Marques et al., 2012; Muradashvili et al., 2015), but distinguished the bacterial wilt symptom from the other wilts. Swanson *et al*, (2007), reported detectable bacterial wilt in all sampled stem sections and was >1X 10^4. This means that a considerable amount of the inoculum was absorbed and the same results were reported

in the present study. On the media, evident fluidal pinkish-red center colonies typical of *R. solanacearum* were observed, and are in tandem with findings of (Chaudhry & Rashid, 2011; Marques et al., 2012; Muradashvili et al., 2015).

Bacterial wilt (*R. solanacearum*) with a very widespread plant host range includes several hundreds of species representing at least 50 families of flowering plants and gymnosperms (Cellier & Prior, 2010; Meng, 2013). It is one of the major diseases in tomato production and other solanaceous plants (Karim et al., 2018; Phoebe et al., 2016; Yuliar et al., 2015). The disease is known to occur in the wet tropics, sub-tropics, and some temperate regions of the world (Champoiseau et al., 2009; Hayward, 1991; Swanson et al., 2007). Specific pathogenic strains for certain hosts may have evolved only in certain parts of the world and are not found elsewhere (Kim et al., 2016). These hosts may only have been susceptible where several environmental factors conducive to disease expression coincided, such as temperature regime, rainfall, soil type and inoculum potential (Kim et al., 2016).

5.2 Identification SSR Markers Linked to Bacterial Wilt Resistance in African Eggplant Accessions

In the present study, 47 accessions were tested for response to bacterial wilt and later subjected to PCR testing for bacterial wilt resistance using 15 SSR markers. Only five (ecm009, emk03O04, ecm001, emi04P17, and SOL5036) showed a considerable level of polymorphism. A study by Dheemanth et al. (2018) reported that resistance markers in tomatoes produced polymorphic bands, while the susceptible varieties gave various amplification patterns. The same was in tandem with their findings were observed during the study.

Marker ecm009 (Lebeau et al., 2013), and emi04P17 (not published) were reported to be associated with bacterial wilt resistance and were present in 10 accessions belonging to different eggplant species. Since the findings in the current study are in tandem with

what (Lebeau et al., 2013), reported, African eggplant accessions are regarded as being resistant. Moreover, accession RV100247 manifested bacterial wilt symptoms and also carried tested markers; this indicated that the resistance was overcome by the disease pressure or maybe they are resistant in different places as this is the case with bacterial wilt brought about by the race identification (Lebeau et al., 2013). Alternatively, it could be due to resistance markers being overcome by bacterial wilt disease or the breakdown of the resistance (Lebeau et al., 2013; Salgon et al., 2017; Siri et al., 2009; Truong et al., 2015). The amplification of the markers was not uniform across all the accessions, which was indicative of resistance diversity among the African eggplant accession.

In a study by Truong et al. (2015), RAPD markers were only associated with resistant parents and amplified the polymorphic fragments alone. With the same markers Truong et al. (2015), reported that 92 tomato lines were evaluated for bacterial wilt resistance and none of them, including the asymptomatic line, carried the markers. Siri (2009), reported the presence of resistant markers in *Solanum commersonii* originating from different locations across Uruguay. Similarly, resistance markers to bacterial wilt were in accessions from Mali, Uganda, and Tanzania which had the lowest disease severity and incidence, hence resistant. This indicates that there is regional instability in the resistance to bacterial wilt as it was reported by earlier researchers (Hayward, 1991). Salgon et al. (2017), resistance to BW is not stable across the geographical regions in that the resistant parent in Indonesia was highly susceptible but with a slower disease progression than the susceptible parent suggesting that QTLS if present acts primarily in the early stages of the infection. Additionally, plant resistance may be affected by changes in location and climate, because of strain differences.

