INHERITANCE OF BACTERIAL WILT (*Ralstonia solanacearum*) RESISTANCE AMONG WILD TOMATO (*Solanum pimpinellifolium*) AND CULTIVATED TOMATO (*Solanum lycopersicon* L.) GERMPLASM IN KENYA

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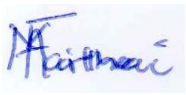
DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented for examination in this or any other university for the award of degree.

Signature  …….. Date: _4_/5_/2022

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Recommendation

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This thesis is dedicated to my beloved parents Jude Mathai and Grace Wangare and my siblings Miriam Nyaguthii, Simon Mathai and Joe Maina for their encouragement, patience and support in my academic life.

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ABSTRACT

Tomato (*Solanum lycopersicon* L.) is the second most important vegetable crop after potato in the world. In Kenya, the crop is ranked high as the most important in the horticultural industry. Tomato production is adversely affected by both abiotic and biotic stresses. Among the biotic stresses, bacterial wilt caused by *Ralstonia solanacearum* is a major disease of tomato causing high reduction in yield and income to farmers in Kenya. The objectives of this study were (i) to identify novel sources of resistance to bacterial wilt from wild tomato germplasm (ii) to determine the mode of gene action controlling bacterial wilt resistance and heritability of the disease in tomato. Two experiments were carried out in the greenhouse at Egerton University in the Department of Horticulture and Soils. In the first experiment, thirty-six tomato genotypes were evaluated for resistance to bacterial wilt in an *alpha lattice* design of four blocks with ten experimental units per block in two replications for two cropping cycles. In the second experiment, eight parents were crossed in North Carolina II mating design to generate sixteen F_1 hybrids. The F_1 hybrids were evaluated alongside the eight parents for bacterial wilt in an *alpha lattice* design of four blocks with six experimental units per block in two replications for cropping two cycles. In the first experiment, significant ($p \leq 0.001$) main effects and interactions were found for all traits measured at 30, 45 and 60 days after inoculation (DAI) except cycle effects for plant survival at 30 DAI and genotype × cropping cycle interaction effects for disease incidence at 45 DAI which were significant at ($p \leq 0.05$) and ($p \leq 0.01$) respectively. Among the genotypes tested for bacterial wilt, KK acc I, NRB, KK acc II, KISII, BRNG acc II and KLF acc III out of thirty-six were resistant with AUDPC value of 0. Cultivar superiority measure based on AUDPC ranked genotypes KK Acc I, NRB, KK Acc II, KISII, BRNG Acc II and KLF Acc III. In the second experiment, testers main effects were significant ($p \leq 0.01$) for AUDPC, disease incidence and plant survival. Lines × testers interaction were significant ($p \leq 0.001$) for AUDPC and disease incidence. Narrow sense heritability estimates of 0.14, 0.16 and 0.20 obtained for AUDPC, disease incidence and plant survival signified that the expression of disease resistance for the traits was dependent on dominance gene action. General predictability ratio of 0.27, 0.29 and 0.50 for AUDPC, disease incidence and plant survival signified the predominance of non-additive genetic variance compared to additive variance in controlling bacterial wilt resistance. The sources of resistance obtained from this study will be deployed in the breeding programmes aimed at developing resistant tomato varieties.

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ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progressive Curve
DAI	Days After Inoculation
DI	Disease Incidence
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GCA	General Combining Ability
GPR	General Predictability Ratio
PS	Plant Survival
QTL	Quantitative Trait Loci
SCA	Specific Combining Ability

CHAPTER ONE
INTRODUCTION

1.1 Background information

Tomato (*Solanum lycopersicon* L.) is native to Central and South America and belongs to the family *Solanaceae* (Ramirez-Ojeda *et al*., 2021). It is the second most consumed vegetable worldwide after potato and a well-studied crop species in terms of genetics, genomics and breeding (Wani, 2011). Tomato is an excellent source of vitamins A and C. It is also a source of many minerals including potassium, calcium, iron, phosphorus and folate (De & De, 2021). The current world production is 180,766, 329 million tonnes per annum with the highest production occurring in China while sub-Saharan Africa contributes only 11, 771,800 tonnes per annum (FAOSTAT, 2019). The average yield of tomatoes in Kenya is estimated to be 83,494 kg ha^{-1} per annum (FAOSTAT, 2019). The production of this vegetable in sub-Saharan Africa is still low compared to the rest of the world against a continuously rising demand. Low production is attributed to biotic and abiotic stresses (Krishna *et al*., 2019). The crop is mainly produced under greenhouse conditions although a few farmers still produce under field conditions (Ochilo *et al*., 2019). Continuous planting of tomato under greenhouse coupled with poor management practices leads to accumulation of pests and diseases thereby impacting negatively on yield (Wayua *et al*., 2020). Lack of resistant tomato varieties to major pests and diseases further aggravates the problem.

Bacterial wilt caused by *Ralstonia solanacearum* is a significant disease of vegetable crops causing losses of 10%-100% per cropping cycle across the world (Radhi *et al*., 2016) with an average loss of 61% being reported in eastern Africa (Mulugeta *et al*., 2020). Studies conducted in Kenya reported high prevalence of bacterial wilt of up to 32% of total diseases of tomatoes (Mwangi *et al*., 2015). Yield losses ranging from 50-100% have also been reported both in the field and in the greenhouse which is attributed to the susceptibility of most of the tomato varieties grown in Kenya to bacterial wilt (Ireri *et al*., 2018).

The *Ralstonia solanacearum* bacterium has been divided into five races and five biovars on the basis of host range variations and metabolic properties, respectively (Aslam *et al*., 2017). The strains of the bacterium affect over 200 plant species in over 50 families (Ahmed *et al*., 2013). The families include vegetables, ornamental crops, fruits, and woody perennial plants. Host plants of global importance include tomato, eggplant (*Solanum melongena* L.), –pepper (*Pepper nigrum*), tobacco (*Nicotiana tabacum*) ginger (*Zingiber officinale),* and groundnut (*Arachis hypogea* L.) (Aslam *et al*., 2017). In sub-Saharan Africa, the pathogen has been reported in Angola, Burkina Faso, Burundi, Congo, Ethiopia, Gabon,

Gambia, Kenya, Madagascar, Somalia, Tanzania, Uganda and Zimbabwe (Afroz *et al*., 2009). Bacterial wilt of tomato is mostly caused by race one and can survive in the soil for a long period of time in association with infested plant debris and because of its wider host range (Yuan *et al*., 2014). Dispersion of this pathogen is through contaminated water sources, infected seedlings and contaminated soils spread through human activity (Manda *et al*., 2020). The disease development is favoured by high temperatures, low soil pH, low soil fertility and moist soil.

The pathogen enters plant roots through natural openings or wounds created by pests such as nematodes (Onduso, 2014). Nematode attacks the roots of tomatoes and feeds on the nutrients thereby creating wounds. As the disease gains entry through the wounds the pathogen multiplies and moves up through the vascular system and finally blocks water transport system thus causes wilting and eventually results in host death (Genin, 2010). It has been reported that most cases of bacterial wilt occur during vegetative to fruiting stages when leaves are green (Acharya *et al*., 2018). Expression of symptoms occurs after 30–35 days following transplanting, inoculation or when flowering begins, and this could be due to increased inoculum load, damage to roots by nematodes or cultural operations (Singh *et al*., 2015). Bacterial wilt attack on tomato farms results to losses of more than 90%. Its manifestation changes with varying conditions and farm management practices (Odoyo, 2016).

