CSIR COLLEGE OF SCIENCE AND TECHNOLOGY

EVALUATION OF TOMATO (*SOLANUM LYCOPERSICUM* L) GENOTYPES FOR BACTERIAL WILT (*RALSTONIA SOLANACEARUM*) RESISTANCE IN GHANA

ISAAC NEWTON BOAKYE-MENSAH

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BY

ISAAC NEWTON BOAKYE-MENSAH

Thesis submitted to the Department of Plant Resources Development of the CSIR College of Science and Technology, in partial fulfillment of the requirements for the award of Master of Philosophy in Plant Breeding and Biotechnology

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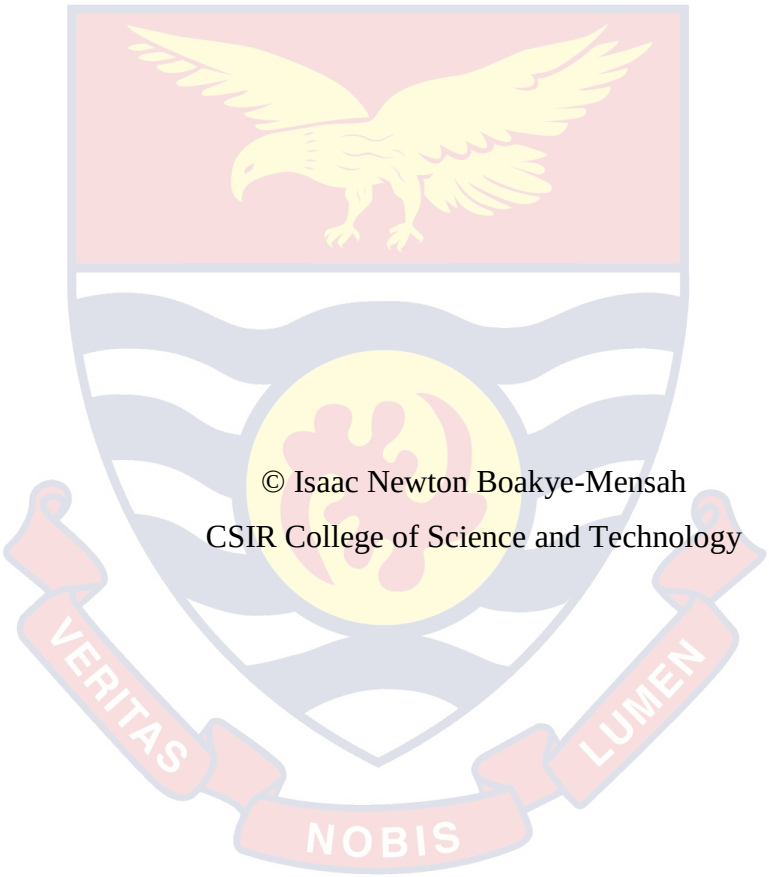

CSIR College of Science and Technology

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my original research work and that no part has been presented for another degree in this College or elsewhere.

Candidate's Signature……..Date……..

Name: Isaac Newton Boakye - Mensah

Supervisors' Declaration

We hereby declare that the preparation and presentation of this thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the CSIR College of Science and Technology.

Principal Supervisor's Signature: ……..Date: ……

Name: Dr. (Mrs.) Ruth Prempeh

Co-Supervisor's Signature: ……Date: ……

Name: Dr. Kingsley Osei

ABSTRACT

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables which ranks second after potato in the world. Its productivity is extremely low due to the effect of pests and diseases. Bacterial wilt, caused by *Ralstonia solanacearum* is one of the most devastating and wide-spread bacterial diseases of tomato. The objective of the study was to identify tomato genotypes resistant to bacterial wilt disease that can be used for future improvement of the crop. The study collected and characterized 20 isolates from some major tomato growing areas in Ghana using both morphological and molecular tools to determine prevalent bacterial wilt strains, screenhouse and field evaluations coupled with marker assisted selection were used to identify resistant materials. In addition, broad sense heritability was estimated for bacterial wilt disease and other economic traits in tomato. From this present study, phylotype II was identified based on the molecular characterization. Four accessions H7996, LA 0442, LA 0443 and LA 0376 were identified to be resistant under both screenhouse and field conditions. For maker validation, six out of the twelve accessions possessed both *Bwr-12* and *Bwr-6* genes which confer resistance to BW disease. High broad-sense heritability was observed for both disease incidence, severity of BW disease, as well as other agronomic characters investigated. The four promising lines identified could be evaluated and subsequently released to farmers. In addition, findings from this study could be used to improve the tomato breeding programme in the country.

KEY WORDS

Bacterial wilt disease

Inoculation

Marker assisted selection

Ralstonia solanacearum

Resistant gene

Tomato

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DEDICATION

To my father, the late Mr. Samuel Boakye-Mensah

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LIST OF ACRONYMS

BW	Bacterial Wilt
Bwr	Bacterial Wilt Resistance
Cells/ml	Cells per milliliter
Cfu/ ml	Colony forming unit per milliliter
Cfu	Colony forming unit
CRBD	Completely Randomized Block Design
CRD	Completely Randomized Design
CTAB	Cetyltrimethylammonium Bromide
Cm	Centimeters
M	Meters
DNA	Deoxyribonucleic Acid
EPS	Exopolysaccharide
Ha	Hectare
Hr	Hour
KOH	Potassium Hydroxide
Mg	Milligram
NA	Nutrient Agar
O.D	Optical Density
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
Rpm	Revolution per minutes
TSS	Total Solid Soluble

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CHAPTER ONE

INTRODUCTION

Background of the Study

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop in most parts of the world and generally a dominant ingredient in thousands of dishes in today's kitchen. According to FAOSTAT (2018), it covered a total production of 182 million metric tons harvested from 4.8 million hectares in 2017. It is a key vegetable crop consumed worldwide in almost every household because of its nutrients and taste. Tomato consumption helps in providing good nutritional balance in the diets of human. It contains vitamins A and C, potassium, phosphorus, magnesium and calcium (Borguini & Torres, 2009; Tambo & Gbemu, 2010). It also contains lycopene, an antioxidant that reduces aging, sunburns and the risk of certain types of cancer (Miller *et al.*, 2002; Perveen *et al*., 2015).

In most West African countries including Ghana, the crop is produced mainly for domestic consumption (Norman, 1992). In addition, its production helps to increase income of farm families. As a highly valued vegetable crop, smallholders have now switched from subsistence to commercial farming, thereby improving their livelihood (Hanson *et al.*, 2016). Nevertheless, production of the crop has not appreciated to its full potential particularly in terms of yields. This has to be improved so as to enhance the livelihoods of farmers and other stakeholders along the tomato value chain. Key limiting factors to the production of tomato include pests and diseases in the nursery and on the field (Prasannath, Dharmadasa, De Costa, & Hemachandra, 2014). Among the several diseases affecting tomato, bacterial wilt disease caused by

Ralstonia solanacearum species complex is a major problem in warm climates worldwide (Daunay, Laterrot, Scott, Hanson, & Wang, 2010; Huerta, Milling, & Allen, 2015). *R. solanacearum* is a rod-shaped bacterium without spore formation and gram negative when stained. It is a facultative parasite of economic importance affecting 200 plant species in over 40 families which include a wide range of ornamentals, weeds and crop plants such as tomato, eggplant, pepper and sweet potato (Pradhanang, Elphinstone, & Fox, 2000; Peeters, Guidot, Vailleau, & Valls, 2013; Lopes, Rossato, & Boiteux, 2015).

Statement of the Problem

The bacterium (*R. solanacearum*) is known to be soil borne with a wide host range causing sudden death and stem rot of plants (Kazuhiro & Caitlyn, 2009). According to Denny (2006), the disease can cause 91 % yield loss based on the tomato variety, soil type, cropping system, climatic conditions and bacterial strains present at the location. The bacterium can survive in soil for extended periods without a host plant (Huang *et al*., 2012). This pathogen primarily attacks host stems and roots leading to browning of the vascular system and rotting of roots due to secondary bacterial infection (Perrier *et al.*, 2016). In addition, the pathogen can persist in uneven stages in water, plant residues and rhizosphere as well as within the host plant (Álvarez, López, & Biosca, 2007; Alvarez, López, & Biosca, 2008; Álvarez, Biosca, & López, 2010) and this makes it very difficult to control. There are many methods of controlling bacterial wilt which include; chemical, cultural and biological methods. However, these methods are not effective and the use of

bacterial wilt-resistant cultivars are considered sustainable approach in managing the disease (Lacombe *et al*., 2010).

Research Objectives

The main objective of the study is to evaluate and identify tomato genotypes resistant to bacterial wilt disease for enhanced productivity. Specifically, the study sought to:

1. characterize bacterial wilt strains in some major tomato growing areas in Ghana,
2. identify and select tomato genotypes resistant to bacterial wilt and
3. estimate heritability for bacterial wilt resistance in tomato.

Research Questions

In achieving the objectives of this study, the research will be addressing the following questions;

1. What bacterial wilt strain is found in the major tomato growing areas within the study area?
2. Are there tomato genotypes resistant to bacterial wilt disease?
3. Is bacterial wilt resistance in tomato heritable?

Hypothesis

1. Bacterial wilt strains in major tomato growing areas in Ghana are unknown.

2. Tomato genotypes resistant to bacterial wilt disease may be present.
3. Bacterial wilt resistance in tomato may be heritable.

Significance of the Study

In Ghana, bacterial wilt disease caused by *R. solanacearum* was first discovered on tomato by Subedi, Gilbertson, Osei, Cornelius, and Miller (2014). The disease was identified in fields of tomato production within the Ashanti (Agogo, Akumadan), Northern (Vea, Tono, Pwalugu), and Brong Ahafo (Tanoso, Tuobodom) regions of Ghana in 2012 (Subedi *et al*., 2014). However, not much research has been conducted regarding the management of the disease. It is important that virulent and prevalent strains of the bacteria are identified to enhance the development of strain specific tomato resistant varieties which will consequently enhance production of the crop. Also, there are no effective control measures practiced by farmers against bacterial wilt disease and this has become one of the most serious drawbacks in tomato production since the disease significantly reduces tomato yields up to 91% leaving the farmer poorer (Denny, 2006). There is therefore the need for an effective and environmentally friendly approach for bacterial wilt management and genetic improvement appears to be the best solution for long term sustainability and effective management of soil borne disease such as bacterial wilt in tomato. Evaluation and identification of varieties resistant to bacterial wilt disease is considered a promising approach to manage the disease since there are no resistant varieties in the country.

Delimitation

The study narrowed its scope to screening for tomato genotypes resistant to bacterial wilt disease within major tomato growing areas in Ashanti, Bono East and Ahafo regions in Ghana.

Limitations

1. The study involved laboratory investigation of primers and other regents which was very expensive. As a result, the inability to use larger samples that might have given a true reflection of the phenomenon.
2. Identification of strains was done using only SSR markers, since I did not have the capacity to identify strains using SNP markers.
3. Delays in arrival of importation of laboratory regents and seeds caused me not to repeat some of the experiment such as the field evaluation.

Organisation of the Study

This study comprises of six chapters. The first chapter generally introduces the whole study by way of background to the study, statement of the problem, research objective, research question, significant of the study, delimitations and limitations, as well as the organisation of the study. Chapter two also deals with the review of related literature on bacterial wilt disease of tomato. Chapter three consists of the research methods, Chapter four covers results, chapter five too contains discussion and last but not least Chapter six consists of a summary of findings, conclusion and recommendations.

CHAPTER TWO

LITERATURE REVIEW

Origin and Distribution of Tomato

Tomato (*Solanum lycopersicum* L.) belongs to the family *Solanacae*. It is believed to have originated from the Andes, in what is now called Peru, Bolivia, Chile and Ecuador, where they grew wild (Grandillo *et al*., 2011; Peralta, Knapp & Spooner, 2007). Throughout Southern Europe, the tomato was quickly accepted into the kitchen. The British, for example, admired tomato for its beauty, but believed it was poisonous, as its appearance was similar to that of the wolf peach (Berry-Ottaway, 2001). Now tomato is accepted in many countries worldwide, including Ghana. The crop was introduced in Ghana in the sixteen century, according to Norman, (1992).

Climatic and Soil Requirements

Tomato is a warm season crop requiring a relatively cool, dry climate for high yield and good quality (Nicola, Tibaldi, Fontana, Crops, & Plants, 2009). It can however survive under moderately low temperature, but are intolerant to very low temperatures. It can grow within a wide range of climatic conditions from temperate to hot and humid tropics with temperatures ranging from 10 ºC to 34 ºC as minimum and maximum temperatures respectively with an optimum around 26 ºC to 29 ºC (Naika, Jeude, Goffau, Hilmi, & van Dam, 2005).

The crop grows well in a well-managed sandy loam and heavy clay loams free of hardpan, nevertheless, best results are achieved in deep, well-

drained loams rich in organic matter and plant nutrients with a pH of 6-7 (Obeng-Ofori *et al*., 2007).