In recent times, SSR markers have been used to characterize eggplant for resistance to plant pathogens (Lebeau et al., 2013; Mutlu et al., 2008; Salgon et al., 2017). Thus there is a relationship between their findings and the findings in the current study. However, these marked genetic advances in the level of resistance of plants to bacterial wilt led to improvements in breeding programs (Aslam et al., 2017; Lebeau et al., 2011, 2013;

Mutlu et al., 2008; Salgon et al., 2017). During the current study, several accessions revealed the presence of bacterial wilt resistance with the tested resistant markers. In another study by Mwaniki, et al. (2016), *S. anguivi* was found susceptible to the three *Fusarium* wilt species while *S. aethipicum* showed considerable resistance to *Fusarium* wilt tested. These findings are in contrast to the current study and indicate the variability in resistance to various pathogens varies among plant species.

Sources of genetic resistance to the bacterial wilt of various crop plants tomato, eggplant, and pepper have been previously studied (Aslam et al., 2017; Cao et al., 2009; Lebeau et al., 2011, 2013). The identification of resistant traits in African eggplant accessions using SSR markers is an important selection of resistance to plant bacterial wilt (Mutlu et al., 2008; Siri et al., 2009). In recent times, SSR markers have been used to characterize plants for resistance to BW (Lebeau et al., 2011, 2013; Salgon et al., 2017).

A wide range of symptoms is caused by the vascular wilt pathogens and some pathogens may cause different symptoms on different host plants. Depending on the pathogen species and host, plants may become stunted, wilt partially or completely, and ultimately die. Plant death may occur within days to weeks or in the case of perennials, months to years. These symptoms tend to be rather unreliable for assessing resistance, since during the study, susceptible eggplant accessions carried bacterial wilt markers, hence the relationship between the amount of pathogen present and the severity of symptoms is often poor (Vale et al., 2001). The amount of tissue affected is in general, a good estimator of the amount of pathogen present. The reliability of this measure of pathogen presence with host resistance varies with host and pathogen but tends to be fairly good in many cases (Vale et al., 2001).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- African eggplant accessions were found to react with bacterial wilt infection but at varying levels. Four classes were identified; resistant (R) - 10 accessions, moderately susceptible (MS) - 13 accessions, susceptible (S) - 12 accessions, and highly susceptible (HS) – 14 accessions.
- Five (5) molecular markers; ecm009, emk03O04, ecm001, emi04P17 and SOL5036 identified and were present in resistant eggplant accessions

6.2 Recommendations

- The African eggplant genotypes identified in this study can be utilized for future breeding programs for bacterial wilt resistance management in solanaceous crops.
- There is a need to carry out a study on the bacterial wilt strains that affected the susceptible accessions and compare them with the existing database.

REFERENCES

Álvarez, B., Biosca, E. G., & López, M. M. (2010). On the life of *Ralstonia solanacearum*, a destructive bacterial plant pathogen. *Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, 267–279.

Aslam, M. N., Mukhtar, T., Hussain, M. A., & Raheel, M. (2017). Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm. *Journal of Plant Diseases and Protection*, *124*(6), 585–590.

Aslam, M. N., Mukhtar, T., Jamil, M., & Nafees, M. (2019). Analysis of aubergine germplasm for resistance sources to bacterial wilt incited by *Ralstonia solanacearum*. *Pakistan Journal of Agricultural Sciences*, *56*(1), 119–122.

Ateka, E. M., Mwangombe, A. W., & Kimenju, J. W. (2001). Reaction of Potato cultivers to *Ralstonia solanacearum* in Kenya. *African Crop Science Journal*, *9*(1), 251–256.

Bacchi, M., Riccardi, P., Fiore, M. C., & Ricciardi, L. (2010). Genetic diversity and characterization of African eggplant germplasm collection. *African Journal Plant Science*, *4*(7), 231–241.

Bainsla, N. K., Singh, S., Singh, P. K., Kumar, K., Kumar Singh, A., & Gautam, R. K. (2016). Genetic Behaviour of Bacterial Wilt Resistance in Brinjal (*Solanum melongena* L.) in Tropics of Andaman and Nicobar Islands of India. *American Journal of Plant Sciences*, *07*(02), 333–338.