The disease is commonly controlled by using different management strategies such as crop sanitation, chemicals, disease-free planting material, solarization, fumigation, flooding and crop rotation (Sood *et al*., 2021). These management practices have been ineffective due to the presence of high inoculum in the soil and the inability to predict survival of the pathogen in the soil (Kago *et al*., 2016; Mwangi *et al*., 2015). The efficacies of such methods are further complicated by the genetic diversity of the pathogen and the existence of a wide host range.

High levels of resistance to *Ralstonia solanacearum* were uncovered in large-fruited tomato breeding lines (Scott *et al*., 2009). Such resistance sources of resistance have been successfully deployed in breeding superior cultivars. For example, bacterial wilt resistant varieties of tomato were released in North Carolina in the USA in 2008 (Panthee & Gardener, 2011). In China, over twenty cultivars with partial resistance to bacterial wilt have been released by various institutes and universities in the past fifty years (Yuqing *et al*., 2018). Improvement of tomatoes for resistance to other major disease such as fusarium wilt, bacterial canker and bacterial spot have also been reported (Wang *et al*., 2013). However,

limited information on breeding for resistance to bacterial wilt of tomato exists in Kenya despite the disease being a major problem. Therefore, the aim of this study was to identify resistant sources from wild genotypes which can be used in the breeding programme for the development of a tomato variety resistant to bacterial wilt.

1.2 Statement of the problem

Ralstonia solanacearum is a soil-borne pathogen that is hard to detect when it infects solanaceous vegetables. It poses serious challenges in its control and management especially in already infected fields. It affects the plant by hindering proper nutrient uptake hence reduction in yield. The pathogen has caused reduction in crop productivity in more than eighty countries throughout the globe with annual losses of over US $1billion every year. In Kenya, it has been reported to cause over 30-50% crop loss for open field production and up to 80-100% under severe conditions in the greenhouses. Most of the varieties of tomato grown in Kenya are susceptible to the disease. The techniques and methods being used in the control such as crop rotation, grafting, chemicals and flooding have been less effective in controlling the pathogen. Use of diverse resistant cultivars is known to be effective against bacterial wilt. However, this method is yet to be deployed. Introgression of new sources of resistance from wild relatives is necessary towards development of bacterial wilt resistant tomato cultivars. Therefore, breeding for resistance offers the most sustainable and cost-effective way to combat the disease.

1.3 Objectives

1.3.1 General objective

To contribute to enhanced production of tomato, nutritional security and income through breeding of bacterial wilt resistant tomato varieties for tomato producers and consumers in Kenya.

1.3.2 Specific objectives

i. To identify bacterial wilt resistant genotypes among wild tomato genotypes collected from diverse agro-ecologies in Kenya.
ii. To determine the mode of gene action underlying the control of bacterial wilt resistance and the heritability of the disease in tomato genotypes.

1.4 Hypotheses

i. There are no bacterial wilt resistant genotypes among wild tomato genotypes collected from different agro-ecologies in Kenya.

ii. There is no gene action involved in the control of bacterial wilt resistance and the heritability of the disease in tomato genotypes.

1.5 Justification

Most tomato farmers in Kenya incur high crop losses arising from bacterial wilt disease. This is due to susceptibility of the varieties grown. Sources of resistance to major diseases have previously been identified from landraces and wild relatives of cultivated crops. In tomato for example, resistance to tomato yellow leaf curl virus disease have been identified from wild tomato species and successfully introgressed to cultivated tomato (Yang *et al*., 2014). Identification of new sources of local resistance tomato genotypes will be a step towards uncovering the potential within the Kenyan germplasm for breeding to develop bacterial wilt resistant tomato in Kenya (Singh *et al*., 2014). However, the use of wild genotypes is prone to introduction of unwanted genetic background through linkage. Tomato production being a key income earner to most families, development of resistant tomato varieties will ensure increased incomes for the farmer and fair prices to the consumer (Wang *et al*., 2013). This management strategy will provide a continuous and increased production per unit area of land with reduced use of pesticides (Jiang *et al*., 2017). The use of resistant varieties is an environmentally friendly strategy for adoption by the farmers (Singh *et al*., 2015). Moreover, it can also be an important component of integrated disease management programme. The knowledge on the mode of gene action governing the inheritance of this resistance is necessary as an integral part in the development of breeding strategies that will provide adequate results with limited resources.

CHAPTER TWO
LITERATURE REVIEW

2.1 Genetics of tomato

Tomato is a member of the *Solanaceae* family and is a diploid plant with 2n=24 chromosomes (Kumar *et al*., 2021). Advances in tomato genetics have been achieved because of mating systems and the controlled hybridization within and among species (Caicedo & Peralta, 2013). The cultivated tomato genome has been selected as a model for the *Solanaceae* family due to its simple diploid genetics, small genome size, short reproduction period and the availability of a great diversity of genetic resources within the cultivated species and in wild related species (Fentik, 2017).

The cultivated tomato is genetically poor in terms of its resistance to pests and diseases. It is estimated that cultivated tomato contains less than 5% of the genetic variation of its relatives (Caicedo & Peralta, 2013). Molecular genetic studies show a shortage of polymorphism in cultivated tomato. Qualitative genes and Quantitative trait loci (QTLs) for the fruits traits have been identified. The most dramatic changes in tomato through domestication are its fruit size. Wild tomato has tiny berries while modern tomato cultivars are large and succulent (Swarup *et al*., 2021).

2.2 Tomato production in Kenya

Agriculture is central to Kenya's economy accounting for 24% of the country's gross domestic product (GDP). In addition, it is estimated that 75% of the population, either directly or indirectly, depends on the sector. Horticultural industry as a sub-sector of agriculture has grown to be an important source of income for smallholder farmers, government revenue, and foreign exchange earnings. Over one million tonnes of vegetables are produced annually (FAOSTAT, 2018).

Smallholder production accounts for 80% of all growers and produces 60% of the horticultural exports. The main horticultural crops produced include vegetables, fruits, herbs, root crops, spices and cut flowers (Ongeri, 2014). Tomato is a popular and extensively cultivated vegetable accounting for 20% by value, of the exotic vegetables (Kaiwart, 2020). Kenya is one of the leading countries in sub-Saharan Africa producing 410,033 tonnes of tomatoes per annum (FAOSTAT, 2018). It is a financially attractive horticultural crop with a potential for high incomes for small-scale farmers and thus, provides a potential source of employment to many rural and semi-urban residents (Kinuthia, 2019).

Production of tomato is mainly carried out by small scale growers with land sizes between 0.5-2.5 ha (Malgie *et al*., 2015). Steady seed supply for production has been achieved by support from seed companies through the supply of a wide range of varieties to meet farmers' demand. Over the years, tomato production has been largely carried out under open field conditions but in the recent past, adoption of greenhouses is on the rise (Geoffrey *et al*., 2014). The greenhouse innovations have attracted more educated youths to horticultural farming (Wachira *et al*., 2014).