Economic Importance, Nutritional and Health Benefits of Tomato

Tomato is a key ingredient in the daily diets of people across all regions in the world and ranks fourth in the world's most important food crops after potato (Peralta & Spooner 2007; Birch *et al*., 2012). The crop is grown for fresh market and processing. However, in most West African countries, it is produced mainly for domestic consumption (Norman, 1992). Tomato production and its consumption help increase income as well as provide good nutritional balance for farm families. The crop contains nutrients such as vitamins A, C and E, beta-carotene, potassium, phosphorus, magnesium and calcium (Tambo & Gbemu, 2010; Perveen *et al*., 2015). It also contains lycopene, which is an antioxidant that reduces the risk of cancer and heart diseases (Miller *et al*., 2002; Ping Chen *et al*., 2015).

Tomato Production in Ghana

In Ghana, tomato is a very important and popular vegetable crop and its cultivation is a key economic activity for low-income farmers (Horna *et al*., 2006). It is eaten by every Ghanaian household almost every day (Osei *et al*., 2010). Furthermore, it is consumed in large amounts and used as flavoring in cooked food and in the raw state as salads. In Ghana, the following locations are noted as the major tomato production areas; Offinso, Techiman, Nkoranza and Wenchi districts (Norman, 1992; Adu-Dapaah & Oppong-Konadu, 2002). According to Eshun, Apori, and Oppong-Anane (2011), production of tomato

in Ghana, covered, about 3,700 hectares of land and accounted for tomato exports of 4,368 metric tonnes valued at $ 427,000 (FAO, 2005). Currently local production does not meet the domestic demand, hence, tomatoes are sometimes imported from Burkina Faso which affects the economy of Ghana (Anaba, G. 2018).

Common tomato varieties cultivated by farmers include; Power Rano, Pectomech, Konkon, Rasta and Wosowoso. Farmers extract seeds from open pollinated varieties and recycle. Power Rano is often the most preferred variety due to its high tolerance and/or resistance to diseases.

Generally, local varieties grow vigorously; fruits are often spherical with low brix, high water content and acidic with a biting taste. According to Robinson and Kolavalli (2010), a range of varieties has emerged over time from uncontrolled crosses due to the open-pollinated nature of varieties cultivated by farmers. Improved varieties include Pectomech, Heinz, and Nimagent F1 (Asuming-Brempong & Boakye, 2008). They have been important drivers of yield improvements in the sector, mostly under irrigated conditions, although they can also do well under rain-fed conditions (Dorward, Galpin, & Shepherd, 2009).

Diseases and Pests of Tomato

Tomato production is affected by numerous biotic and abiotic factors. The biotic factors are mainly pests and diseases and they are the major constraints in all tomato producing areas. Foliar diseases and soil-borne pathogens are of high economic importance in tomato production (Gilardi, Gullino, & Garibaldi, 2018; Hassanein, Abou Zeid, Youssef, & Mahmoud,

2008). Soil temperature and moisture of 25 ºC – 30 ºC and 90 % relative humidity (RH) respectively plays a significant role in the development of diseases. However, since tomato optimum growing temperature falls within that range, the crop grows under high disease pressure (Horvath *et al*., 2012).

Diseases of tomato by pathogenic genera ranges from bacteria, fungi, virus and nematodes (Agrios, 2005; Dean *et al*., 2012; Kumar, 2017). The most prevalent diseases of tomato in Ghana include tomato yellow leaf curl virus, bacterial wilt (*Ralstonia solanacearum*), southern blight (*Sclerotium rolfsii*), Fusarium wilt (*Fusarium oxysporum*), damping-off (*Pythiumdebaryanum*) at the nursery stage, leaf mosaic (*Tomato mosaic virus*), anthracnose (*Colletotrichum lindemuthianum*), nematodes attack and fruit blossom end-rot (*Alternaria altemata*) (Oduro, 2000; Offei, Cornelius, & Sakyi-Dawson, 2008; Osei *et al*., 2012).

Usually, tomato farmers often use pesticides to control diseases and pests for higher gain, nevertheless in most cases these do not provide adequate protection. In addition, pesticides are environmentally unfriendly and harmful to users (Jagtap & Kamble, 2010; Loganathan & Murugan, 2017).

Distribution, Ecology and Host Range of *Ralstonia Solanacearum*

Ralstonia solanacearum is the causative agent of bacterial wilt disease. It is a soil-borne bacterium with complex species. *R. solanacearum* species complex has been grouped into five races, six biovars and four phylotypes (Hayward, 1991; Safni *et al.*, 2014). It is widely spread in temperate, tropical and subtropical regions where low and high temperatures prevail (Hayward, 1994). It is known to attack over 200 plant species in over 40 families,

including ornamentals, weeds and crop plants such as tomatoes, sweet potato, egg plants and peppers (Lopes *et al*., 2015). A close inspection of the base of the stem of an infected plant when cut vertically reveals brown lesions. The disease causes sudden flaccidity of leaves, leading to leave yellowing and death of the whole plant. Wilting and plant death result from a blockage of vascular tissues and decay of the stem at the soil line upwards. The bacterium is spread by infested soil on cultivating tools and machinery used on uninfected land areas, infected transplants, infected running water and dams used for irrigation.

Diagnostic Features of Bacterial Wilt (*Ralstonia solanacearum*)

Signs and symptoms of bacterial wilt disease

Bacterial wilt disease can be associated with sudden wilting of green leaves, yellowing of the foliage and finally browning to death. In addition, sings of *R. solanacearum* include a progressive discoloration of the vascular tissue, portions of the pith and cortex, as disease develops and finally complete necrosis development. Also, slimy viscous ooze appears upon transverse sectioning of an infected plant stem at the point corresponding to the vascular bundles when placed in clear water. At a developed stage in a host plant, the plant collapse and dies because of degradation of blocked xylem vessels and destruction of surrounding tissues. *R. solanacearum* can infect undisturbed roots of susceptible hosts through microscopic wounds caused by the emergence of lateral roots. Breaking of root tips during removal of seedlings for transplanting, feeding activities by nematodes, insects attack, and agricultural equipment also cause wounding on roots creating entry points for

the pathogen. Bacteria then colonize the cortex and advance towards the xylem vessel, from where they rapidly spread in the plant to cause wilting since there is cessation of water-nutrient flow from the soil to the plant.

Management of Bacterial Wilt Disease in Tomato

Bacterial wilt is very difficult to control, thus there is no single control strategy that can completely prevent the losses it cause (Siri, Sanabria, & Pianzzola, 2011; Gutarra, Herrera, Fernandez, Kreuze, & Lindqvist-Kreuze, 2017). According to Huang *et al*. (2012), the bacterium can survive in soil for extended periods without a host plant. Though managing this disease is difficult, there are some control measures practiced by farmers for higher gain, which include cultural practices and use of resistant varieties. According to Lacombe *et al*. (2010), the use of resistant cultivar is considered as a sustainable approach to manage bacterial wilt epidemics. Although the effectiveness of bacterial wilt management rests on the use of resistant cultivars, a cultivar resistant to *R. solanacearum* and good horticultural characteristics has been a challenge for many years.

Cultural management of bacterial wilt

Culturally, bacterial wilt is very difficult to control most especially in already established fields. It can however be used to totally control the disease in uninfected fields and use of pathogen free seeds and seedlings. In addition, seedbeds should be made on land of no history of the disease. On the other hand, crop rotation using a non-susceptible crop such as maize, rice, amaranth, garlic, cauliflower, mung beans and lettuce may help to impede the

development and spread of the pathogen in an infested field. Avoiding movement of water, equipment and soil from infested fields to non-infested ones would also reduce the spread of bacteria into non-infested fields. Fields should not be over-irrigated, because excess soil moisture favors disease build-up. Generally, there can be an appreciable reduction of bacterial wilt disease incidence and severity, allowing the disease to be manageable if cultural practices are cautiously used.

Management of bacterial wilt by means of resistant varieties

Tomato varieties resistant to bacterial wilt are the most effective way to manage the disease. Research has led to the development of tolerant varieties worldwide. However, most of these varieties are not commercial varieties with good fruit characteristics (Hanson *et al.*, 2016). Examples of some tolerant varieties include 'Arthaloka' in Indonesia, 'Delta' in Thailand, and 'Taichung AVRDC 4' in Taiwan.

In order to develop tomato varieties with bacterial wilt resistance with good fruit characteristics, hybridization and or grafting can be considered. Tomato varieties with good resistance but poor horticultural traits can be used as rootstock or crossed with commercial varieties within a specific location. Cultivars such as 'Hawaii 7996' and resistant eggplant rootstocks could be used for tomato improvement. (McAvoy *et al*., 2012; Namisy *et al*., 2019)

Breeding for Resistance

Breeding for resistance does not necessarily require a good source of resistance and the time needed for a good response to selection depends on the

initial frequencies of desirable traits in the target population. High level of genetic frequencies for desired traits depends on screening for parents with high resistance. The screening process requires a good method for inoculum isolation and multiplication, a good experimental design and an efficient inoculation technique to assess the different responses of the material to be screened (Sere, Onasanya, Afolabi, & Abo, 2005).

Resistance mechanism

Metabolites required for growth and maintenance of cellular function helps to promote defense mechanism in plants against insects and diseases. These secondary metabolites of polyphenols in resistant plants prevent the bacteria movement in the plant system by acting as repellents. Inhibitor extracts, tyloses and gums in resistant plants also act like filters, thereby preventing bacteria movement inside the plant system. Steroidal glycoalkaloid like α tomatine is produced in higher concentration in resistant plants, compared to susceptible plants. During plant–pathogen co-evolution, plants developed quantitative resistance (French, Kim, & Pascuzzi, 2016; Yang *et al*., 2017) to bacterial wilt disease. The quantitative resistance could inhibit the multiplication of *R. solanacearum* in plants, which usually leads to a reduction in symptom expression but not absence of the disease.

Expression of defense mechanisms in tomato

Tomato plants respond to *R. solanacearum* infection by up regulating genes for the salicylic acid (SA) and ethylene (ET) defense pathways. Quantitative RT-PCR gene expression analysis in susceptible and resistant

tomato plants infected with *R. solanacearum* revealed little or no activation of the jasmonic acid (JA) pathway genes Pin-2 and LoxA Milling, A., Babujee, L., & Allen, C. (2011). However, both PR-1b and Osm, which are ET-induced, and GluA and PR-1a, which are regulated by the SA pathway, were expressed at significantly higher levels in plants with pathogen cell densities 36108 CFU/g, relative to water inoculated controls. (Milling, A., Babujee, L., & Allen, C. (2011) Hanemian, M., Barlet, X., Sorin, Yadeta, Keller, Favery, & Deslandes, (2016))

Resistant tomato plants, activated the SA and ET defense pathways more rapidly than a susceptible cultivar. BW-resistant H7996 responded to large populations of *R. solanacearum* strains by increasing expression of genes in the ET and SA signaling pathways by two to three orders of magnitude. Defense genes in H7996 were noticeably induced even at lower pathogen cell densities (16107 CFU of GMI1000/gm stem and 36108 CFU of UW551/gm stem). In contrast, susceptible cultivar Bonny Best had no detectable defense response to 16107 CFU/gm. This is in agreement with the general observation that disease-resistant plants have faster and stronger defense responses (Milling, Babujee, & Allen, 2011; Pradhan *et al*., 2017).

Inoculation Techniques

Inoculation of cotyledons is done by wounding. This can be either by needle puncture or carborundum to introduce the pathogen. This technique is recognized to be very effective in different conditions. Stem inoculation is achieved through the injection of bacterial suspension with a fine hypodermic

syringe into the vascular tissue of the stem. The technique has been proven effective.

Inoculation through leaves and petioles

The technique consists of wounding the leaf surface using sand paper or needle after which the inoculum is sprayed directly on the wounded leaves. The petiole at its attachment point to the stem is wounded and the inoculum is sprayed or applied on the wounds. This method is not always successful under dry conditions since the inoculum drop can dry before getting inside the plant. It is however always successful through feeding activities of insect pests (Chinchilla-Ramírez *et al*., 2020).

Root inoculation through infested soils

This method is an effective inoculation technique where bacterial suspensions (50 ml of $8x10^6$ cfu/ml) of inoculum solution are used to water seedlings during transplanting. Hanson *et al*. (2016) used the root injury technique to inoculate tomato plants by applying the bacteria suspension on cut lateral roots of 4-6 weeks old plants.

Inoculation by dipping roots in bacterial suspension

The method consists of dipping plant roots in the bacterial suspension before transplanting in the field or in greenhouses. Its effectiveness is achieved by wounding the roots through cutting 1-2 cm of the roots before transplanting. In addition, the roots must be planted immediately after inoculation to prevent them from drying (Hanson *et al*., 2016; Nawangsih, Damayanti, Wiyono, & Kartika, 2011).