Caguiat, X. G. I., & Hautea, D. M. (2014). Genetic diversity analysis of eggplant (*Solanum melongena* L.) and related wild species in the philippines using morphological and SSR markers. *Sabrao Journal of Breeding and Genetics*,

46(2), 183–201.

Cao, B. H., Lei, J. J., Wang, Y., & Chen, G. J. (2009). Inheritance and identification of SCAR marker linked to bacterial wilt-resistance in eggplant. *African Journal of Biotechnology*, *8*(20), 5201–5207.

Castillo, J. A., & Greenberg, J. T. (2007). Evolutionary dynamics of *Ralstonia solanacearum*. *Applied and Environmental Microbiology*, *73*(4), 1225–1238.

Cellier, G., & Prior, P. (2010). Deciphering phenotypic diversity of Ralstonia solanacearum strains pathogenic to potato. *Phytopathology*, *100*(11), 1250–1261.

Chakravarty, G., & Kalita, M. C. (2011). Management of bacterial wilt of brinjal by *P. fluorescens* based bioformulation. *Journal of Agricultural and Biological Science*, *6*(3), 1–11.

Champoiseau, P. G., Jones, J. B., & Allen, C. (2009). *Ralstonia solanacearum* Race 3 Biovar 2 Causes Tropical Losses and Temperate Anxieties . *Plant Health Progress*, *10*(1), 35 - 44

Chaudhry, Z., & Rashid, H. (2011). Isolation and characterization of *Ralstonia solanacearum* from infected tomato plants of Soan Skesar valley of Punjab. *Pakistan Journal of Botany*, *43*(6), 2979–2985.

De Morais, T. P., Lopes, C. A., Tebaldi, N. D., & Luz, J. M. Q. (2015). Ocorrência e diversidade de populações de *Ralstonia solanacearum* no Brasil. *Bioscience Journal*, *31*(6), 1722–1737.

Dheemanth, T. L., Nazeem, P. A., Sadhan Kumar, P. G., K. Mathew, S., & Amaranatha Reddy, M. (2018). Validation of SSR Markers for Imparting Disease

Resistance in Tomato (*Solanum lycopersicum* L.). *International Journal of Current Microbiology and Applied Sciences*, *7*(1), 1513–1522.

FOASTAT. (2020). *Production / Yield quantities.*

Gbonamou, M., N'Guessan, A. C., Kone, D., & Bamba, M. (2021). Spread of bacterial wilt disease of potato in the highlands of Fouta Djalon, Republic of Guinea. *International Journal of Biological and Chemical Sciences*, *14*(9), 3353–3360.

Gildemacher, P. R., Demo, P., Barker, I., Kaguongo, W., Woldegiorgis, G., Wagoire, W. W., Wakahiu, M., Leeuwis, C., & Struik, P. C. (2009). A description of seed potato systems in Kenya, Uganda and Ethiopia. *American Journal of Potato Research*, *86*(5), 373–382.

Gopalakrishnan, C., Singh, T. H., & Artal, R. B. (2014). Evaluation of eggplant accessions for resistance to bacterial wilt caused by *Ralstonia solanacearum* (E . F . Smith) Yabuuchi et al., *J. Hortl. Sci*. *9*(2), 202–205.

Hayat Bhatti, K., Kausar, N., Rashid, U., Hussain, K., Nawaz, K., & Hussain Siddiqi, E. (2013). Effects of biotic stresses on egg plant (*Solanum melongena* L.). *World Applied Sciences Journal*, *26*(3), 302–311.

Hayward, A. C. (1991). Bacterial Wilt Caused by pseudomonas solanacearum. *Annual Review Phytopathology*, *29*, 65–87.

Huet, G. (2014). Breeding for resistances to *Ralstonia solanacearum*. *Frontiers in Plant Science*, *5*, 1–5.