2.3 Constraints associated with tomato production in Kenya

Tomato industry is faced with a diverse range of constraints despite its contribution to alleviate poverty (FAOSTAT, 2018). The key constraints are abiotic and biotic factors. Abiotic factors include high temperature, erratic rainfall, and poor soils while biotic factors comprise of pests, fungal bacterial and viral diseases. The major pests attacking the crop are whiteflies, nematodes, spider mites, thrips, leaf miners, African bollworms and aphids (Wright *et al*., 2016).

Diseases are the principal challenge to tomato production. They are prevalent in lowlands, highlands, tropics and can cause 15-95% crop loss (Nguetti *et al*., 2018). Some of the major diseases affecting tomatoes in Kenya include; early and late blight (*Alternaria solani* and *Phytophthora infestans*, fusarium wilt (*Fusarium oxysporum*), yellow leaf curl virus, tobacco mosaic virus, septoria leaf spot (*Septoria lycopersici*), powdery mildew (*Pseudoidium neolycopersici*), bacterial canker (*Clavibacter michiganensis*) and bacterial spot (*Xanthomonas vesicatoria*) (Singh *et al*., 2014). In their effort to control pests and diseases, farmers use chemicals excessively, with up to over 40 applications per season recorded in tomato fields (Asante *et al*., 2013). The overdependence on pesticide use causes health risk to growers and consumers and degrades the environment (FAOSTAT, 2018). Farmers have reported health complications arising from poor handling of pesticides during application (Marete *et al*., 2020).

2.3.1 Biotic stresses on tomato

Biotic stresses affecting tomato production include insect pests, diseases, nematodes and competing weeds. The main insect pests attacking tomatoes are: African bollworms *Helicoverpa armigera*, which feed on the flowers and fruits (Ekesi *et al*., 2009); whitefly *Bemisia tabaci* cause damage by sucking plant sap and transmitting viral diseases such as yellow leaf curl virus (Kinuthia, 2019); thrips *Frankliniella occidentalis* feed on flowers and

young fruit by sucking the sap from the broken cells (Tonnang *et al*., 2015). The major bacterial diseases of tomatoes include bacterial wilt that is caused by *Ralstonia solanacearum* and bacterial spot caused by *Xanthomonas campestris* while the fungal diseases including damping off disease caused by *Rhizoctonia solani* and early and late blight caused by *Alternaria solani* and *Phytophthora infestans* (Ahmed *et al*., 2013). Of these diseases, bacterial wilt is the most serious hindrance to the cultivation of tomatoes (Shen *et al*., 2021).

2.4 Bacterial wilt disease of tomato

2.4.1 Causal agent of bacterial wilt

Ralstonia solanacearum is an important pathogen of diverse crops (He *et al*., 2020). It was first reported at the end of the 19th Century on potato, tobacco, tomato and groundnut in Asia, southern USA and South America (Fajinmi & Fajinmi, 2010). The pathogen is a gram-negative, rod shaped, largely aerobic bacterium that is 0.5-0.7 by 1.5-2.0µm in size and is able to survive for long at -80°C in liquid culture broth containing 40% glycerol. It is a worldwide quarantine pest and it has been listed as a select agent plant pathogen under the Agricultural Bioterrorism Act of 2002 in the USA (Kamuyu, 2017).

The origin of the pathogen is not clear, but Hayward (1991) suggested that it comes before the geological separation of the continents as the bacterium has been found in South America and Indonesia. Strains of *Ralstonia solanacearum* have been classified as races and biovars (Kamuyu, 2017). Race one has a very wide host range and is endemic to the southern United States, Africa, Asia and South America. Race two is known to attack bananas (*Musa acuminata*) and is mainly found in Central America and Southeast Asia. Race three is distributed worldwide and has been associated with potato. Race four affects ginger mainly in Asia and Hawaii and race five affects mulberries in China (Jibat *et al*., 2018).

2.4.2 Symptoms and signs of *Ralstonia solanacearum*

The first visible symptoms of bacterial wilt are usually seen on the leaves of plants. These symptoms consist of wilting of the youngest leaves at the ends of the branches during the hottest part of the day (Vanitha *et al*., 2009). The stem near the root produces many adventitious root buds and roots indicating infection to the vascular bundle (Pollard, 2013). When the stem of a wilted plant is cut across, the pith has a darkened, water-soaked appearance. There is greyish slimy ooze on pressing the stem indicating the presence of bacterial cells from vascular bundles (Kanyua, 2018). In later stages of the disease, decay of the pith may cause extensive hollowing of the stem and permanent wilting occurs due to a

massive invasion of the cortex which may result to water-soaked lesions on the external surface of the stem. In some cases, the plant may have latent infection where none of these symptoms, even under typical environmental conditions that are ideal for the pathogen are expressed (Aribaud *et al*., 2014).

2.4.3 Etiology of tomato bacterial wilt

The pathogen *Ralstonia solanacearum* moves to the host plant, attaches to the roots of the plant, infects the cortex and colonizes the xylem; an action that requires secretion of cell wall degrading enzymes (Fajinmi & Fajinmi, 2010). After destroying the host, the bacterial returns to the environment and is likely to survive in the soil, water or reservoir plants (Alvarez *et al*., 2010). Within the xylem vessels the pathogen moves throughout the stem to the upper part of the plant while it is multiplying. As extensive multiplication takes place in the xylem, wilting of the host plant occurs due to clogging of the vessels and eventually the plant collapses and dies (Genin, 2010). When the roots of the wilted plants decay the bacteria are released back into the soil and the infestation by root-knot nematodes accelerates the disease.

2.4.4 Epidemiology of tomato bacterial wilt

Ralstonia solanacearum is a soil borne and waterborne pathogen; the bacterium can survive and spread for various periods of time in infested soil or water, which can form a reservoir source of inoculum (Fajinmi & Fajinmi, 2010). The pathogen is mainly dispersed through soil and enters roots through wounds or natural opening (Kamuyu, 2017). Neighbouring plants can be infected via root contacts from infected plants. The pathogen can be dispersed into a clean field through contaminated water sources, contaminated seedlings, as well as humans or machinery carrying infested soils. High temperatures of 30-35ºC play a major role in pathogen growth and disease development (Alvarez *et al*., 2020). The presence of root-knot nematode (*Meloidogyne incognita*) favours disease development by creating wounds on the roots which increase the number of sites for bacterial entry (Kamuyu, 2017). Other factors that may affect pathogen survival in soil and water may also favour disease development. These factors include; soil type and structure, soil moisture content, organic matter in soil, water pH and salt (Zheng *et al*., 2014). Similarly, infected semi-aquatic weeds may also play a major role in disseminating the pathogen by releasing bacteria from roots into irrigation waters.

2.5 Breeding options for resistance to bacterial wilt

Bacterial wilt of tomato has received great attention from breeders worldwide. A lot of efforts have been put towards screening for sources of resistance and developing resistant cultivars. Genes conferring resistance to bacterial wilt of tomato have been identified by Parveen *et al*. (2006). The identified resistance sources have been either directly used in tomato production or used as resistance donors in breeding programmes (Hanson *et al*., 2016). Four tomato genotypes resistant to *Ralstonia solanacearum* were identified from two hundred and eighty-five accessions (Kim *et al*., 2018). Similarly, Kumar *et al.* (2018) identified seven tomato genotypes with resistance to bacterial wilt out of fifty-seven genotypes evaluated. Through breeding efforts, cultivars resistant to *Ralstonia solanacearum* have been developed. However, the developed varieties are restricted to specific climatic conditions, locations, soil characteristics and strains of the pathogen (Hutton *et al*., 2012).