Genes Involved in Bacterial Wilt Resistance

Markers for bacterial wilt resistant genes in tomato

Resistant genes perform a key role in the combat against pathogens. Generally, resistant cultivars perform better than susceptible cultivars under infestation. However, variation in environmental conditions and specie complexity may lead to breaking of resistance and lowering of performance. One of the best method for screening tomato lines for BW resistance is by using molecular tools because of its precision. Several markers such as SSRs (simple sequence repeats) and SNP (single nucleotide polymorphism) have been used to identify genes in tomato that confer resistance to bacterial wilt. Two genes *Bwr-6* and *Bwr-12* has been identified in tomato that confers resistance to bacterial wilt disease. These two genes have proven to be resistant to *Ralstonia solanacearum* species in many locations worldwide.

According to Carmeille *et al*. (2006), these genes (*Bwr-6* and *Bwr-12*) are associated with the resistance observed in tomato accession (Hawaii 7996). Quantitative trait loci (QTL) associated with these genes in tomato have been reported on chromosomes 4, 6, 11 and 12. QTLs on chromosomes 6 and 12 have however been reported to be present in stable resistant tomato accessions widely (Wang *et al*., 2013). These QTLs were confirmed in Hawaii 7996 under different environmental conditions. Several SSR markers associated with *Bwr-12* and *Bwr-6* genes have been developed for marker assisted selection for bacterial wilt resistance in tomato (Wang *et al*. 2013).

Heritability of Genes

Heritability is the degree of correspondence between phenotypic and genotypic values. It also determines the extent by which genes are transferred to successive generations, indicating the level of resemblance of progenies to their parents (Luna *et al*., 2012). Knowledge on heritability of a trait helps the plant breeder in imagining the behaviour of successive generations and predicts the response to selection. Heritability is a key concept, particularly in selective plant breeding and it is important because it is used in estimating physiological traits in recombinant inbred lines in tomato crop (Flowers *et al*., 2005). In addition, it has broad applications across a range of disciplines, from evolutionary biology, agriculture to human medicine (Wray, & Visscher, 2008).

Heritability can be either broad-sense or narrow-sense. Broad-sense heritability expresses the proportion of phenotypic variance attributed to genetic variance whereas narrow-sense heritability is the proportion attributable to additive gene effects and the extent to which genes are transmitted from parents to their progenies (Falconer, 1989; Lebeau *et al*., 2013).

CHAPTER THREE

RESEARCH METHODS

Experimental Site

The study was conducted at the CSIR-Crops Research Institute (CSIR-CRI) research station, Kwame Nkrumah University of Science and Technology (KNUST) and Ministry of Food and Agriculture (MoFA) at Bechem district in the Ahafo region.

Field experiment was carried out at Bechem MoFA demonstrational field. This field is a hotspot for bacterial wilt disease and is characterized by a bimodal rainfall pattern with a major season starting from April and ending in July, followed by minor season which begins from September and ends in November. Also, isolation and microbial identification were carried out at the KNUST Microbiology Laboratory whiles molecular and greenhouse screenings were conducted at the CSIR- CRI at Fumesua and Kwadaso respectively.

Characterization of Bacterial Wilt Strains

Sampling of bacteria associated with bacterial wilt disease

Tomato plants showing symptoms of bacterial wilt were randomly sampled by uprooting them together with the rhizosphere soil of tomato farms in the Ashanti (Agogo and Akomadan), Bono East (Tuabodom) and Ahafo (Bechem) regions. The infected roots and rhizosphere soil were used for the isolation of the bacteria.

Morphological Characterization

Isolation and morphological identification of bacteria

To confirm *R. solanacearum* as the causative agent of tomato wilt, the sampled tomato plants showing symptoms of bacterial wilt disease collected from farms within the study area were used to isolate the microorganism *R. solanacearum*.

The isolation was done by using serial dilution (1mL / 10mL) of infected roots and rhizosphere soil and the bacteria was grown on a Nutrient Agar (NA). One gram of the sample was dissolved in 10 ml of sterile water to make a suspension. Portion of the suspension was inoculated on NA by streaking and were incubated at 37 °C for 24 h.

After preparing the serial dilutions, identification was conducted by grouping and counting colonies using the colony counter. Each colony was sub-cultured in a 9 cm petri dish containing NA to obtain pure cultures using the single hyphal tip technique. Cultures were then maintained on peptone slants for further studies.

Slides for microscopic examination of the bacteria were prepared by gram staining and examined under a compound microscope at high power objective (400 X) magnification. The identification of the bacteria was based on colour and morphology (Kwak *et al*., 2018).

Molecular Identification of Bacteria Isolates

Deoxyribonucleic acid (DNA) extraction

Twenty pure cultures of bacteria grown in petri dishes were used for the extraction using the Cetyltrimethylammonium bromide (CTAB) protocol

(Dwimartina, Arwiyanto, & Joko, 2017; Grover, Chakrabarti, Azmi, & Khurana, 2012). A spatula was used to pick bacteria colonies into a 2 ml sterile eppendorf tube and suspended in 400 ml normal saline. The mixture was vortexed for 20 sec and centrifuged at 13,000 rpm for 10 min. After centrifugation, supernatant was carefully decanted leaving the pellets at the base. The pellets were then washed five times with normal saline to remove the slimy exopolysaccharide (EPS). The harvested pellets were re-suspended in 400 ml CTAB lysis buffer, vortexed and incubated in a water bath at 65 ℃ for 2 h with intermittent shaking by inverting the tube every 20 min. After which equal volumes of phenol chloroform isoamyl alcohol (25:24:1) solution was added, vortexed and centrifuged at 4 ℃ at 13,000 rpm for 1 min. The supernatant was carefully transferred into a new 1.5 ml eppendorf tube and an equal volume of 400 ml of absolute ethanol was added and incubated at 4 ℃ for 10 min to allow precipitation. It was then centrifuged at 13,000 rpm for 10 min and the supernatant was discarded leaving the DNA pellet to dry.

Pellet was then dissolved in 40 ml of DNase free water and stored at -20 ℃. The quality and quantity of the nucleic acid was checked on 0.8 % agarose gel and Nanodrop 2000c Spectrophotometer (Thermo Scientific) respectively.

Phylotype identification

For strain identification, PCR was conducted with four primers (Table 1). A reaction mixture of 10 µl containing 1 µl of 10X Dream taq Buffer Master mix, 0.4 µl of 10 mM, dNTPs, 0.5 µl each of forward and reverse primer, 0.2 µl of Tag polymerase, 2 µl of DNA and 5.4 µl nuclease free water

was prepared and PCR conducted in a thermocycler (GeneAmp cycler, Singapore). The following thermal cycle conditions were used: initial denaturation at 96 °C for 5 minutes; then 30 cycles of denaturation at 94 °C for 15 seconds, annealing at 59 °C for 30 seconds and extension at 72 °C for 30 seconds, then a final extension at 72 °C for 10 minutes (Fegan & Prior, 2005: Fonseca *et al*., 2014).

Table 1 - *List of SSR Primers used for Bacterial Phylotype Identification*

Primer	Primer Sequence (5' – 3')	Expected Band Size (bp)	Phylotype
Nmult:21:1F	CGTTGATGAGGCGCGCAATTT	144	Phylotype I (Asia)
Nmult:21:2F	AAGTTATGGACGGTGGAAGTC	372	Phylotype II(America)
Nmult:23:AF	ATTACGAGAGCAATCGAAAGATT	91	Phylotype III (African)
Nmult:22:InF	ATTGCCAAGACGAGAGAAGTC	213	Phylotype IV(Tropical)
Nmult:22:RR	TCGCTTGACCCTATAACGAGAGTA		

Source: Fegan and Prior (2005)

Molecular Screening for Bacterial Wilt Resistance in Tomato

Two sets of SSR markers (SLM 12 and SLM 6) that confer resistance to BW disease associated with genes *Bw 6* and *Bw 12* were used. The SSR set SLM 12 is made up of two primer pairs and is associated with gene *Bwr-12* whilst SLM 6 is made up of seven primer pairs and associated with gene *Bwr-6* (Table 3).

Germplasm used for molecular screening

This activity consisted of twelve accessions (LA 0376, LA 0442, LA0443, LA 2701, AVTO 1717, AVTO 1713, H7996, Power, Petofake, CRI-ATS 06, CRI-P005 and Konkon) (Table 10) and 25 FI individuals (Table 11). The F1 population was previously generated using an NC2 mating design where five resistants' (LA 0376, LA 0442, LA0443, LA 2701 and H7996) and five susceptible (Power, Petofake, CRI-ATS 06, CRI-P005 and Konkon) parents were used to generate 25 families.

Screening for bacterial wilt disease resistance

Deoxyribonucleic acid (DNA) extraction

Genomic DNA was extracted using modified CTAB Protocol (Turaki *et al*., 2017). Fresh young tomato leaves were sampled and kept on ice to prevent denaturation. Two hundred milligrams of (200 mg) leaf sample were weighed into 2 ml eppendorf tube and kept in liquid nitrogen. The frozen leaf sample was ground to fine powder and 1 ml of freshly prepared CTAB extraction buffer was added under a fume hood. The preparation was shaken vigorously and samples were incubated in a water bath at 65 °C for 25 min, with 5 min interval gentle mixing by inverting the tubes. Samples were then allowed to cool for 3 min, centrifuged at 13,000 rpm for 10 min at room temperature.

Six hundred microlitres (600 ml) of the aqueous phase was transferred into a new 2 ml tube and 600 µl of Chloroform: Isoamyl alcohol (24:1) was added under a fume hood. The preparation was mixed gently by inversion

until the mixture turned milky. It was then centrifuged at 13,000 rpm for 10 min at room temperature.

The above step was repeated and 550 µl of the upper layer was carefully pipetted and dispensed into a clean 2 ml tube. Then, 825 µl of ethanol and 27.5 µl of 3M sodium acetate were added. The solution was mixed 10 times by inversion and centrifuged at 13,000 rpm for 10 min at room temperature. After centrifugation, DNA pellet was washed in 1ml 70 % ethanol, transferred into a 1.5 ml tube and centrifuged again at 13,000 rpm for 5 min. The ethanol was discarded and the DNA pellet dried for 30 min. The pellet was then dissolved in 500 µl 1X TE buffer and 10 µl Ribonuclease (RNase) A was added and incubated at 37 °C for 1 hr with occasional shaking. Ten microlitres of 3 M sodium acetate and 800 µl absolute ethanol was added and mixed gently by inversion. The mixture was incubated at -20 °C for 1 hr and centrifuged at 13,000 rpm for 10 min at room temperature and the supernatant discarded. Pellets were washed with 800 µl of 70 % ethanol and centrifuged at 13,000 rpm for 5 min. Ethanol was discarded and pellets dried at room temperature for 30 to 60 min. Finally, DNA was dissolved in 50 µl of 1X TE buffer and DNA quality checked on 0.8 % ethidium bromide stained agarose gel.

Polymerase chain reaction (PCR)

PCR was conducted using SSR markers (Table 3) associated with *Bwr-12* and *Bwr-6* genes that confer resistance to bacterial wilt in tomato (Wang *et al.*, 2013). The reaction mixture consisted of 10 ng DNA, 0.5 µM each of forward and reverse primers, 10 mM of dNTPs, 10X Dream taq Buffer, PCR

water, and 0.2 Unit of One Taq DNA polymerase (New England). The following cycling conditions were used: an initial denaturation at 94 °C for 10 min, 30 cycles of 94 °C for 30 s, annealing at 55 °C for 45 s, extension at 72 °C for 45 s, and final extension at 72 °C for 7 min. PCR fragments were then separated by electrophoresis using a 1.5 % ethidium bromide stained agarose gel. Amplicons were visualized under UV transilluminator (Alpha Imager Protein Simple, USA) and scored using a binary scoring system where zero signifies absence of the gene and one signifies presence of the marker.