Karim, Z., Hossain, M., & Begum, M. (2018). *Ralstonia solanacearum*: A threat to potato production in Bangladesh. *Fundamental and Applied Agriculture*,

3(1), 407 - 421.

Kim, S. G., Hur, O. S., Ro, N. Y., Ko, H. C., Rhee, J. H., Sung, J. S., Ryu, K. Y., Lee, S. Y., & Baek, H. J. (2016). Evaluation of resistance to *Ralstonia solanacearum* in tomato genetic resources at seedling stage. *Plant Pathology Journal*, *32*(1), 58–64.

Knapp, S., Vorontsova, M. S., & Prohens, J. (2013). Wild Relatives of the Eggplant (*Solanum melongena* L.: Solanaceae): New understanding of species names in a complex group. *PLoS ONE*, *8*(2) 1 - 12.

Kumar, D., Saleem Dar, M., Khan, K., Kumar Choudhary, D., Srinagar, R., (2018). *Ralstonia solanacearum*: A wide spread and global bacterial plant wilt pathogen. *Journal of Pharmacognosy and Phytochemistry JPP*, *7*(72), 85–90.

Lebeau, A., Daunay, M. C., Frary, A., Palloix, A., Wang, J. F., Dintinger, J., Chiroleu, F., Wicker, E., & Prior, P. (2011). Bacterial wilt resistance in tomato, pepper, and eggplant: Genetic resources respond to diverse strains in the *Ralstonia solanacearum* species complex. *Phytopathology*, *101*(1), 154–165.

Lebeau, A., Gouy, M., Daunay, M. C., Wicker, E., Chiroleu, F., Prior, P., Frary, A., & Dintinger, J. (2013). Genetic mapping of a major dominant gene for resistance to *Ralstonia solanacearum* in eggplant. *Theoretical and Applied Genetics*, *126*(1), 143–158.

Mamphogoro, T. P., Babalola, O. O., & Aiyegoro, O. A. (2020). Sustainable management strategies for bacterial wilt of sweet peppers (*Capsicum annuum*) and other Solanaceous crops. *Journal of Applied Microbiology*, *129*(3), 496–508.

Manda, R. R., Addanki, V. A., & Srivastava, S. (2020). Bacterial wilt of solanaceous crops. *International Journal of Chemical Studies*, *8*(6), 1048–1057.

Marques, E., Uesugi, C. H., Ferreira, M. A. S. V., & de Rezende, D. V. (2012). Characterization of isolates of *Ralstonia solanacearum* biovar 2, pathogenic to eucalyptus "urograndis" hybrids. *Tropical Plant Pathology*, *37*(6), 399–408.

Meng, F. (2013). *Ralstonia Solanacearum* Species Complex and Bacterial Wilt Disease. *Journal of Bacteriology & Parasitology*, *04*(02), 2–5.

Mew, T. ., & Ho, W. . (1977). Effect of soil temperature on resistance of tomato cultivars to bacterial wilt *Phytopathology*, *67*, 909–911.

Muradashvili, M. T., Meparishvili, G. V, Tediashvili, M. I., & Britain, G. (2015). Phenotypic properties of Georgian isolates of *Ralstonia solanacearum*. *Annals of Agrarian Science*, *13*(3), 20–25.

Muthoni, J., Shimelis, H., Melis, R., & Kinyua, Z. M. (2014). Response of potato genotypes to bacterial wilt caused by *Ralstonia solanacearum* (Smith)(Yabuuchi et al. year) in the tropical highlands. *American Journal of Potato Research*, *91*(2), 215–232.

Mutlu, N., Boyaci, F. H., Göçmen, M., & Abak, K. (2008). Development of SRAP, SRAP-RGA, RAPD and SCAR markers linked with a *Fusarium* wilt resistance gene in eggplant. *Theoretical and Applied Genetics*, *117*(8), 1303–1312.

Mwaniki, K. P., Birech, R., Wagara, I. N., Kinyua, Z. M., Schulte-Geldermann, E., & Freyer, B. (2016). Distribution, prevalence and incidence of potato bacterial wilt in Nakuru County , Kenya. *International Journal of Innovative*

Research and Development, *5*(1), 435–442.