Gene silencing has been deployed to induce resistance to bacterial wilt in tomato (Chen *et al*., 2009). Virus-induced gene silencing (VIGS) pathways were involved in the control of bacterial wilt resistance. Defined sequence fragments obtained from a group of genes predicted to be involved in ethylene (ET) and salicylic acid (SA) signalling transduction pathways were subjected to a tomato cultivar with stable resistance to bacterial wilt and their effect on resistance was determined (Jiang *et al*., 2017). The results indicated that silencing some of the genes caused significant decrease in bacterial proliferation in stem bases.

Sources of genetics of resistance to bacterial wilt have been reported and the genetics of resistance have been studied widely (Ji *et al*., 2009). It has been suggested that genetic control of resistance to bacterial wilt disease varies with the source of resistance as well as the environmental conditions (Aslam *et al*., 2017). Diagnostic molecular markers have been developed to facilitate in marker-assisted breeding of tomato resistant to bacterial wilt (Abebe *et al*., 2020). The use of molecular markers in separating bacterial wilt resistance from undesirable horticultural characteristics and to pyramid resistance characteristics from multiple sources has been reported in the United States of America. A highly resistant cultivar against bacterial wilt was developed through the use of molecular markers by introgression of two complementary and co-dominant resistance genes (Miao *et al*., 2009). However, the virulence nature of the pathogen renders resistance ineffective. So far, the tomato breeding programmes in Kenya are yet to embrace the use of molecular markers.

Mutation breeding has been reported as an option in developing resistant genotypes against bacterial wilt disease of tomato according to Jyothi and Santhosha (2012). It creates

variation in constitutive gene expression of gene regulation sequences. O'Herlihy *et al*. (2012) successfully created genetic variability for bacterial wilt disease of tomato through mutation breeding. However, mutations in genes that govern bacterial wilt resistance, lack of stability of the mutant lines in hot climates and lack of suitability of mutants for the agronomic traits of interest complicate the breeding process hence need to be addressed (Singh *et al*., 2015).

Soma clonal variations have been useful sources for obtaining the necessary genetic variation for mitigating losses caused by bacterial wilt of tomato (Singh *et al*., 2015). In vitro screening of the genotypes against *Ralstonia solanacearum* is done using bacterial populations which generates resistant varieties. The best cultivars are then cultured in vitro and selected for further improvements for the desired characteristics. Nonetheless, the stability of the soma clones generated against the pathogen remains to be an issue under discussion

2.5.1 Gene action controlling resistance to bacterial wilt

The presence of pathogen race and strain diversity makes it necessary to search for a durable sources of resistance among landraces and wild genotypes (Kim *et al*., 2016). Neto *et al.* (2002) studied the inheritance of resistance to bacterial wilt in tomato plants using bi-parental populations and reported successful introgression of resistance genes into adapted genetic backgrounds of tomato. Similarly, Sharma and Verma (2004) reported the significance of additive × dominance type of interaction in the inheritance of bacterial wilt resistance on four tomato crosses in India.

A study conducted by Osiru *et al*. (2008) found significant general combining ability (GCA.) and specific combining ability (SCA.) effects for resistance to bacterial wilt. Significance of two major genes with segregation independent additive effects, plus polygenes with additive and dominance effects were important for inheritance of resistance to *Ralstonia solanaecearum* in tomato cultivars (Da-Silvia *et al.,* 2018). Diallel analysis of bacterial wilt resistance on tomatoes derived from different sources revealed high GCA for bacterial wilt resistance (Hanson *et al*., 1998). New tomato lines possessing bacterial wilt disease resistance were developed by repeated backcrosses (Ano *et al*., 2004). From thirteen tomato hybrids evaluated for yield and bacterial wilt resistance, the resistant hybrid had the highest number of fruits per plant. Similarly, crosses between large fruited, bacterial wilt resistant lines and several F_1s resulted in recovery of desirable lines with resistance to bacterial wilt, big fruit size, improved yield and good horticultural traits (Scott *et al*., 2009).

Bacterial wilt disease was found to be controlled by single dominant gene from studies involving segregating F_2 populations derived from a cross between tolerant × susceptible lines (Acharya *et al*., 2018). The backcross generations confirmed monogenic inheritance of bacterial wilt tolerance in tomato.

2.5.2 Identification and introgression of resistance genes from wild tomato into adapted cultivars

Wild tomatoes have been utilized for many years in breeding programmes to improve the cultivated tomato. Breeders have transferred resistant alleles from wild species into the cultivated tomato (Gill *et al*., 2019). Resistance to plant pathogens has been identified from several sources of wild tomato germplasm (Brozynska *et al*., 2016). Resistance to tomato yellow leaf curl virus was discovered in several wild tomato species, including *Solanum pimpinellifolium, Solanum peruvianum, Solanum chilense, Solanum habrochaites,* and *Solanum cheesmaniae* (Yang *et al*., 2014). Five resistance loci to tomato yellow leaf curl virus have been introgressed from wild tomato species to cultivated tomato. Robert *et al.* (2001) found black mold resistance in a wild tomato and quantitative trait loci for resistance were mapped in an interspecific population. Five QTLs for black mold resistance were selected for introgression into cultivated tomato using marker assisted selection (MAS) in backcross and selfing generations. Three of the five QTLs introgressed showed a significant and positive effect on black mold resistance.

A study conducted by Francis *et al*. (2001) identified sources of resistance from wild tomato species that could easily be crossed to cultivated tomato to control bacterial canker and from their study, the F_1s expressed resistance to bacterial canker. Wild tomato accessions had high levels of early blight (Chaerani & Voorrips, 2006). Resistant genes to late blight of tomato were identified and introgressed from wild tomato species into cultivated tomato by Demissie (2019). Similarly, genes for monogenic resistance to powdery mildew in tomato have been identified (Faino *et al*., 2012). A resistance locus from wild tomato species which conferred resistance to bacterial spot disease of tomato was identified through molecular markers by Sharlach *et al*. (2013). Genes conferring resistance to fusarium wilt were also successfully introgressed into cultivated tomato from wild tomato species by aid of molecular markers (Gonzalez-Cendales *et al*., 2016).

Genes conferring resistance to late blight of tomato for introgression into cultivated tomatoes were identified in wild tomato species (Arafa *et al*., 2017). A wild tomato accession was identified as a source of resistance for introgression into cultivated tomatoes for

Parietaria mottle virus disease (Parrella, 2020). Introgression of genes from wild species has also been achieved in other crops where new leaf rust resistant gene from wild wheat species were successfully transferred into wheat through marker assisted breeding (Narang *et al*., 2020). Even though wild tomato species are a rich source of desirable genes and characteristics for key agronomic traits, their utilization has been limited in the breeding programmes due linkage drag associated with transfer of undesirable traits (Nowicki *et al*., 2012).