Table 2 - *List of SSR Primers Associated With the Bacterial Wilt Resistance in Tomato*

Marker	R-gene	Repeat motif	Forward primer (5' to 3')	Reverse primer (3' to 5')	Expected product size (bp)
SLM 12–2	*Bw-12*	(TA)11	ATCTCATTCAACGCACACCA	AACGGTGGAAACTATTGAAAGG	209
SLM 12–10	*Bw-12*	(AT)21	ACCGCCCTAGCCATAAAGAC	TGCGTCGAAAATAGTTGCAT	242
SLM 6–124	*Bw-6*	(TAT)10	CATGGGTTAGCAGATGATTCAA	GCTAGGTTATTGGGCCAGAA	292
SLM 6–118	*Bw-6*	(AAT)18	TCCCAAAGTGCAATAGGACA	CACATAACATGGAGTTCGACAGA	183
SLM 6–119	*Bw-6*	(AT)24	GCCTGCCCTACAACAACATT	CGACATCAAACCTATGACTGGA	255
SLM 6–136	*Bw-6*	(AT)37	CCAGGCCACATAGAACTCAAG	ACAGGTCTCCATACGGCATC	290
SLM 6–17	*Bw-6*	(TA)12	TCCTTCAAATCTCCCATCAA	ACGAGCAATTGCAAGGAAAA	186
SLM 6–94	*Bw-6*	(TA)33	CTAAATTTAAATGGACAAGTAATAGCC	CACGATAGGTTGGTATTTTCTGG	276
SLM 6-110	*Bw-6*	(ATT)32	AGAATGCGGAGGTCTGAGAA	ATCCCACTGTCTTTCCACCA	274

Source: Fegan and Prior (2005)

Evaluation of Tomato Genotypes for Bacterial Wilt Resistance

Screening was conducted under screen house conditions and on the field for the assessment of BW resistance in some selected tomato accessions. Plant materials used for the study comprised thirteen (13) tomato lines from the United States of America, Taiwan and Ghana (Table 2).

Table 3 - *Tomato Accessions, Sources and Their Respective Countries of Origin*

Accession	Source	Country
LA 0376	University of California at Davis	USA
LA 2701	University of California at Davis	USA
LA 0716	University of California at Davis	USA
LA 0442	University of California at Davis	USA
LA 0443	University of California at Davis	USA
Hawaii 7996	AVRDC	Taiwan
AVTO 1713	AVRDC	Taiwan
AVTO 1717	AVRDC	Taiwan
CRI-ATS 06	CSIR-CRI	Ghana
CRI-P005	CSIR-CRI	Ghana
Power	LOCAL FARMER	Ghana
Konkon	LOCAL FARMER	Ghana
Petofake	LOCAL FARMER	Ghana

AVRDC=Asian Vegetable Research and Development Centre;
CSIR=Council for Scientific and Industrial Research; CRI=Crops Research Institute

Source: Field data (2019)

Screening of tomato accessions for bacterial wilt resistance in the screenhouse

Screening in the screen house was conducted in pots at temperatures and relative humidity ranging from 21 °C - 38 °C and 21 % - 85 % respectively. The experiment was carried out within four weeks' duration.

Nursery and management

Plastic seed trays were filled with universal potting medium obtained from Dizengoff Ghana Ltd. A seed was carefully put in each cell and watered daily till seedlings emerged (Figure 1).

After emergence of seedlings, watering was done as and when needed to avoid damping-off. Both insecticide Emamectin Benzoate and fungicide Mancozeb Super were timely applied to keep the seedlings healthy.

A B C

Figure 1: Nursed tomato accessions used for the study (A, B and C)

Preparation of inoculum

Pure culture of *R. solanacearum* isolated from freshly wilted tomato plants was cultured on a nutrient broth for 48 hours at 30 ^{0}C. The bacterial suspension was prepared by adjusting the inoculum dosage to an optical density of 0.3 at 600 nm (10^{8} cfu/ml) using spectrophotometer, after which suspension was used to inoculate tomato seedlings using the root dip method (Dubey, Tripathi, Tak, & Devi, 2020; Hanson *et al*., 2016).

Inoculation and transplanting of tomato seedlings

Root tips of three weeks old seedlings were cut to about 10 mm creating a wound or an entry point for the inoculum (Figure 2). The seedlings were then dipped in the bacterial suspension for 15 min before transplanting into a pot containing sterilized loamy soil. For the controls, wounded root tips were dipped in distilled water for 15 min without using the bacterial suspension. Regular cultural practices and plant protection measures were carried out as recommended. The experiment was laid in a Completely Randomized Design (CRD) with three replications having ten (10) plants per replication. Bacterial wilt resistant tomato accession H7996 and susceptible tomato accession Petofake were used as checks.

Figure 2: Seedlings inoculated with bacterial suspension

Field screening of tomato accessions for bacterial wilt resistance

With the field screening, only 12 accessions (LA 0376, LA 0442, LA0443, LA 2701, AVTO 1717, AVTO 1713, H7996, Power, Petofake, CRI-ATS 06, CRI-P005 and Konkon) were used, this is because one of the introduced accessions (LA0716) did not show resistance to the BW disease when evaluated at the screen house, it was therefore excluded from the field evaluation. This activity was conducted in a bacterial wilt hot spot at Bechem in the Ahafo region of Ghana.

Seeds were nursed in plastic seed trays where all nursery cultural practices and plant protection measures were carried out to raise healthy seedlings. Three weeks old seedlings were inoculated with pure isolates of *R. solanacearum* using the same protocol used for the screen house experiment. Inoculation of seedlings was conducted by the root dip method. The experimental area was slashed, ploughed and harrowed so as to pulverize the soil to have a good tilth. Individual plots were labeled and seedlings from trays were arranged according to their labels to avoid mix-up during the transplanting process. Twenty-one days old healthy seedlings were

transplanted in the field at one seedling per hill using a Randomized Complete Block Design (RCBD) with three replications. Seedlings were gently removed and planted on their respective plots followed by watering.

Transplanting was done late in the afternoon to enhance growth of seedling and avoidance of heat shock. A spacing of 80 cm × 40 cm was used with three rows having eight plants per row accommodating 24 plants per plot. In field management, regular cultural practices and plant protection measures were carried out as recommended.

Fertilization and earthing-up

A week after transplanting, Mono Ammonium Phosphate (MAP) granules were mixed in water and applied to seedlings (sprayed) at 50 g per 15 L of water to promote root establishment. In addition, an insecticide, Dean (Emamectin Benzoate + Imidacloprid) was sprayed at 40 ml per 15 L to avoid cricket and other insects from attacking the seedlings. After a week, Yara Winner N: P: K (supplied by Weinco Gh Ltd) was applied at a rate of 5 g per plant. Fungicide Diathane (Dihiocarbomate) and Yara Nitrabor (Sulphate of Ammonia) were also applied two weeks later at 225 g per 15 L and 5 g per plant respectively. Subsequently, an insecticide, Protect (Emamectin Benzoate) and a fungicide Victory [Mancozeb (ethylene-bis-dithiocarbamate) + Metalaxyl (N-(2,6-dimethylohenyl)-N-(methoxyacetyl)] were sprayed at 20 g per 15 L alternatively to control insect pests and diseases at weekly intervals. Four weeks after transplanting, earthing-up of plants was carried out to support plants and increase moisture retention.

Weed management

Weeding was done manually with a hoe and by hand picking regularly to maintain a clean field. Weedicide (Glyphosate) was applied at 200 ml per 15 L around the boundary at an appropriate distance from the experimental field to prevent damage to the crop, keep the surroundings clean and reduce insect pest accumulation around the field.

Data Collection

In the screenhouse experiment, wilting of plants was recorded within four weeks of incubation after inoculation. Plants showing signs and symptoms of bacterial wilt were selected and scored for disease incidence and severity.

In the field experiment, six plants were randomly selected from the middle row of each plot and tagged for data collection. Data collected included; incidence and severity of bacterial wilt, days to 50 % flowering, days to fruit set, plant height, stem girth, number of fruits per plot, weight of fruits, and brix (Total soluble solids). Plant height was measured with a tape measure. Number of days to 50 % flowering and fruit setting were counted (from the first day till when half of the plant population flowered and set fruit respectively) while number of fruit per plot were counted at harvest. The weight of fruits per plant was determined with a measuring scale (Compact 11lbs/5kg 5000g/1g weight electronic digital kitchen diet food postal scale). Total soluble solid (TSS) of fruits was determined with a hand held brix refractometer (Tester meter with ATC 0 - 32 %).

Data Analysis

Data was analyzed after the collection and organization of needed parameters. Disease incidence was calculated using a disease index percentage

$$(\%) = \frac{\text{Number of wilted plant}}{\text{Total number of inoculated plants}} \times 100$$

Whilst severity was evaluated based on a 1 - 5 disease scale (Aslam, Mukhtar, Hussain, & Raheel, 2017).

Where:

1 = no symptoms of wilting

2 = 1 to 25 % of plants wilting

3 = 26 to 50 % of plants wilting

4 = 51 to 75 % of plants wilting

5 = 76 to 100 % of plants wilting

Disease severity index was scored at weekly interval within a period of four weeks after inoculation on a scale of 1–5 and calculated by the Formula:

$$\frac{\text{Score x Number of plants scored}}{\text{Total number of plants x Highest score}} \times 100$$

(Nakaho *et al*., 2004).

Percentage of wilted plants based on incidence and severity were transformed using square root transformation and subjected to analysis of variance (ANOVA) to evaluate the levels of resistance among the accessions using GenStat version 14 software. Means were separated using the Standard Error of Difference (Sed) test at $P < 0.05$.

Also, data collected on, days to 50 % flowering, days to fruit setting, plant height, stem girth, number of fruits per plant, weight of fruits, and brix (Total soluble solids) were subjected to analysis of variance (ANOVA) to evaluate their economic importance among the accessions using GenStat version 14 software. Means were separated using the Standard Error of Difference (Sed) test at $P < 0.05$. From the ANOVA, variance components were calculated and used to estimate broad-sense heritability (H^2_b) by using the formula ($H^2_b = V_G / V_P$) according to Johnson, Robison, and Comstock (1955).

Where:

H^2_b = Broad-sense heritability

V_G = Genetic variance

V_P = Phenotypic variance

CHAPTER FOUR

RESULTS

Morphological Characterization of Bacterial Wilt Strains

A total of 20 samples were collected from Brong East, Ahafo and Ashanti regions to identify the different bacterial wilt strains prevalent in the two regions. Five different isolates, all negative gram stained and rod shaped were identified. They presented varying purity levels ranging from 1.23 to 2.09 (Table 4). White Irregular (WI) isolates dominated the samples representing 30 % followed by White Circular Small (WCS) 20 %, Cream Irregular (CI) 20 %, Irregular (I) 20 % and White Circular Large (WCL) constituted 10 %, the least encountered in the samples.

Table 4 - *Bacterial Wilt (Suspected R. Solanacearum) Isolates Sampled From The Ashanti, Bono East and Ahafo Regions*

Sample Code	Regional Code	Status	Source	Purity	Gram Stain Test	Cell Appearance
1	A1	WCL	Plant	1.67	- ive	Rod shaped
2	A 2	WCS	Soil	1.82	- ive	Rod shaped
3	A 3	WI	Plant	2.04	- ive	Rod shaped
4	A 4	CI	Soil	2.02	- ive	Rod shaped
5	A 5	CI	Plant	2.07	- ive	Rod shaped
6	B 6	WI	Soil	2.01	- ive	Rod shaped
7	B 7	WCS	Soil	2.06	- ive	Rod shaped

Regional code: A = Ahafo; B = Bono East and Ash = Ashanti. Bacteria status: WCL = White Circular Large; WCS = White Circular Small; WI = White Irregular; CI = Cream Irregular; I = Irregular

Source: Field data (2019)

Table 4 - *Continued*

Sample Code	Regional Code	Status	Source	Purity	Gram Stain Test	Cell Appearance
8	B 8	WI	Plant	1.95	- ive	Rod shaped
9	B 9	I	Soil	2.07	- ive	Rod shaped
10	B 10	WCS	Plant	2.09	- ive	Rod shaped
11	Ash 1	WI	Soil	2.02	- ive	Rod shaped
12	Ash 2	I	Plant	2.03	- ive	Rod shaped
13	Ash 3	WCL	Plant	2.02	- ive	Rod shaped
14	Ash 4	CI	Soil	1.88	- ive	Rod shaped
15	Ash 5	I	Plant	1.99	- ive	Rod shaped
16	Ash 6	I	Soil	1.98	- ive	Rod shaped
17	Ash 7	WI	Soil	2.07	- ive	Rod shaped
18	Ash 8	WI	Plant	1.23	- ive	Rod shaped
19	Ash 9	CI	Soil	1.77	- ive	Rod shaped
20	Ash 10	WCS	Plant	1.89	- ive	Rod shaped

Regional code: A = Ahafo; B = Bono East and Ash = Ashanti. Bacteria status: WCL = White Circular Large; WCS = White Circular Small; WI = White Irregular; CI = Cream Irregular; I = Irregular

Source: Field data (2019)

Molecular Characterization

Phylotype identification

Out of the four phylotype specific primers used, only one primer (Nmult21:2F) showed amplification for eleven of the isolates (Figure 3). This primer is specific for *R. solanacearum* strain phylotype II. Bacterial isolates collected from the three regions (Ashanti, Bono East and Ahafo) of Ghana

were therefore identified as Phylotype II (from America) of *R. solanacearum*. None of the isolates belong to either Phylotype I, III or IV.