Namisy, A., Chen, J. R., Prohens, J., Metwally, E., Elmahrouk, M., & Rakha, M. (2019). Screening cultivated eggplant and wild relatives for resistance to bacterial wilt (*Ralstonia solanacearum*). *Agriculture (Switzerland)*, *9*(7), 1–11.

Nunome, T., Negoro, S., Kono, I., Kanamori, H., Miyatake, K., Yamaguchi, H., Ohyama, A., & Fukuoka, H. (2009). Development of SSR markers derived from SSR-enriched genomic library of eggplant (*Solanum melongen*a L.). *Theoretical and Applied Genetics*, *119*(6), 1143–1153.

Oliveira, I. T., Lopes, C. A., & Moura, A. B. (2014). Fruit yield and bacterial wilt symptoms on eggplant genotypes grown in soil infested with *Ralstonia solanacearum*. *Horticultura Brasileira*, *32*(4), 453–457.

Osei, M. K., Banful, B., Osei, C. K., & Oluoch, M. O. (2010). Characterization of African eggplant for morphological characteristics. *4*(3), 33 - 37.

Paudel, S., Dobhal, S., Alvarez, A. M., & Arif, M. (2020). Taxonomy and phylogenetic research on *Ralstonia solanacearum* species complex: A complex pathogen with extraordinary economic consequences. *Pathogens*, *9*(11), 1–26.

Phoebe, K. M., Mathew, M. A., Isabel, N. W., Joseph, N. W., & Schroers, H. J. (2016). Response of African eggplants to *Fusarium spp*. and identification of sources of resistance. *African Journal of Biotechnology*, *15*(11), 392–400.

Plazas, M., Andújar, I., Vilanova, S., Gramazio, P., Her-, F. J., & Prohens, J. (2014). Morphological diversity in Gboma eggplant (*Solanum macrocarpon*) as assessed with conventional and tomato analyzer descriptors, *72*(2), 355-356.

Pradhanang, P. M., Elphinstone, J. G., & Fox, R. T. V. (2000). Sensitive detection of

Ralstonia solanacearum in soil: A comparison of different detection techniques. *Plant Pathology*, *49*(4), 414–422.

Reddy, A. C., Venkat, S., Singh, T. H., Aswath, C., Reddy, K. M., & Reddy, D. C. L. (2015). Isolation, characterization and evolution of NBS-LRR encoding disease-resistance gene analogs in eggplant against bacterial wilt. *European Journal of Plant Pathology*, *143*(3), 417–426.

Reynolds, T. W., Waddington, S. R., Anderson, C. L., Chew, A., True, Z., Cullen, A., Reynolds, T. W., Anderson, C. L., Chew, A., & True, Z. (2015). Environmental impacts and constraints associated with the production of major food crops in Sub-Saharan Africa and South Asia, *Food Security*. 7,795–822.

Salgon, S., Jourda, C., Sauvage, C., Daunay, M. C., Reynaud, B., Wicker, E., & Dintinger, J. (2017). Eggplant resistance to the *Ralstonia solanacearum* species complex involves both broad-spectrum and strain-specific quantitative trait loci. *Frontiers in Plant Science*, *8*, 1–21.

Sanchez Perez, A., Mejia, L., Fegan, M., & Allen, C. (2008). Diversity and distribution of *Ralstonia solanacearum* strains in Guatemala and rare occurrence of tomato fruit infection. *Plant Pathology*, *57*(2), 320–331.

Sanders, E. R. (2012). Aseptic laboratory techniques: Plating methods. *Journal of Visualized Experiments*, *63*, 1–18.

Savary, S., Ficke, A., Aubertot, J. N., & Hollier, C. (2012). Crop losses due to diseases and their implications for global food production losses and food security. *Food Security*, *4*(4), 519–537.