2.6 Genotype-by-environment interaction and stability of resistance

Bacterial wilt disease resistance in tomato is controlled quantitatively hence influenced by both gene expression and environmental conditions including soil moisture, soil temperature and soil pH. Seasonal variations are important in the study of resistance to diseases (Burdon *et al*., 2016). Genotypic variation for resistance to bacterial wilt was reported among tomato genotypes screened across two cropping seasons (Ganiyu *et al*., 2017). A study by Asiimwe *et al*. (2013) identified a tomato genotype with both high fruit yields and stable resistance to bacterial wilt across six locations. Tomato progenies from a cross between two parents were found to have greater resistance to *Ralstonia solanacearum* across diverse environments (Mendes *et al*., 2018).

The role of genotype-by-environment interaction in expression of resistance to major diseases has been reported in a number of major crops. Zeng *et al*. (2017) identified rice (*Oryza sativa* L.) genotype with a stable resistance to sheath blight across six diverse environments indicating the significance of genotype-by-environment interaction in resistance expression. Similarly, peanut (*Arachis hypogaea*) genotypes with stable resistance to late leaf spot and rust were found across locations (Chaudhari *et al*., 2019). Cassava (*Manihot esculenta*) genotypes with resistance to cassava brown streak disease and cassava mosaic were identified across different agro-ecological zones (Maroya *et al*., 2012; Pariyo *et al*., 2015). Muthoni *et al*. (2015) identified one potato (*Solanum tuberosum* L.) family with the most stable resistance to bacterial wilt disease across four environments. Pereira *et al.* (2018) also identified bean (*Phaseolus vulgaris* L.) cultivars with stability for resistance to rust, anthracnose, fusarium wilt, angular leaf spot and common bacterial blight when evaluated in different locations Tekalign *et al*. (2017) reported two faba bean (*Pisum sativum* L.*)* genotypes with moderate levels of resistance to chocolate disease across six locations. Field pea (*Pisum sativum*) genotypes were identified for stable resistance to rust in multi-locations and years (Arpita *et al.,* 2019). Stable resistance was also reported in sweet potato

(*Ipomea batatas* L.) for sweet potato virus disease (Ngailo *et al*., 2019). Mungbean (*Vigna radiata* L.) genotypes with high and stable resistance to common bacterial blight were identified across semi-arid production environments (Tollo *et al*., 2020).

2.7 Combining ability

Combining ability analysis is defined as an estimation of the value of genotypes based on offspring performance in a definite mating design (Fasahat *et al*., 2016). From a line by tester mating design, Asati and Singh (2011) reported parental combinations of tomato lines which could be used as potential donors in breeding programmes to generate populations of resistant cultivars for deployment of bacterial wilt resistance in target areas. Similarly, tomato parental lines were identified as good general and specific combiners for bacterial wilt resistance demonstrating the role of both additive and non-additive gene action in the inheritance of the disease (Narayan *et al*., 2018). A number of tomato F_1 hybrids produced from diverse tomato line crosses in a line by tester mating design were reported to have moderate to high levels of resistance to bacterial wilt (Kathimba, 2021). Three tomato lines were identified as potential donors for resistance to tomato yellow leaf curl virus disease in a half-diallel mating design (Pandiarana *et al*., 2015). Similarly, three parental lines expressed good general combining ability (GCA) for resistance to tomato yellow leaf curl virus disease (Kaushik & Dhaliwal, 2018).

Studies in other crops revealed the role of additive and non-additive gene action in the inheritance of disease resistance. Low GCA and specific combing ability (SCA)values obtained from soybean (*Glycine max* L.) bi-parental crosses demonstrated the significance of both additive and non-additive gene action in the inheritance of soybean rust resistance (Kiryowa *et al.*, 2008). A study in hot pepper (*Capsicum annum* L.) revealed one genotype as a good general combiner for reduced bacterial wilt disease incidence (Nsabiyera *et al*., 2013). In a line by tester mating design, the potential of eggplant (*Solanum melongena* L.) parental lines in generating populations for development of bacterial wilt disease resistant genotypes was demonstrated (Chattopadhyay *et al*., 2012). Three parents were identified as donor parents for the development of hybrids resistant to bacterial wilt disease in brinjals (Lalramhlim *et al*., 2019). Combing ability for bacterial wilt resistance was revealed from chilli (*Capsicum frutescens* L.) genotypes crossed in a half-diallel mating design through artificial inoculation under fields conditions (James *et al*., 2017). Superior hybrids generated from a full-diallel mating design of rice were selected for development of resistant progenies for bacterial late blight (Habarurena *et al*., 2012). Parental lines with negative GCA values

and families with negative SCA values were selected for breeding for resistance to rice yellow mottle virus disease (Suvi *et al*., 2021). Good general combiners were identified for the development of grey leaf spot disease resistant cultivars in maize (*Zea mays* L.) (Tilahun *et al*., 2017). Another study on maize identified good general and specific combiners for maize streak virus disease resistance from a full-diallel mating design (Ige *et al*., 2017; Nyaligwa *et al*., 2018). Muhinyuza *et al*. (2016) identified potato families with resistance to late blight for further evaluation and release.

CHAPTER THREE

RESISTANCE OF WILD TOMATO (*Solanum pimpinellifolium*) AND CULTIVATED TOMATO (*Solanum lycopersicum*) GENOTYPES TO BACTERIAL WILT

Abstract

Bacterial wilt caused by *Ralstonia solanacearum* is a devastating soil-borne disease of tomato in Kenya. The little information which exists on sources of resistance has hindered progress on improvement of tomato genotypes for resistance to bacterial wilt. Wild species have previously been used as sources of genes for resistance to major diseases of tomato. The objective of this study was to identify novel sources of resistance to bacterial wilt from wild tomato species. Thirty-six tomato genotypes consisting of twenty-eight wild and eight cultivated tomatoes were screened in the greenhouse at the department of Crops, Horticulture and Soils, Egerton University. The trial was planted in an *alpha lattice* design for two cropping cycles. Genotype main effects were significant ($p \leq 0.001$) for area under disease progress curve (AUDPC), plant survival, disease incidence, average fruit weight and fruit yield. Effects due to cropping cycles were significant ($p \leq 0.001$) for AUDPC and plant survival at 30 days after inoculation (DAI), AUDPC, plant survival and disease incidence at 45 and 60 DAI and ($p \leq 0.05$) for plant survival at 30 DAI. Genotype × cropping cycle interaction effects were significant ($p \leq 0.001$) for AUDPC at 30 DAI, AUDPC and plant survival at 45 DAI and AUDPC at 60 DAI and ($p \leq 0.01$) for disease incidence at 45 DAI. Means for AUDPC ranged from 0-1575, disease incidence ranged from 0-100 and plant survival ranged from 0-100 in the two cropping cycles. Genotypes KK 1, NRB, KK acc II, KISII, BRNG acc II and KLF acc III expressed high and stable resistance to bacterial wilt in both cropping cycles. The sources of resistance identified will potentially be deployed in breeding programmes involved in developing new resistant tomato varieties against bacterial wilt.

3.1 Introduction

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most major constraints in tomato production worldwide (Elnaggar *et al*., 2018). The disease is mostly severe in tropical regions of the world where it is favoured by factors such as virulent pathogen, favourable climate, susceptible host and sufficient time for development of symptoms (Singh *et al*., 2014). Bacterial wilt has been reported to cause yield losses of up to 100% under rain-fed conditions (Costa *et al*., 2019). Apart from quality down grade of tomatoes, the control of bacterial wilt disease increases the cost of production which arises mainly from the

application of copper based fungicides (Nion &Toyota, 2015). *Ralstonia solanacearum* has been considered as the most destructive pathogen due to the persistence of the bacterium in the soil and crop debris, prevalence in a wide range of temperatures, pH, moisture and relative humidity (Ignatius, 2018). Nearly all stages of growth in tomatoes are affected by bacterial wilt with the maximum severity being at flowering and fruiting stages (Acharya *et al*., 2018).