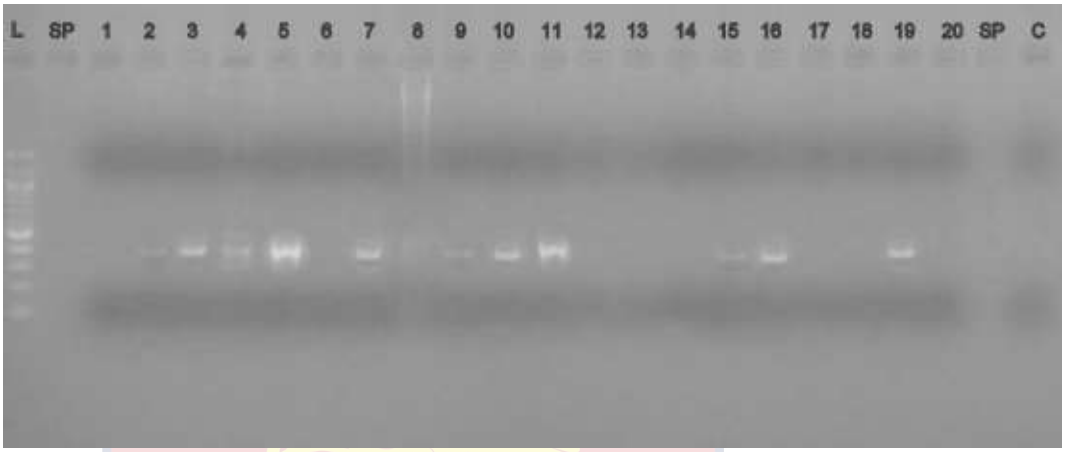

Figure 3: PCR amplifications obtained using Phylotype specific primer Num 21: 2F for confirmation of strain identity of the Bacterial isolate. L= 100bp Ladder, SP= Space and 1 to 20 = samples (Bacterial wilt isolates)

Screenhouse Evaluation of Tomato Accessions for BW Resistance

The results revealed that among the accessions, there were no significant ($p>0.05$) differences at week one of incubation but there were significant ($p< 0.05$) differences among the accessions at weeks two, three and four weeks after inoculation (Tables 5 and 6). Week four after inoculation, the foreign accessions (H7996, AVTO1713, AVTO1717, LA0442, LA0443, LA2701 and LA0376) apart from LA 0716 showed significantly ($p< 0.05$) lower incidence and severity of wilt whiles all the local checks showed higher incidence and severity of wilt. In addition, there were no significant ($p>0.05$) differences among LA 0716 and the local accessions (Petofake, Power, Konkon, CRI-ATS 06 and CRI-P005).

From Table 5, accessions H7996, LA 0442 and LA 0443 showed the lowest level of incidence (2.40) at the end of the experiment which were

statistically ($p>0.05$) not different from LA 0376 (2.83), AVTO 1717 (3.67) and LA 2701 (4.00). However, LA 0716 (7.71), CRI-P005 (8.70), Konkon (8.94), Power (9.05) and CRI-ATS 06 (9.31) recorded high incidence levels and were also not significantly ($p>0.05$) different from Petofake (9.68) which was used as a susceptible check.

The results from Table 6 also indicated that H7996 which was used as a resistant check showed the lowest level of severity (4.48) which was statistically the same as LA 0442 and LA 0443 (4.48) and were also not significantly ($p>0.05$) different from LA 0376 (5.35), AVTO 1717 (7.23) and LA 2701 (7.90). However, LA 0716 (15.36), CRI-P005 (17.36), Konkon (17.84), Power (18.06) and CRI-ATS 06 (18.58) showed very high levels of severity and were not significantly ($p>0.05$) different from Petofake (19.33) which was used as a susceptible check.

Table 5- *Incidence of Bacterial Wilt at Different Incubation Periods for 13 Tomato Accessions*

Accessions	1WAP	2WAP	3WAP	4WA
H7996	0.71	0.71	0.71	2.40
LA0376	0.71	0.71	2.83	2.83
LA 2701	0.71	0.71	2.83	4.00
LA 0716	0.71	1.55	5.47	7.70
LA 0422	0.71	0.71	1.55	2.40
LA 0443	0.71	0.71	1.55	2.40
AVTO 1717	0.71	0.71	0.71	3.67

WAP = week after planting

Source: Field data (2019)

Table 5- *Continued*

Accessions	1WAP	2WAP	3WAP	4WA
AVTO 1717	0.71	0.71	0.71	3.67
AVTO 1713	0.71	1.55	2.40	5.47
CRI-ATS06	0.71	4.43	6.55	9.31
CRI-P005	0.71	3.25	6.22	8.70
Power	0.71	5.29	6.84	9.05
Konkon	0.71	4.53	6.67	8.94
Petofake	0.71	5.47	7.33	9.68
Grand mean	0.71	2.33	3.97	5.89
Sed	0	1.01	1.31	1.06
Fpr	*	<0.001	<0.001	<0.001

WAP = week after planting

Source: Field data (2019)

Table 6 - *Severity Index of Bacterial Wilt at Different Incubation Periods for 13 Tomato Accessions*

Accessions	1WAP	2WAP	3WAP	4WAP
H7996	0.71	0.71	0.71	4.48
LA0376	0.71	2.59	5.35	5.35
LA 2701	0.71	0.71	5.35	7.90
LA 0716	0.71	2.59	10.87	15.36
LA 0422	0.71	0.71	2.59	4.48
LA 0443	0.71	0.71	2.59	4.48

WAP = week after planting

Source: Field data (2019)

Table 6 - *Continued*

Accessions	1WAP	2WAP	3WAP	4WAP
AVTO 1717	0.71	0.71	0.71	7.23
AVTO 1713	0.71	2.59	4.48	10.87
CRI-ATS06	0.71	8.77	13.05	18.58
CRI-P005	0.71	6.22	12.38	17.36
Power	0.71	10.50	13.62	18.06
Konkon	0.71	8.98	13.21	17.84
Petofake	0.71	10.87	14.61	19.33
Grand mean	0.71	4.36	7.66	11.64
Sed	0	2.26	2.74	2.22
Fpr	*	<0.001	<0.001	<0.001

WAP = week after planting

Source: Field data (2019)

Field Screening for Bacterial Wilt Resistance in Tomato Accessions

It was revealed from the results that, LA 0442, LA 0443, LA 0376, AVTO 1713, AVTO 1717, and LA 2701 recorded lower levels of incidence ranging from 2.10 to 12.50 (Table7) which were statistically the same as the resistant check (H7996). However, CRI-P005, CRI-ATS 06, Power and Konkon recorded high incidence levels ranging from 22.92 to 33.33 which were significantly ($p<0.05$) lower than the susceptible check (Petofake) which recorded the highest level of incidence of 52.08 (Table 7).

Results from Table 7 revealed that, LA 0442 and LA 0443 recorded lower severity index of 2.15 and 3.60 respectively. Though they were lower

than the resistant check (H7996), there was no significant differences among them and the resistant check (H7996) as well as LA 0376 which recorded severity index of 4.29. Accessions LA 2701, AVTO 1713, AVTO 1717, CRI-P005, CRI-ATS 06, Power and Konkon had indices ranging from 6.42 to 23.98 and were significantly ($p<0.05$) lower than the susceptible check (Petofake) which recorded the highest index of 28.5.

Table 7 - *Assessment of Bacterial Wilt Incidence and Severity Index for 12 Tomato Accessions*

Accessions	WI	WS
CRI-ATS06	27.10	19.69
AVTO1713	8.30	8.08
AVTO1717	12.50	9.00
H7996	4.20	4.29
Konkon	33.30	23.18
LA 0376	6.20	4.29
LA 0442	2.10	2.15
LA 0443	4.20	3.60
LA 2701	12.50	6.42
CRI-P005	22.90	19.12
Petofake	52.10	28.50
Power	29.20	21.40
Grand mean	17.90	12.48
Sed	5.51	1.894
Fpr	< 0.001	< 0.001

WI= wilt incidence WS= wilt severity

Source: Field data (2019)

Days to 50 % flowering

It was observed that LA 0443 and LA 0376 which produced flowers in 28 and 29 days respectively and were significantly ($p<0.05$) different from accessions CRI-ATS 06 (42 days), LA 2701 (37 days), LA 0442 (33 days) and the resistant check H7996 which produced 50 % flowering at 38 days. However, H7996 was not different from the susceptible check Petofake (41 days) and two other local accessions (Konkon and CRI-P005) which recorded 41 and 39 days respectively. On the other hand, Power recorded a maximum number of 43 days to produce flowers which was not significantly ($p>0.05$) different from the susceptible check Petofake and two other resistant accessions AVTO 1717 and AVTO 1713 (Figure 4).

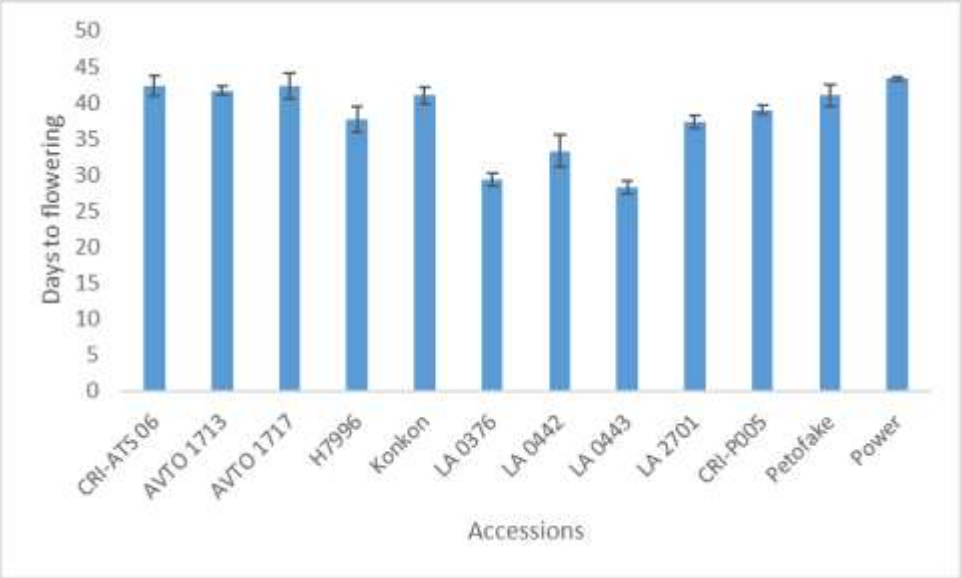

Figure 4 - A histogram of 50 % flowering of 12 tomato accessions

Days to 50 % fruit setting

Results from Figure 5 showed that Power used a maximum number of 56 days in producing 50 % fruit setting which was statistically the same as

CRI-ATS 06, AVTO 1713, AVTO 1717, Konkon and the susceptible check Petofake which had 50 % fruit setting at 55, 55, 55, 53 and 52 days respectively. However, LA0376 and LA 0443 used a minimum number of 46 days to produce 50 % fruit setting which were statistically the same as LA 0442, CRI-P005 and LA 2701 which used 48, 49 and 50 days for fruit setting respectfully but significantly ($p<0.05$) lower than the resistant check H7996 which used 51 days to produce fruit set.

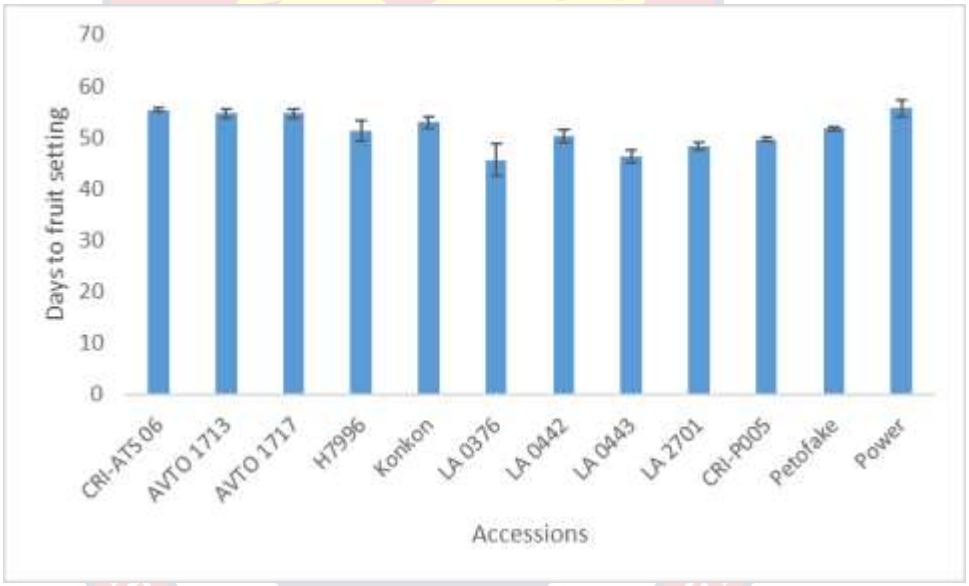

Figure 5 - A histogram of 50 % fruit set of 12 tomato accessions

Plant height

From Figure 6, accession LA 0443 recorded the tallest mean height of 71.00 cm, which was statistically similar to both the resistant and susceptible checks (H7996 (67.67 cm) and Petofake (65.00 cm) respectively), LA0376 (69.00 cm), and LA0442 (68.00 cm). However, they varied significantly from the other accessions. AVTO 1717 recorded the shortest mean height of 44.00 cm which was statistically similar to Power (47.67 cm), ATS 06 (50.67 cm)

and LA 2701 (45.67 cm) but was significantly lower than both the resistant and susceptible (H7996 and Petofake) checks and accessions AVTO 1713, Konkon and CRI-P005 which recorded 59.00 cm, 57.00 cm and 53.67 cm respectively.