Sękara, A., Cebula, S., & Kunicki, E. (2007). Cultivated eggplants – origin, breeding

objectives and genetic resources, a review. *World*, *19*(1), 97–114.

She, X., Yu, L., Lan, G., Tang, Y., & He, Z. (2017). Identification and genetic characterization of *Ralstonia solanacearum* species complex isolates from cucurbita maxima in China. *Frontiers in Plant Science 8,* 1 - 11.

Siljak-yakovlev, S., & Paris-sud, U. (2001). Collonier C ., Karden M ., Fock I ., Mariska I ., Servaes A ., Vedel F ., Siljak-Yakovlev S ., Souvannavong V ., Ducreux G ., Sihachakr D . 2001 - Source of resistance against *Ralstonia solanacaerum* somatic hybrids of eggplant (*Solanum melongena* L .) with *Solanum aethiopicum*, *160,*301 - 313.

Singh, T. H., Reddy, D. C. L., Reddy, C. A., Sadashiva, A. T., Pandyaraj, P., & Manoj, Y. B. (2019). Evaluation of *Solanum* species and eggplant cultivated varieties for bacterial wilt resistance. *Journal of Horticultural Sciences*, *14*(1), 13–19.

Siri, M. I., Galván, G. A., Quirici, L., Silvera, E., Villanueva, P., Ferreira, F., Franco Fraguas, L., & Pianzzola, M. J. (2009). Molecular marker diversity and bacterial wilt resistance in wild *Solanum commersonii* accessions from Uruguay. *Euphytica*, *165*(2), 371–382.

Swanson, J. K., Montes, L., Mejia, L., & Allen, C. (2007). Detection of latent infections of *Ralstonia solanacearum* race 3 biovar 2 in geranium. *Plant Disease*, *91*(7), 828–834.

Taher, D., Solberg, S., Prohens, J., Chou, Y. Y., Rakha, M., & Wu, T. H. (2017). World vegetable center eggplant collection: Origin, composition, seed dissemination and utilization in breeding. *Frontiers in Plant Science*, *8,* 1–12.

Tessema, L., Seid, E., Woldegiorgis, G., & Sharma, K. (2020). Current status of bacterial wilt *(Ralstonia solanacearum)* disease in major seed potato (Solanum tuberosum L.) growing areas of Ethiopia. *Journal of Plant Pathology & Microbiology*, *11*(5), 1–8.

Truong, H. T. H., Kim, S., Tran, H. N., Nguyen, T. T. T., Nguyen, L. T., & Hoang, T. K. (2015). Development of a SCAR marker linked to bacterial wilt *(Ralstonia solanacearum)* resistance in tomato line Hawaii 7996 using bulked-segregant analysis. *Horticulture Environment and Biotechnology*, *56*(4), 506–515.

Ubisi, N. R., Mafongoya, P. L., Kolanisi, U., & Jiri, O. (2017). Smallholder farmer's perceived effects of climate change on crop production and household livelihoods in rural Limpopo province, South Africa. *Change and Adaptation in Socio-Ecological Systems*, *3*(1), 27–38.

Uwamahoro, F., Berlin, A., Bucagu, C., Bylund, H., & Yuen, J. (2018). Potato bacterial wilt in Rwanda: occurrence, risk factors, farmers' knowledge and attitudes. *Food Security*, *10*(5), 1221–1235.

Vale, F. X. R. Do, Parlevliet, J. E., & Zambolim, L. (2001). Concepts in plant disease resistance. *Fitopatologia Brasileira*, *26*(3), 577–589.

Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D., & Prior, P. (2009). Epidemiological evidence for the emergence of a new pathogenic variant of *Ralstonia solanacearum* in martinique (French West Indies). *Plant Pathology*, *58*(5), 853–861.

Xi'ou, X., Bihao, C., Guannan, L., Jianjun, L., Qinghua, C., Jin, J., & Yujing, C. (2015). Functional Characterization of a Putative Bacterial Wilt Resistance Gene (RE-bw) in Eggplant. *Plant Molecular Biology Reporter*, *33*(4), 1058–1073.