In Kenya, yield losses of up to 50% in the field and 100% in both the greenhouse and high tunnel, have been attributed to bacterial wilt disease of tomato (Ireri *et al*., 2018). At present, both small-scale and large-scale tomato producers in Kenya largely rely on soil solarization and copper-based fungicide application, crop rotation and field sanitation to control the disease (Panth *et al*., 2020). The use of copper-based fungicides in management of bacterial wilt is ineffective, hazardous to the environment and costly to the majority of small-scale farmers (Ignatius, 2018). Besides, there is limited information on conventional bactericides which are known to provide effective control against the disease (Malgie, 2015). A wide range of integrated management options for bacterial wilt such as avoiding wounds in the stem, removing sick plants, grafting, use of coco-peat growing media, supplementing irrigation with sea water and crop protection methods have been employed (Singh *et al*., 2015). However, these methods have not been significant in the control of bacterial wilt. Crop rotation with non-host crop is one of the recommended control options. It has been reported that a single year crop rotation can significantly reduce the impact of bacterial wilt but two or more years of rotation is recommended (Wright *et al*., 2015) Nonetheless, farmers are unable to adapt to the effective crop rotations due to limited land availability (Ayana & Fininsa, 2017). Overall, the available strategies have not provided an effective control against the soil-borne pathogen and the farmers continue to experience high yield losses from the disease.

Tomato is largely grown by small-scale farmers in Kenya (Ayana & Fininsa, 2017). Currently, nearly all the commercial varieties of tomato grown in Kenya are introduced mostly from Europe and less adapted to the local conditions thus suffer greatly from bacterial wilt attack. This is further aggravated by lack of a formal breeding programme to address the problem. Reliance on chemical control and cultural practices has not been effective (Ignatius, 2018). Studies to screen tomato germplasm have identified QTLs linked to bacterial wilt resistance (Kumar *et al*. 2018). However, bacterial wilt still remains a challenge in tomato production and information on stability of the identified QTLs and their utilization in breeding for resistance is limited. Consequently, identification of novel sources of resistance

is a viable strategy which offers the possibility of introgression of resistance genes into adapted cultivars (Beaver & Osomo 2009). Deployment of resistant varieties is a realistic alternative method that can be used to manage bacterial wilt disease. Adoption of resistant varieties is an affordable and environmentally friendly approach (Aslam *et al*., 2017). Tomato varieties with stable resistance can be employed as an important component in an integrated disease management programme of bacterial wilt (Singh *et al*., 2015). Wild germplasm act as a repository for novel genes for improvement of many modern crop species for a number traits of economic importance (Priyanka *et al*., 2021). Despite the problem of linkage drag being responsible for introducing unwanted genetic background that lower quality of the adapted crop, wild germplasm has been successfully used as donor parents in backcrossing schemes to transfer useful resistance genes. For example, Lebeau *et al*. (2011) reported a wild tomato species *Solanum pimpinellifolium* as being an important source to bacterial wilt resistance. The current study was therefore conducted to identify novel sources of resistance to bacterial wilt for breeding of improved resistant tomato varieties.

3.2 Materials and methods

3.2.1 Experimental site

The study was conducted in the greenhouse at Egerton University, Njoro Campus in the Department of Crops, Horticulture and Soils in Kenya. The site lies approximately at 35°55'58.0''E and 0°22'11.0''S and an altitude of 2238 m above the sea level. The area is situated in the lower highland agro-ecological zone 3 (LH 3) (Jaetzold & Schmidt, 2012). It has a tropical climate and the temperatures ranges between 15°C- 21°C. The area receives a mean annual rainfall of 1200 mm. The distribution of the rain is bimodal with long rains between April and August and short rains between October and December, yearly. The soils at the experimental site are well drained, sandy loams which are dark brown in colour characterized by a pH of 6 (Jaetzold & Schmidt, 2012). Njoro was chosen as a study site because it is one of the hot spots of *Ralstonia solanacearum* (Kago *et al*., 2016).

3.2.2 Plant material

A set of thirty-six tomato genotypes comprising 28 wild genotypes collected from different sites across the country in regions where there is predominance of wild tomato diversity along with eight commercial tomato varieties were assembled (Table 3.1). The selected commercial varieties for the study were pure line varieties which are widely preferred for cultivation by the farmers.

Table 3.1. List of tomato germplasm evaluated for bacterial wilt

S/No	Germplasm	Source	Geographical coordinates
Wild			
1	MK	Makueni County	1°48' 0"S and 37°37'11.0"E
2	BRNG acc1	Baringo County	0°1'12"S and 35°58'12"E
3	NKR acc I	Nakuru County	0°22' 11.0"S and 35°58.0"E
4	KSM	Kisumu County	0°8' 29"S and 34°58' 0"E
5	TVT	Taita Taveta County	03°24' 6.95"S and 38°21' 50.47"E
6	TRNZ	Transzoia County	03°24' 6.95"S and 38°21' 50.47"E
7	NYR	Nyeri County	0°28' 7"N and 36°59' 54"E
8	NKR acc II	Nakuru County	03°24' 6.95"S and 38°21' 50.47"E
9	KK acc I	Kakamega County	0°04' 60"N and 34°00' 0.00"E
10	NRB	Nairobi County	-1°14' 15"S and 36°49' 14.99"E
11	KK acc II	Kakamega County	0°04' 60"N and 34°00' 0.00"E
12	KISII	Kisii County	0°38' 0"S and 34°47' 0"E
13	KLF acc I	Kilifi County	3°2' 0"S and 40°8' 0"E
14	KLF acc II	Kilifi County	4°25' 0"S and 39°29'0"E
15	BRNG acc II	Baringo County	0°28' 0"N and 35°59'0"E
16	BRNG acc III	Baringo County	0°43' 0"N and 35°44' 0"E
17	NYM	Nyamira County	0°38' 52.8"N and 34°58' 1.2"E
18	KITUI	Kitui County	1°18' 30"S and 37°59' 30"E
19	NKR acc III	Nakuru County	0°12' 60"N and 36°15' 60"E
20	UAG	Uasin Gishu County	0°12' 55.42"N and 35°25' 53.83"E
21	KRC	Kericho County	0°15' 27"S and 35°27' 2"E
22	MCK acc I	Machakos County	-1°31' 0.01"S and 37°16' 0.01"E
23	BRNG acc IV	Baringo County	0°03' 5.69"N and 35°43'50.81"E
24	BRNG acc V	Baringo County	0°2' 40"N and 35°31' 38"E
25	NKR acc IV	Uasin Gishu County	0°03' 5.69"N and 35°43' 50.81"E
26	KLF acc III	Kilifi County	-3°51' 51.99"S and 39°27' 59.99"E
27	MCK acc II	Machakos County	-1°31' 0.01"S and 37°16' 0.01"E
28	KLF acc IV	Kilifi County	4°25' 0"S and 39°29' 0"E
Cultivated			