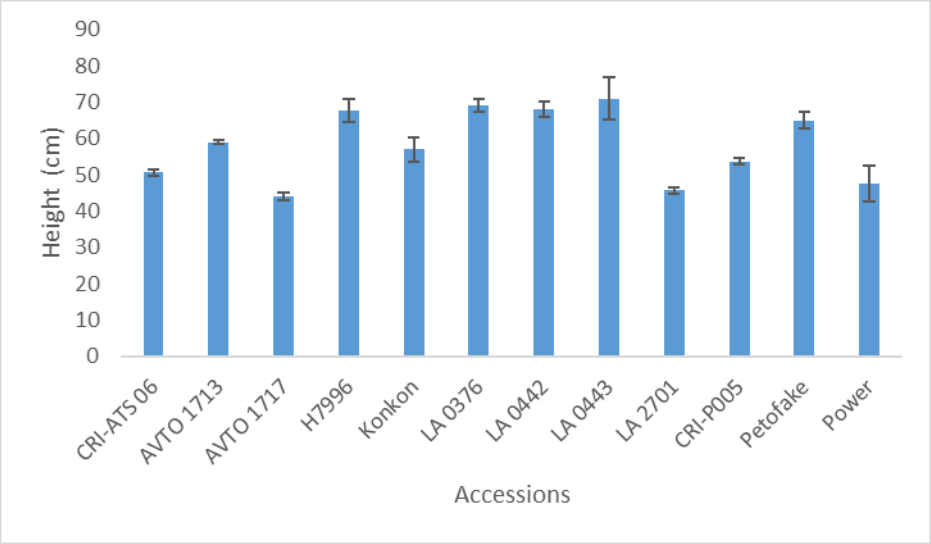

Figure 6 - A histogram of plant height of 12 tomato accessions

Stem girth

Results in Figure 7 showed that CRI-ATS06 recorded the largest mean stem girth (1.27 cm) which was not significantly different ($p>0.05$) from the susceptible check Petofake (1.17 cm) but significantly ($p<0.05$) larger than the resistant check H7996 (0.87 cm). However, LA 0376 recorded the smallest stem girth of 0.07 cm which was significantly ($p<0.05$) lower than both the susceptible (Petofake) and the resistant (H7996) checks.

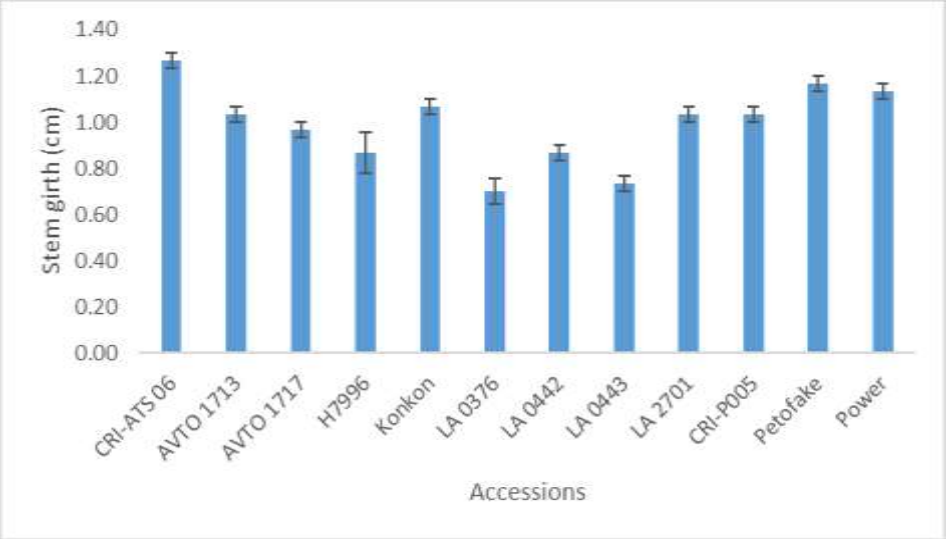

Figure 7 - A histogram of stem girth of 12 tomato accessions

Yield Performance of Accessions

From Figure 8, accession LA 0442 produced the highest total yield of 50.67 t/h which was significantly ($p<0.05$) higher than both the resistant H7996 (24 t/h) and the susceptible Petofake (17.67 t/h) checks. However, accession LA 0443 produced the second highest yield (34 t/h) which was significantly lower than the yield of LA 0442 but statistically higher than the resistant (H7996) and the susceptible (Petofake) checks as well as other accessions including CRI-ATS06, CRI-P005, LA 2701, AVTO 1717, Konkon, Power and AVTO 1713 which produced 23, 18, 16.33, 15.67, 15, 13.67 and 12.67 t/h respectively.

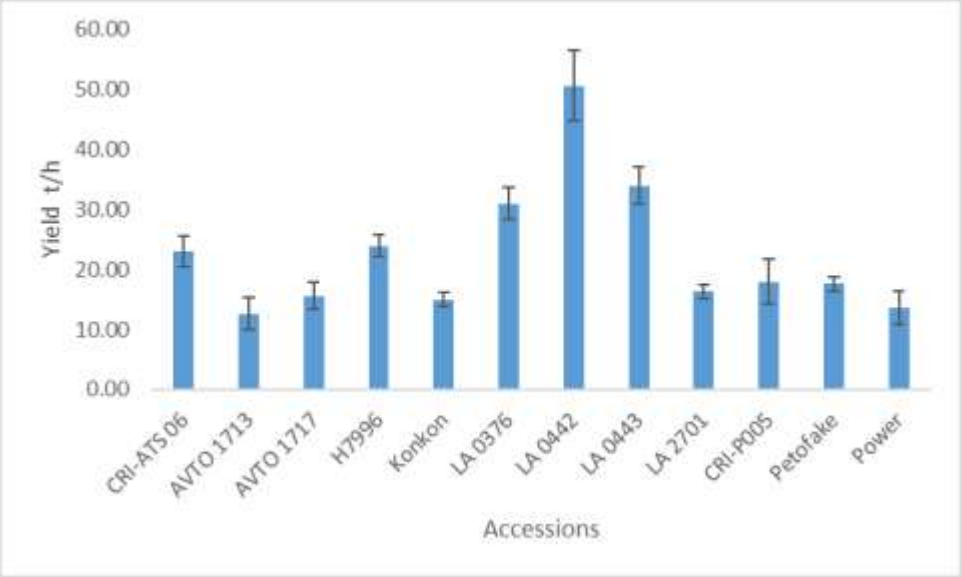

Figure 8 - A histogram of yield performance of 12 tomato accessions

Total soluble solids (brix)

Figure 9 presents the results of total soluble solids. LA 2701 produced the lowest brix of 2.27 % which was statistically the same as LA 0442 and the resistant check H7996 which produced 2.37 % each. Accession AVTO 1717 also produced the highest brix level of 3.37 %, which was significantly ($p<0.05$) different from the susceptible check Petofake (2.60 %) and the other accessions LA 0443, CRI-P005, Konkon, Power, AVTO 1713, CRI-ATS06 and LA 0376 which produced brix levels ranging from 2.60 % to 3.17 %.

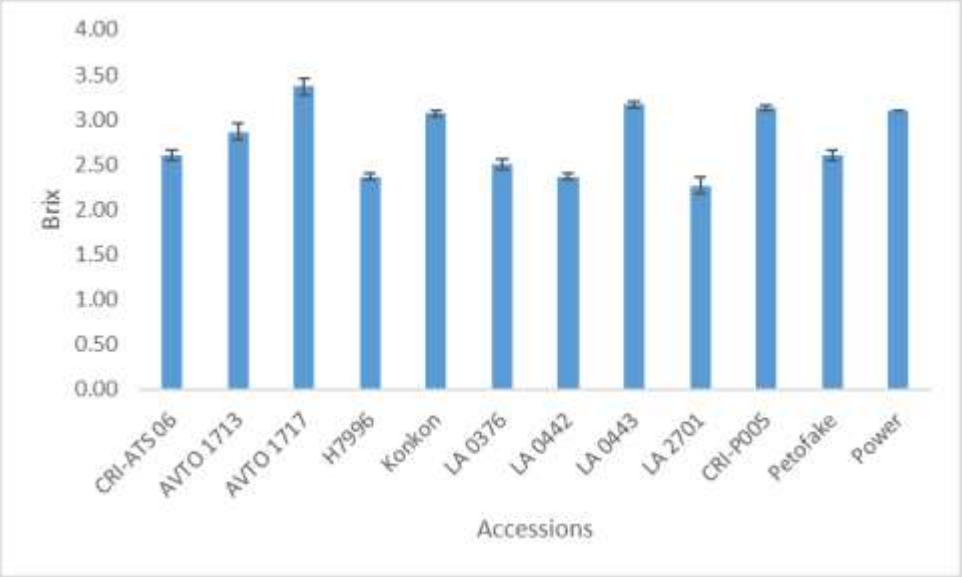

Figure 9 - A histogram of total soluble solid (TSS) of 12 tomato accessions

Locule numbers

Results in Figure 10 revealed that two of the varieties (CRI-ATS06and Power) recorded significantly high locule numbers (6 each). However, they were not significantly ($p>0.05$) different from Petofake and CRI-P005 which recorded 5 locules each. Three of the accessions (LA 0442, LA 0443, and LA 0376) developed significantly ($p<0.05$) lower locules numbers (2 each) which were not different from accessions H7996, LA 2701 and AVTO 1713 which developed 3 locules per fruit. In addition, two other accessions (Konkon and AVTO 1717) developed significantly high number of locules (4 each) which were higher than the resistant check (H7996) but significantly lower than the susceptible check (Petofake).

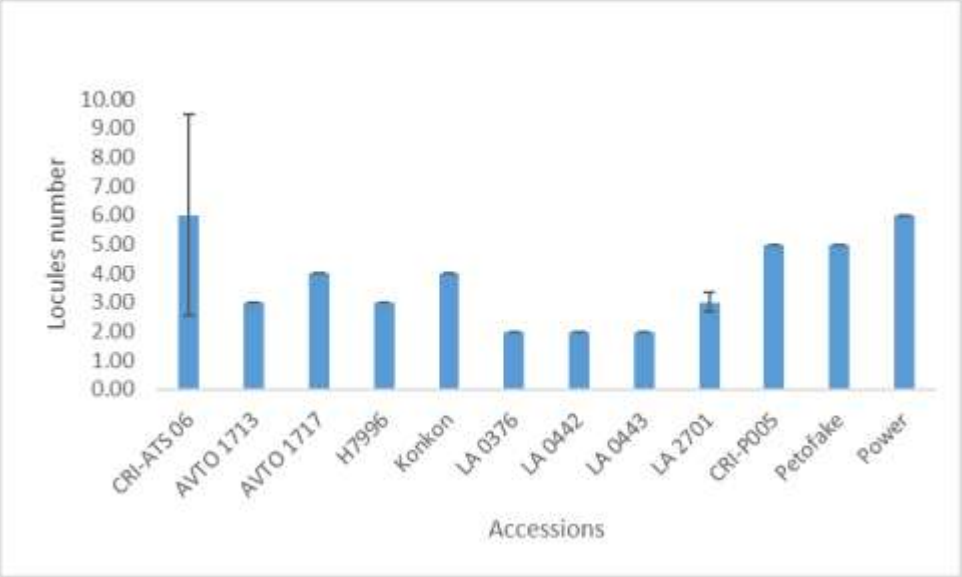

Figure 10 - A histogram of locules number developed by 12 tomato accessions

Screening of Molecular Markers for Bacterial Wilt Resistance in Tomato

A total of 37 individuals (comprised of 12 accessions and 25 F1's) were screened for BW resistance using two SSR marker sets (SLM 12 and SLM 6). SLM 12 set consist of (SLM 12-2 and SLM 12-10) whiles SLM 6 set is made up of (SLM 6-124, SLM 6-118, SLM 6-119, SLM 6-136, SLM 6-17, SLM 6-94 and SLM 6-110).