Xue, Q. Y., Yin, Y. N., Yang, W., Heuer, H., Prior, P., Guo, J. H., & Smalla, K. (2011). Genetic diversity of *Ralstonia solanacearum* strains from China assessed by PCR-based fingerprints to unravel host plant- and site-dependent distribution patterns. *FEMS Microbiology Ecology*, *75*(3), 507–519.

Yuliar, Asi Nion, Y., & Toyota, K. (2015). Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum. Microbes and Environments*, *30*(1), 1–11.

APPENDICES

Appendix I: African Eggplant Accessions Used in the Present Study

S/no	Accession Number	Origin	Family
1	RV100246	Unknown	*Solanum aethiopicum*
2	RV100245	Mali	*Solanum aethiopicum*
3	RV100334	Mali	*Solanumaethiopicum*
4	RV100352	Uganda	*Solanum aethiopicum*
5	RV100328	Mali	*Solanum aethiopicum*
6	RV100330	Mali	*Solanum aethiopicum*
7	RV100264	Mali	*Solanum aethiopicum*
8	RV100432	Unknown	*Solanum spp*
9	RV100445	Unknown	*Solanum spp*
10	RV100333	Mali	*Solanum aethiopicum*
11	RV100185	Gabon	*Solanum aethiopicum*
12	RV100259	Senegal	*Solanumaethiopicum*
13	RV100250	Mali	*Solanumaethiopicum*
14	RV100453	Unknown	*Solanumspp*
15	RV100342	Cameroon	*Solanum aethiopicum*
16	GBK50591	Unknown	*Solanum spp*
17	RV100452	Unknown	*Solanum spp*
18	RV100270	Mali	*Solanum aethiopicum*
19	RV100201	Malawi	*Solanum aethiopicum*
20	RV100455	Unknown	*Solanum spp*

21	RV100332	Bukina Faso	*Solanum aethiopicum*
22	RV100247	Mali	*Solanum aethiopicum*
23	RV100447	Mali	*Solanum spp*
24	RV100335	Cameroon	*Solanum anguivi*
25	RV100161	Tanzania	*Solanum aethiopicum*
26	RV100242	Mali	*Solanum aethiopicum*
27	RV100234	Mali	*Solanum aethiopicum*
28	RV100438	Unknown	*Solanum aethiopicum*
29	RV100240	Mali	*Solanum aethiopicum*
30	RV100218	Unknown	*Solanum aethiopicum*
31	RV100364	Uganda	*Solanum anguivi*
32	RV100263	Mali	*Solanumaethiopicum*
33	RV100239	Mali	*Solanum aethiopicum*
34	RV100271	Mali	*Solanum aethiopicum*
35	RV100169	Tanzania	*Solanum aethiopicum*
36	RV100268	Mali	*Solanum aethiopicum*
37	RV100386	Ivory Coast	*Solanum aethiopicum*
38	RV100377	Uganda	*Solanum aethiopicum*
39	RV100265	Mali	*Solanum aethiopicum*
40	RV100261	Mali	*Solanum aethiopicum*
41	RV100217	Mali	*Solanum aethiopicum*
42	RV100327	Mali	*Solanum aethiopicum*
43	RV100511	Tanzania	*Solanum aethiopicum*

44	RV100360	Uganda	*Solanum anguivi*
45	RV100190	Tanzania	*Solanum anguivi*
46	RV100458	Unknown	
47	RV100 331	Unknown	

African eggplant accessions are used for screening for resistance. 47 accessions obtained from 4 species were used to identify their reaction to bacterial wilt infection.