Table 3.2. Contd…

29	Rionex	Continental Seed Company
30	M-82	Kenya Seed Company
31	Money Maker	Kenya Seed Company
32	Cal-J	Kenya Seed Company
33	Marglobe	Kenya Seed Company
34	Riogrande	Kenya Seed Company
35	Red Diamond	Continental Seed Company
36	Oxyly	Royal Seed Company

NRB Nairobi, KLF Kilifi, NYM Nyamira, UAG Uasin Gishu, KRC Kericho, MCK Machakos,

3.2.3 Collection, isolation and preservation of *Ralstonia solanacearum* inoculum

Samples of five infected tomato plants showing bacterial wilting symptoms were collected from individual farms in Subukia, Nakuru County in Kenya for isolation of the pathogen. Geographical locations of the farms were recorded using the Global Position System. A quick field ooze test was carried out to distinguish *Ralstonia solanacearum* from vascular wilts that are caused by fungal pathogens. The stems of diseased tomato plants showing typical symptoms of bacterial wilt were cut using sterilized scalpel blades. The cut ends of the stem were placed in test tubes containing sterile distilled water. The presence of the pathogen was confirmed by the proliferation of fine milky white strands when the infected tissue is placed in water. These white strands are as a result of masses of bacteria, which come out of the margins of the cut portions within ten minutes (Rohini *et al*., 2017).

The infected tomato plants collected from the field were washed under running tap water to remove sand and soil. Vascular tissues were extracted with a new sterile scalpel blade into sections of about 10 cm in length from collar region of wilted plants (Ahmed *et al*., 2013). The tissues were surface sterilized for thirty seconds in 1% sodium hypochlorite solution, 70% ethyl alcohol followed by three repeated washings in sterile distilled water and blot dried. The stem sections weighing one gram were macerated in a test tube containing 10 ml of clean sterile distilled water to create a stock solution. The stock solution was serially diluted by adding 1 ml of bacterial solution to eight test tubes each containing 9 ml of sterile distilled water. Each test tube was vortexed and allowed to settle for at least ten minutes.

Isolation of the bacterium was done following streak plate method as described by Grover *et al*. (2012) on to 2, 3, 5 Triphenyl Tetrazolium Chloride (Kelman's TZC agar) medium

(glucose 5 g, peptone 10 g, casein hydrolysate 1 g, agar 18 g, distilled water 1000 ml), 5 ml of TZC solution filter sterilized was added to the autoclaved medium to give a final concentration of 0.005%) according to the procedure of Seleim *et al.* (2014). One loopful of bacterial suspension was taken from the eight test tubes and streaked on pre sterilized moisture free plates. The plates were incubated upside down in an incubator at 28ºC ± 2ºC for 24-48 hours. Single virulent colonies from the medium were characterized by dull white colour fluid with irregular round and light pink centres and these were further streaked on TZC plates to obtain pure culture of the isolates. The pure culture was transferred to 5 mL of sterile double distilled water in screw capped bottles where they were stored for experimental use under refrigeration at –20ºC for maintenance of virulence.

3.2.4 Experimental procedure

Thirty-six genotypes were sowed in a nursery for 5 weeks before transplanting. Five hundred kg of forest soil was sterilized by autoclaving in an autoclave at 121ºC for 1 hour. The soil was placed in plastic pots measuring 25 cm in diameter and 30 cm in height. The seedlings were transplanted at three-leaf stage in the pots with row spacing of 40 cm and plant to plant distance of 30 cm. Six pots were used for each entry per experimental unit giving a total of six plants per plot. The experimental design was an *alpha*-lattice design of 9 blocks and 4 units within the blocks, in two replicates. During transplanting Di-Ammonium Phosphate was applied at the rate of 5 g per pot to supply 62.5 kg N/ha and 95 kg P/ha. Three weeks after transplanting Calcium Ammonium Nitrate (CAN) was applied at the rate of 5 g per pot to supply 250 kg Ca/ha. Magnesium was applied at the rate of 5 g per pot to supply 60 kg Mg/ha. Three weeks after application of CAN, Nitrogen, Phosphate, Potassium (NPK) compound fertilizer was applied at the rate of 5 g per pot to supply 100 kg N/ha, 60 kg P/ha and 60 kg K/ha foliar fertilizers were used as supplements. Evisect (Thiocyclam hydrogen oxalate, water soluble powder, Arysta Life Science, BP Noguères France) was applied at the rate of 500 kg/ha for protection against white flies. Confidor (Imidacloprid 700 kg/ha; Bayer Crop Science AG, Kalser-Wilhelm-Allee 1, Germany) was applied at the rate of 100 mL ha-1 for protection against aphids, thrips and leaf miners. Ridomil Gold (Metalaxyl-M 40 g/kg+Mancozeb-640 g/kg, United States) was applied at the rate of 2.5 kg/ha to control downy mildew, early and late blight fungal diseases. Token (Azoxystrobin 259g/l + Difeconazole 125g/l, Osho Chemicals Industries Limited, Nairobi Osho Compl) was applied at the rate of 2 kg/ha) to control early and late blight. No control measures were taken against bacterial wilt disease. The crop was kept free of weeds by manual weeding. Pruning was

done regularly by manual removal of proliferating branches. For training of the plants, staking was done by use of sticks. Irrigation was done regularly once in a day during cloudy conditions and twice a day during dry periods of sunshine.

3.2.5 Plant inoculation with bacterial wilt pathogen

Seedlings were inoculated 14 days after transplanting using cultured pathogen. Before applying the inoculum, the main stem was incised on either side to a depth of 5-6 cm using a sterile scalpel thereby causing injury to the secondary roots (Mwangi *et al*., 2008; Onduso, 2014). Thereafter, 30 mm of the standardized bacterial suspension containing 1×10^9 colony forming units per ml inoculation of *R. solanacearum* was poured over the roots (Singh *et al*., 2018). After inoculation, the plants were watered at alternative days to maintain a high soil moisture for the development of the disease.

3.2.6 Data collection

All plants in each experimental unit were used for evaluation of host plant response to disease. The visual disease scoring using a scale of 0-5 as described by Kempe and Sequeira (1983) (Table 3.2) was deployed to record disease scores at three different crop growth stages *viz.*, 30, 45 and 60 days after inoculation (DAI).

Figure 3.1. Disease severity scale of Bacterial wilt on tomato (R-Resistant, MR-Moderately Resistant, MS-Moderately Susceptible, S-Susceptible and HS-Highly Susceptible)

Table 3.3. Disease rating scale for bacterial wilt

Rating scale	Description	Disease reaction
0	No symptoms	Highly resistant
1	1 to 25% leaves wilted	Resistant
2	26 to 50% leaves wilted	Moderately resistant
3	51 to 75% leaves wilted	Moderately susceptible
4	75% but less than 100% of leaves wilted	Susceptible
5	All leaves wilted and plant dead	Highly susceptible

Source: Moussa *et al*. (2017).

The disease evaluation data were summarized using the percent disease severity (PDS) formula as described by Pranamika and Saikia (2013) and expressed as the area under the disease progress curve (AUDPC). AUDPC was estimated following Wilcoxson *et al*. (1975) as;

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

Where y_i is the % disease severity on the i^{th} scoring; t_i is the number of days from sowing to i^{th} scoring; n is the total number of scores.