From Table 8, four of the accessions (Petofake, CRI-ATS06, Konkon and CRI-P005) did not amplify for any of the markers. Accessions AVTO 1713 and Power amplified for only one marker under SSR SLM 6 marker set while H7996, AVTO 1717, LA0376, LA0443, LA2701 and LA0442 amplified for both SSR marker sets SLM 12 and SLM 6. However, two accessions; LA2701 and LA0442 had the highest number of amplicons (four each) while H7996, LA 0443 and LA0376 had three each, followed by AVTO 1717 with two amplicons. Under SSR marker set SLM 6, the accessions Power, AVTO1717 and AVTO1713 amplified for only one marker, LA0376, LA0443

and H7996 amplified for two markers whiles accessions LA2701 and LA0442 did amplified for only three markers each.

Table 8 - *Scores For Nine (9) Tomato Bacterial Wilt Resistant Ssr Marker For 12 Tomato Accessions*

Accessions	SSR Marker									Total Score
	SLM 12-2 Score	SLM 12-10 Score	SLM 6-124 Score	SLM 6-118 Score	SLM 6-119 Score	SLM 6-136 Score	SLM 6-17 Score	SLM 6-94 Score	SLM 6-110 Score	
LA 0376	+	-	-	-	-	+	+	-	-	3
LA 2701	+	-	-	+	-	-	+	-	+	4
LA 0442	+	-	-	-	+	-	+	-	+	4
LA 0443	+	-	-	-	-	-	+	-	+	3
H7996	+	-	-	+	-	-	+	-	-	3
AVTO1717	+	-	-	-	-	-	+	-	-	2
AVTO1713	-	-	-	+	-	-	-	-	-	1
Petofake	-	-	-	-	-	-	-	-	-	0
Power	-	-	-	-	-	+	-	-	-	1
CRI-ATS06	-	-	-	-	-	-	-	-	-	0
Konkon	-	-	-	-	-	-	-	-	-	0
CRI-P005	-	-	-	-	-	-	-	-	-	0

+: presence of marker -: absence of marker. Source: Field data (2019)

Table 9 presents the progenies with bacterial wilt resistance gene. Out of the 25 progenies screened, nine were successful, as they carried the resistance gene. This means that the resistant gene has been introgressed into the local accessions. This has helped in identifying successful crosses that would be evaluated and subsequently be used for further studies.

Table 9 - *Scores for Bacterial Wilt Resistance SSR Markers for 25 Tomato F1's Individuals*

Susceptible (Female)	Resistant (Male)	Progeny (F1)	Score
Petofake	LA 0376	LA 0376 X Petofake	+
Power	LA 0376	LA 0376 X Power	+
CRI-ATS 06	LA 0376	LA 0376 X CRI-ATS 06	-
Konkon	LA 0376	LA 0376 X Konkon	+
CRI-P005	LA 0376	LA 0376 X CRI-P005	-
Petofake	LA 2701	LA 2701 X Petofake	-
Power	LA 2701	LA 2701 X Power	-
CRI-ATS 06	LA 2701	LA 2701 X CRI-ATS 06	+
LA 2701	LA 2701	LA 2701 X Konkon	-
CRI-P005	LA 2701	LA 2701 X CRI-P005	-
Petofake	LA 0442	LA 0442 X Petofake	++
Power	LA 0442	LA 0442 X Power	-
CRI-ATS 06	LA 0442	LA 0442 X CRI-ATS 06	-
Konkon	LA 0442	LA 0442 X Konkon	-
CRI-P005	LA 0442	LA 0442 X P005	++
Petofake	LA 0443	LA 0443 X Petofake	-
Power	LA 0443	LA 0443 X Power	-
ATS 06	LA 0443	LA 0443 X ATS 06	-
Konkon	LA 0443	LA 0443 X Konkon	-
P005	LA 0443	LA 0443 X P005	-
Petofake	H7996	H7996 X Petofake	+
Power	H7996	H7996 X Power	+
ATS 06	H7996	H7996 X ATS 06	-
Konkon	H7996	H7996 X Konkon	+
P005	H7996	H7996 X P005	-

Binary score: - absence of *Bwr* marker + presence of *Bwr-6* marker, ++ presence of *Bwr-12*

Source: Field data (2019)

From the results, the twelve genotypes were grouped into three based on their reaction to BW disease (Figure 11). Group 1 consists of susceptible accessions (CRI-P005, Konkon, Petofake and CRI-ATS06), Group 2 consists of moderately resistant accessions (Power, AVTO 1717 and AVTO 1713) with a maximum of two amplicons and Group 3 consists of resistant accessions (LA 0442, LA 0443, LA 2701, LA 0376 and H7996) with either three or four amplicons.

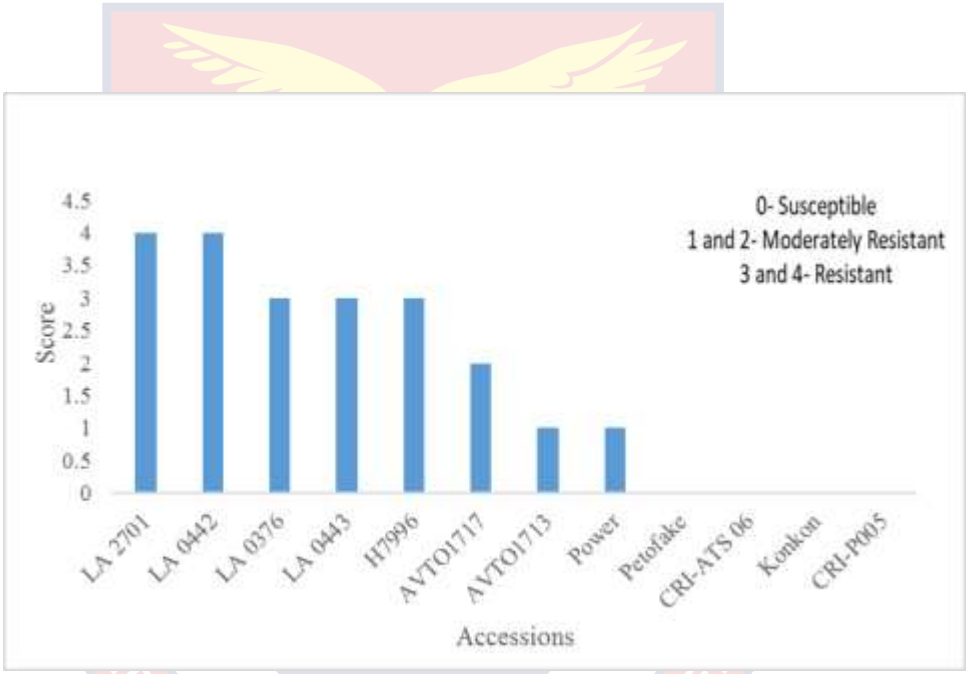

Figure 11 - Grouping of accessions based on genotypic scores with SSR SLM 6 and SLM 12 primers. (Degree of susceptibility and resistance)

Heritability

Broad-sense heritability (H^2_b) of nine (9) economic traits of tomato is presented in Table 10. The magnitude of heritability is classified as high (>50), moderate (20- 49) and low (~ 0-19). Broad-sense heritability estimate was high ranging from 82.84 % to 99.27 % for all the traits studied (Bacterial wilt incidence and severity, days to 50 % flowering, days to 50 % fruit setting, plant height at 50 % fruit setting (cm), stem girth at 50 % fruit set (cm), yield

(t/h), Brix (%) and locule number). Locules number recorded the highest heritability (99.27 %), followed by TSS (97.79 %). in addition, days to 50 % flowering, stem girth at 50 % fruit setting, yield, plant height at 50 % fruit setting, bacterial wilt incidence, and bacterial wilt severity index recorded an estimated broad-sense heritability of 94.37 %, 94.14 %, 92.95 %, 91.38 %, 91.36 % and 90.42 % respectively. Days to 50 % fruit setting recorded the lowest heritability of 82.84 %. Though days to 50 % fruit setting recorded the lowest heritability, its magnitude was classified as high.

Table 10 - *Broad-Sense Heritability Estimates of Nine (9) Traits of Tomato Accessions*

Trait	Broadsense (H^2_b)
Bacterial wilt incidence	91.36
Bacterial wilt severity	90.42
Days to 50 % flowering	94.37
Days to 50 % fruit set	82.84
Stem girth at 50 % fruit set (cm)	94.14
Plant height at 50 % fruit set (cm)	91.38
Yield (t/h)	92.95
SS (Brix %)	97.79
Locule number	99.27

Source: Field data (2019)

CHAPTER FIVE

DISCUSSION

This study confirms *R. solanacearum* as the causal organism of bacterial wilt disease affecting production of tomato in Ashanti, Bono East and Ahafo regions in Ghana. The bacteria have been reported by Subedi *et al*. (2014), as the cause of tomato wilt in Ghana. According to Elings, Saavedra and Nkansah (2015), tomatoes have a high capital input, however high returns could be easily achieved due to high market demands for good and quality of the crop. Bacterial wilt disease (*R. solanacearum*) is among the key limiting factors in the production of tomato.

Morphological and Molecular Characterization

Five different isolates, all negative gram stained and rod shaped were identified. White Irregular (WI) isolates dominated the samples representing 30 % followed by White Circular Small (WCS) 20 %, Cream Irregular (CI) 20 %, Irregular (I) 20 % and White Circular Large (WCL) constituted 10 %, the least encountered in the samples. It is a soil-borne bacterium and survives in soil, water, and plant materials for prolonged periods (Lopez & Biosca, 2004) and thus to prevent the spread of pathogens, it is important to use clean seeds, soil, water and tools in the production of the crop (Meng, 2013).

From this study, bacterial wilt strains collected from Ashanti, Bono East and Ahafo regions of Ghana were identified as Phylotype II (from America). This is in agreement to studies by Fegan and Prior (2005) who classified bacterial wilt strains into four Phylotypes (Phylotypes I, II, III and IV) although Phylotypes I, III and IV strains were not identified in this current

study. Subedi *et al*., (2014) identified Phylotypes I and III on tomato as the first report of *R. solanacearum* in Ghana and according to Sarfo (2018), Phylotypes I, III and IV was identified from samples from greenhouse within Southern Ghana. The introduction of a BW disease strains could be as a results of exchange of infected planting materials (seeds and substrate) by *R. solanacearum* in the country (Garcia *et al*., 2013). However, bacteria inoculum may be found in contaminated irrigation water and spread from one community to the other (Waiganjo *et al*., 2006). Also, in other studies, seeds have been noted to be a potential source of the disease (Abdurahman *et al*., 2017).

Tomato Genotypes Resistant to Bacterial Wilt

Reaction of tomato genotypes to the bacterial wilt disease is determined by the incidence and severity levels. The lower the level of bacterial wilt disease incidence and severity, the higher the resistance (Li *et al*., 2015). From this current study, accessions LA 0442, LA 0443, LA 0376 and H7996 recorded the lowest levels of incidence and severity of the BW disease. The variation in the level of BW incidence and severity index between these tomato accessions observed in this study could be due to the differences in their genetic makeup as reported by Abebe *et al.* (2020) who screened 27 cultivars for resistance to bacterial wilt disease and identified resistant cultivars with low mean disease severity scores. Comparing the resistance level using the susceptible check Petofake as a reference, accessions H7996, LA 0442 and LA 0443 were of higher resistance to BW disease followed by LA 0376, AVTO 1717 and LA 2701.With the molecular

screening for BW resistance, accessions H7996, LA 0442, LA 0443, LA 0376, AVTO 1717, and LA 2701 amplified for both SSR marker sets (SLM 12 and SLM 6), an indication that they possess both *Bwr-12* and *Bwr-6* genes that confer resistance to the disease. They also showed a significantly lower level of incidence and severity of wilt compared to accessions AVTO 1713 and Power which amplified for only one SSR marker set for the *Bwr-6* gene and accessions Konkon, CRI-P005 and CRI-ATS 06 which did not show any amplification for any of the SSR marker set used. This clearly shows that BW resistance may be controlled by polygenes (*Bwr-12* and *Bwr-6*) and this is in agreement to report by Neto, da Silveira, de Souza, Nogueira, and André (2002) and Costa *et al*. (2019) that the inheritance of bacterial wilt resistance in tomato plants under conditions of naturally infested soil is polygenic in nature, with partial dominance of the alleles. This shows that in breeding for BW resistance in tomato, gene pyramiding may be the appropriate strategy to use for durable resistance. In a previous study by Carmeille *et al*. (2006), *Bwr-12* and *Bwr-6* genes were detected in most of the resistant genotypes. This current results are in consonance with a similar study by Wang *et al.* (1998) who reported that H7996 expressed the highest level of resistance to bacterial wilt in tomato.

It is important to note that, accessions LA 0442 and LA 0443 are resistant to BW disease and therefore could be used as parental lines to improve BW susceptible varieties.

In addition, results of this study revealed that among the varieties showing resistance to BW, disease incidence and severity showed a significant level of stability both in the screen house and in the field evaluation. This is in

line with report by Wang *et al*. (2013), who examined some inbred lines derived from H7996.