Appendix II: Bacterial Wilt Resistance Markers for 47 African Eggplant Accessions

		SSR Markers					Origin	Species
S/No.	**Accession**	**ecm009**	**emk03O04**	**ecm001**	**emi04P17**	**SOL5036**		
1	RV100438	0	1	1	1	1	Unknown	*Solanum aethiopicum*
2	RV100185	1	0	1	0	1	Gabon	*Solanum aethiopicum*
3	RV100334	0	1	1	1	1	Mali	*Solanum aethiopicum*
4	RV100234	0	0	0	0	0	Mali	*Solanum aethiopicum*
5	RV100453	1	1	1	1	1	Unknown	*Solanum sp*
6	RV100246	1	1	1	1	1	Unknown	*Solanum aethiopicum*
7	RV100458	0	0	0	0	0		
8	RV100364	1	0	0	0	0	Uganda	*Solanum anguivi*
9	RV100245	0	0	0	0	0	Mali	*Solanum aethiopicum*
10	RV100360	1	1	1	1	1	Uganda	*Solanum anguivi*
11	RV100242	1	0	1	1	1	Mali	*Solanum aethiopicum*
12	RV100445	1	1	1	1	1	Unknown	*Solanum aethiopicum*
13	RV100217	1	0	0	1	1	Mali	*Solanum aethiopicum*
14	RV100268	1	1	1	1	1	Mali	*Solanum aethiopicum*
15	RV100352	1	1	1	1	1	Uganda	*Solanum aethiopicum*

16	RV100259	1	0	1	0	0	Senegal	*Solanum aethiopicum*
17	RV100190	0	1	1	1	1	Tanzania	*Solanum anguivi*
18	RV100455	1	1	1	1	0	Unknown	*Solanum sp*
19	RV100265	0	1	1	1	1	Mali	*Solanum aethiopicum*
20	RV100239	0	1	0	0	1	Mali	*Solanum aethiopicum*
21	RV100328	0	0	0	0	0	Mali	*Solanum aethiopicum*
22	RV100327	0	0	0	0	1	Mali	*Solanum aethiopicum*
23	RV100240	0	0	0	0	0		
24	RV100270	0	0	1	0	1	Mali	*Solanum aethiopicum*
25	RV100452	0	0	1	0	1	Unknown	*Solanum sp*
26	RV100432	0	0	1	0	0	Unknown	*Solanum sp*
27	RV100218	0	0	0	0	0		*Solanum aethiopicum*
28	RV100201	0	0	0	0	0	Malawi	*Solanum aethiopicum*
29	RV100331	0	0	0	0	0		
30	RV100264	0	0	0	0	0	Mali	*Solanum aethiopicum*
31	RV100447	0	0	0	0	0	Mali	*Solanum sp*
32	RV100161	0	0	0	0	0	Tanzania	*Solanum aethiopicum*
33	RV100247	0	0	0	0	0	Mali	*Solanum aethiopicum*
34	RV100377	0	0	1	0	1	Uganda	*Solanum aethiopicum*
35	RV100250	0	0	0	0	0	Mali	*Solanum aethiopicum*
36	RV100333	0	1	0	0	0	Mali	*Solanum aethiopicum*

37	RV100330	1	1	1	1	1	Mali	*Solanum aethiopicum*
38	RV100332	0	0	0	0	0	Bukina Faso	*Solanum aethiopicum*
39	RV100342	0	0	0	0	0	Cameroon	*Solanum aethiopicum*
40	RV100511	0	0	1	0	0	Tanzania	*Solanum aethiopicum*
41	RV100263	1	0	0	0	0	Mali	*Solanum aethiopicum*
42	GBK050572	0	0	0	1	0		
43	RV100271	0	0	0	0	0	Mali	*Solanum aethiopicum*
44	RV100335	0	0	0	0	0	Tanzania	*Solanum sp*
45	RV100386	0	0	0	0	0	Ivory Coast	*Solanum aethiopicum*
46	RV100169	0	0	0	0	1	Tanzania	*Solanum sp*
47	RV100261	0	0	1	1	1	Mali	*Solanum aethiopicum*

Resistance makers present (1) and absent (0) in 47 African eggplant accessions. The resistance was concentrated in accessions that did not have a clear origin while the majority of accessions tested originated from Mali.