Flowering in tomato is very important because it has a greater impact on the potential yield of the crop. A high rate of flower bud drop may result in a drastic reduction of the yield and this can be of a greater disadvantage in cultivars for single harvest. Flowering differed significantly among tomato accessions studied which agrees with previous studies by Wang *et al.* (1998). The days required for 50% flowering were minimum (28 and 29 days) in LA 0443 and LA 0376 respectively while the flowering delayed in CRI-ATS06, AVTO1713, AVTO1717 and Petofake (42, 42, 42 and 41 days respectively). From the results, it could be observed that BW severity did not influence the number of days to 50 % flowering. The resistant check H7996 used 38 days to attain 50% flowering which was comparable to the susceptible check Petofake which used 41 days.

Fruit setting is important in tomato crop since it gives an idea on the estimated yield of the crop if all conditions remain constant. Therefore, fruit setting may contribute to yield, thus the higher the number of fruits set, the higher the yield and vice versa. In this current study, 50 % fruit setting was considered, whilst in an earlier study by Hussain *et al.* (2001) fruit setting initiation was considered. 50 % fruit setting and fruit setting initiation are critical in tomato production and both parameters can be used in determining the potential yield of the crop. Hussain *et al.* (2001) reported that the cultivar "Samarzano" took a maximum of 27 days to initiate fruit setting while "Nadir" took a minimum of 16 days. In this study, Power used a maximum number of 56 days in producing 50 % fruit setting which was statistically the same as the

bacterial wilt susceptible check Petofake which had 50% fruit setting at 55 days. However, LA 0376 and LA 0443 used a minimum number of 46 days to produce 50 % fruit setting which was significantly ($p<0.05$) lower than the bacterial wilt resistant check H7996 which used a number of 51 days to fruit set. In both studies, the differences in time to initiate fruit setting and attaining 50 % fruit setting might be due to the genetic make-up of the different experimental materials used.

The results on plant height were expected as different accessions exhibited different plant heights.

The tallest accession LA 0443 was 38 % taller than the shortest accession AVTO 1717. The reasons for the differences in height might be due to genetic, biotic and abiotic factors. Similarly, differences in stem girth could be attributed to the genetic constitution of the accessions as well as the environmental effects. The largest stem girth in CRI-ATS 06 was approximately 94 % larger than the smallest girth in LA 0376.

The yield results from this study also revealed the importance of obtaining high yielding varieties from accessions that are resistant to BW disease. These accessions could subsequently be used in future improvement programs to develop tomato varieties with BW resistance. However, variations observed in yielding ability exhibited by accessions in the study could be attributed to the number of flowers set that developed into fruits and preserved by the crop till harvest. This result is similar to reports on differences in fruit yield among tomato varieties (Das, 2017; Gongolee, Osei, Akromah, Nyadanu, & Aboagye, 2015; Melomey, 2018)

In a similar study, Hussain *et al.* (2001) reported the highest yield of 41.45 t/h in "Tanja" and the lowest of 26.07 t/h was recorded in "Rio Grande". In the current study apart from the highest yielding accession LA 0442 (50.67 t/h), the rest of the accessions recorded very low yields. For instance, the lowest yielding accession was AVTO 1713 which yielded 12.67 t/h. Thus, the highest yielding accession, LA 0442 over-yielded by approximately 300 %. The significant differences in yield might be attributed to genetic factors as well as the effect of bacterial wilt disease in some of the accessions.

°Brix is a measure of the Total Soluble Solids (TSS) content in the tomato or tomato product and it relates to the taste. TSS in tomatoes is mainly sugars (fructose). A tomato juice, which is assessed as having 20 °Brix, has 200 g/litre of soluble sugars. Tomatoes for processing require a minimum Brix of 4.5 (Korob, 2020). The highest yielding Brix AVTO 1717 out-yielded LA 2701 the lowest yielding accession by approximately 32.64 %. However, virtually all the accessions studied fell below the minimum processing Brix requirement level of 4.5.

In tomato, the number of locules (cavities containing seeds that are derived from carpels) varies from two to 10 or more. Locule number is controlled by quantitative trait loci (Munos *et al.,* 2011). The number of locules in tomato affects the fruit size, shape and the incidence of malformation (Li *et al.,* 2019). Therefore, the higher the locule number, the less attractive the tomato crop and the lower the price. In this study, it was observed that locules number ranges from 2 to 6. Fruits with locule numbers two looked smaller whiles' fruits with locules numbers three and four looked

bigger in sizes. However, fruits having more than four locules were the biggest in size.

The good qualities observed in all the resistant accessions based on their agronomic parameters project the potential of developing a cultivar resistant to BW disease *(R. solanacearum*) with good horticultural characteristics (Li *et al.,* 2015; Wang *et al.,* 2000). This would help reduce the challenge of managing BW disease and increase the productivity of tomatoes in disease prone areas in the country.

Broad-sense Heritability

According to Kaushik, Tomar and Dixit (2011), heritability estimates are better indicators of heritable proportion of variation. Nevertheless, this does not generally mean high genetic gain for a specific trait. Low heritability of a trait is an indication that environmental factors strongly influenced the trait hence breeding for such traits will be difficult. In that same vein, traits with high heritability can easily be passed on to successive generations (Fahliani *et al*., 2010; Ghosh & Sharma, 2012).

The results obtained from the study revealed high broad-sense heritability for both disease incidence and severity of bacterial wilt, days to 50 % flowering, days to 50 % fruit setting, plant height (cm), stem girth (cm), yield (t/h), TSS (Brix) and locule number. This is an indication that influence of environmental conditions was relatively lower, thus variations being observed were largely due to genetic factors. This means that these traits can easily be bred for and phenotypic selection will be reliable as well.

The results from this study are in agreement with Bahmankar, Reza Raij. Reza Seloki, and Shirkool (2014) who reported high broad-sense heritability for plant height and days to flowering. It also confirms earlier reports by Haydar *et al*. (2007) and Mohamed *et al*. (2012) for plant height and days to flowering; Kumar (2010) for days to flowering and TSS (brix). Mehta and Asati (2008) also observed high broad-sense heritability for plant height and TSS. Additionally, Kumar *et al*. (2013) reported high broad-sense heritability for plant height and fruit diameter while Islam *et al*. (2012) recorded high broad-sense heritability for days to flowering.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

A laboratory, screenhouse and a field investigative study was conducted on bacterial wilt disease of tomato caused by *R. solanacearum* and their prevalent strains within Ashante, Bono Eat and Ahafo regions in Ghana.

The study was achieved by collection and characterization of bacterial wilt strains in the major tomato growing areas within the study regions. Also, identification and selection of resistant tomato genotypes to bacterial wilt disease were carried out. In the experiment, three screening approaches was investigated (under screenhouse conditions, laboratory using marker assisted selection and field conditions).

It was observed that four tomato genotypes (H7996, LA 0442, LA 0443 and LA 0376) showed higher level of resistance to bacterial wilt disease at the end of the screening. The study results, however, showed that all the local tomato genotypes used in the experiment were susceptible to the bacterial wilt disease which causes a great reduction to the potential yield of the crop.

Conclusions

In characterizing bacterial wilt strains, 20 isolates were obtained from tomato producing areas within Ashanti, Bono East and Ahafo regions in Ghana. Strains from the three regions (Ashanti, Bono East and Ahafo) of Ghana were identified as Phylotype II strain (from America) and was for the first time identified to be present in the study area.

In efforts to identify tomato genotypes resistant to bacterial wilt disease in Ghana, 13 tomato accessions were evaluated in the screen house and on the field. Six accessions; LA 0442, LA 0443, LA 0376, LA 02701, AVTO 1717 and the resistant check H7996 were found to possess both *Bwr-12* and *Bwr-6* genes which confer resistance to BW disease. These are promising lines that can be used in the tomato improvement program for introgression into susceptible genotypes.

Out of the six promising lines, four (H7996, LA 0442, LA 0443 and LA 0376) were identified as possessing significant levels of stability both in the screen house and in the field. It was revealed that none of the local materials was significantly resistant comparable to the selected four genotypes.

In estimating heritability for the accessions, high broad-sense heritability was revealed for both disease incidence and severity of bacterial wilt, as well as all the other agronomic characters investigated. This is an indication that the crop can be improved without the influence of environmental conditions since variations being observed were largely due to genetic factors. Therefore, these traits can easily and reliably be bred for phenotypic selection.

Recommendations

From this study, I recommend the following:

1. Further studies should be conducted on characterization of bacterial wilt strains in other tomato growing areas in the country.

2. The four superior genotypes (H7996, LA 0442, LA 0443 and LA 0376) identified should be evaluated across different environments to determine their performance.
3. These superior genotypes should be used as parental lines to introgress bacterial wilt resistant genes via pyramiding into bacterial wilt susceptible genotypes
4. For bacterial wilt resistance breeding, both conventional and molecular screening should be used to facilitate the development of durable resistance for bacterial wilt.
5. Only primers that amplified for the study under the two SSR marker sets for bacterial wilt resistance should be used for molecular screening to cut down cost if same tomato accessions are to be used in any further studies.

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APPENDICES

Appendix 1 - *ANOVA Table of Wilt Incidence for Week One of Incubation for Screenhouse Trial*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	12	1.7306E-29	1.4421E-30		
Residual	26	0.0000E+00	0.0000E+00		
Total	38	1.7306E-29			

Appendix 2 - *ANOVA Table of Wilt Incidence for Week Two of Incubation for Screenhouse Trial*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	12	137.234	11.436	7.41	<.001
Residual	26	40.114	1.543		
Total	38	177.348			

Appendix 3 - *ANOVA Table of Wilt Incidence For Week Three of Incubation for Screenhouse Trial*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	12	236.874	19.740	7.69	<.001
Residual	26	66.767	2.568		
Total	38	303.641			

Appendix 4 - *ANOVA Table of Wilt Incidence for Week Four of Incubation for Screenhouse Trial*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	12	333.855	27.821	16.65	<.001
Residual	26	43.447	1.671		
Total	38	377.302			

Appendix 5 - *ANOVA Table of Severity index for Week One of Incubation for Screenhouse Trial*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	12	1.7306E-29	1.4421E-30		
Residual	26	0.0000E+00	0.0000E+00		
Total	38	1.7306E-29			

Appendix 6 - *ANOVA Table of Wilt Severity index for Week Two of Incubation for Screenhouse Trial*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	12	600.895	50.075	6.56	<.001
Residual	26	198.337	7.628		
Total	38	799.233			

Appendix 7 - *ANOVA Table of Wilt Severity index for Week Three of Incubation for Screenhouse Trial*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	12	1038.03	86.50	7.69	<.001
Residual	26	292.43	11.25		
Total	38	1330.45			

Appendix 8 - *ANOVA Table of Wilt Severity index for Week Four of Incubation for Screenhouse Trial*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	12	1382.769	115.231	15.54	<.001
Residual	26	192.792	7.415		
Total	38	1575.561			

Appendix 9 - *ANOVA Table of Wilt Incidence for Field Trial*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	93.32	46.66	1.03	
Genotype	11	7668.19	697.11	15.33	<.001
Residual	22	1000.43	45.47		
Total	35	8761.94			

Appendix 10 - *ANOVA Table of Wilt Severity index for field trial*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	41.485	20.742	3.85	
Genotype	11	2803.791	254.890	47.36	<.001
Residual	22	118.404	5.382		
Total	35	2963.680			

Appendix 11 - *ANOVA Table of Yield in ton/ha*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	10.72	5.36	0.21	
Genotype	11	4078.31	370.76	14.18	<.001
Residual	22	575.28	26.15		
Total	35	4664.31			

Appendix 12 - *ANOVA Table of Days to 50% Flowering*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	22.222	11.111	2.50	
Genotype	11	867.889	78.899	17.75	<.001
Residual	22	97.778	4.444		
Total	35	987.889			

Appendix 13 - *ANOVA Table of Days to 50% Fruit Set*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	9.722	4.861	0.80	
Genotype	11	389.222	35.384	5.83	<.001
Residual	22	133.611	6.073		
Total	35	532.556			

Appendix 14 - *ANOVA Table of Total Soluble Solids (Brix)*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.026667	0.013333	1.42	
Genotype	11	4.676667	0.425152	45.26	<.001
Residual	22	0.206667	0.009394		
Total	35	4.910000			

Appendix 15 - *ANOVA Table of Locules Number*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.22222	0.11111	2.20	
Genotype	11	76.30556	6.93687	137.35	<.001
Residual	22	1.11111	0.05051		
Total	35	77.63889			

Appendix 16 - *ANOVA Table of Plant Height (cm)*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	28.22	14.11	0.57	
Genotype	11	3184.31	289.48	11.60	<.001
Residual	22	549.11	24.96		
Total	35	3761.64			

Appendix 17 - *ANOVA Table of Stem Girth (cm)*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.020556	0.010278	2.00	
Genotype	11	0.962222	0.087475	17.06	<.001
Residual	22	0.112778	0.005126		
Total	35	1.095556\			