Plant survival was calculated using the formula described by Jyoti *et al*. (2015) as;

$$\text{PS} = \frac{\text{NHP}}{\text{NPE}} \times 100 \quad \ldots\ldots (2)$$

Where NHP is the number of healthy plants, NPE is the number of plants established.

Disease incidence was calculated using the formula described by Gashaw *et al*. (2014) as;

$$\text{DI} = \frac{\text{NIP}}{\text{NPA}} \times 100 \quad \ldots\ldots (3)$$

Where NIP is the number of infected plants, NPA is the total number of plants assessed.

Average fruit weight was computed by taking the total fruit weight of each plant divided by the total number of fruits from each plant. Fruit yield was determined by adding the weight of the total fruits from different pickings from each of the selected and tagged plants.

3.2.7 Data analyses

Data for AUDPC were log transformed while data for disease incidence and plant survival were arcsine square root transformed to obtain a normal frequency distribution. The

analysis of variance was done using Genstat 15th edition (VSN International, Hemel Hempstead, UK). The statistical model for the analysis was;

$$Y_{ijkl\,m} = \mu + C_j + R_l + B_{k(l)} + G_i + GC_{ij} + \varepsilon_{ijklm} \quad \ldots\ldots (4)$$

Where; Y_{ijklm} is the observed performance from each experimental unit; C_j is the effect due to j^{th} cropping cycle; R_l is the effect due to l^{th} replicate; $B_{k\,(l)}$ is the effect due to k^{th} block within the l^{th} replicate; G_i is the effect due to i^{th} genotype; GC_{ij} is the effect due to interaction between the genotype and the cycle; ε_{ijklm} is the random error component.

Genotypes, cropping cycles and replications were considered as fixed effect while blocks were considered as random effects. Mean separation was performed using least significant difference (LSD) test at $p \leq 0.05$ given as:

$$\text{LSD} = \frac{t(s\sqrt{2})}{\sqrt{n}} \quad \ldots\ldots (5)$$

Where t is tabulated t value, s is standard deviation of all the plots and n is number of observations.

A Pearson correlation coefficient analysis was done using Genstat 15th edition (VSN International, Hemel Hempstead, UK) by applying the following formula:

$$r = \frac{n\Sigma xy - (\Sigma x)(\Sigma y)}{\sqrt{[n(\Sigma x^2 - (\Sigma x^2)]\,[n\Sigma y^2 - (\Sigma y^2]}} \quad \ldots\ldots (6)$$

Where r is the Pearson's correlation coefficient, n is the number of samples, x is the dependable variable and y is the independent variable.

Standard error of the mean was calculated using the following formula:

$$Standard\ error\ of\ the\ mean = \frac{sd}{\sqrt{n}} \quad \ldots\ldots (7)$$

Where sd is the standard deviation and n is the number of samples.

Genotypic stability for AUDPC was estimated using cultivar superiority as described by Lin and Binns (1985). The superiority of performance for each genotype was estimated as the distance mean square (MS) from the maximum response genotype in each cropping cycle and was determined as;

$$P_i = \left[n(\bar{X}_{i.} - \bar{M})^2 + \sum_{j=1}^{n}\left(X_{ij} - \bar{X}_{i.} - M_j + \bar{M}\right)^2\right]/(2n) \quad \ldots\ldots (8)$$

Where, P_i is the superiority measure of the i^{th} genotype, n is the number of cropping cycles, X_{ij} is performance of the i^{th} genotype in the j^{th} cycle and M_j is the maximum cropping cycle response.

Genotypic superiority was based on P_i values which represent mean square (MS) of the effect due to genotype$[n(\bar{X}_{i.} - \bar{M})^2]$, GCI $\left[\sum_{j=1}^{n}\left(X_{ij} - \bar{X}_{i.} - M_j + \bar{M}\right)^2\right]$ and the general adaptability of the genotype (Lin & Binns, 1988; Lin & Binns, 1985). Pairwise genotype-by-cropping cycle interaction (GCI) MS between maximum and test genotype were used to eliminate the possibility of discarding genotypes with specific adaptability. Critical values for significance of P_i and GCI were the product of pooled residual MS from combined analyses of variance and tabulated F-values for corresponding degrees of freedom (df), where the df for P_i and GCI were n and n-1, respectively (Lin & Binns, 1988). The Finlay and Wilkinson (1963) regression coefficients (b_i) on cropping cycle mean indicated the general response pattern among genotypes and were used to protect against discarding genotyped with narrow adaptability.

3.3 Results

3.3.1. Analysis of variance and phenotypic performance

Data for Area Under Disease Progress Curve (AUDPC), disease incidence and plant survival were reported for two cropping cycles. However, data for yield and quality traits were reported for the first cropping cycle only and this was due to high disease pressure experienced during the second cropping cycle which caused plant mortality of up to 100% for more than half of the genotypes tested before crop maturity. Significant ($p \leq 0.01$) variation among the genotypes was observed across the cropping cycles for AUDPC, disease incidence and plant survival at 30, 45 and 60 days after inoculation (DAI) (Table 3.3). Cropping cycles effects were significant ($p \leq 0.001$) for AUDPC and plant survival at 30 DAI, AUDPC, plant survival and disease incidence at 45 and 60 DAI and ($p \leq 0.05$) for plant survival at 30 DAI. Effects due to interaction between genotypes and cropping cycles were significant ($p \leq 0.001$) for AUDPC at 30 DAI, AUDPC and plant survival at 45 DAI and AUDPC at 60 DAI and ($p \leq 0.01$) for disease incidence at 45 DAI. Similarly, genotype main effects were significant ($p \leq 0.001$) for average fruit weight and fruit yield at 60 DAI. (Table 3.4). AUDPC values of 0-150, 151-300, 301-500 and > 500 represented very low, low, moderate and high levels of resistance, respectively.

Genotypes expressed variation for disease measurements as well as yield and yield related traits in different cropping cycles (Table 3.5 and 3.6). Sixteen genotypes out of 36 exhibited resistances to bacterial wilt with mean AUDPC scores of ≤ 500, and plant survival of 100 % at 45 DAI. There was a trend of high disease pressure in the second cropping cycle with mean AUDPC of 642 and 734 at 45 and 60 DAI compared to the first cropping cycle

with mean AUDPC of 535 and 714 at 45 and 60 DAI. In contrast, the plant survival was higher in the first cropping cycle with 50% and 28%of the plants surviving at 45 and 60 DAI compared to the second cropping when only 38% and 20% of the plants survived (Table 3.5).

High genotypic variation was displayed among the genotypes for disease resistance traits, maturity yield and yield related traits among the genotypes. In general, the wild genotypes were more resistant compared to the cultivated genotypes due to their low mean values for AUDPC and disease incidence and high mean values for plant survival. However, cultivated genotypes had higher mean values for average fruit weight and fruit yield as compared to the wild genotypes (Table 3.6). Significant differences ($p \leq 0.05$) between wild and cultivated genotypes was observed for disease traits as average weight and fruit yield (Table3.6)

Table 3.4. Mean squares for AUDPC, disease incidence and plant survival of tomato genotypes evaluated for two cropping cycles in the greenhouse at Egerton University, Njoro in 2020

Source of variation	df	30 days after inoculation			45 days after inoculation			60 days after inoculation		
		AUDPC	PS	DI	AUDPC	PS	DI	AUDPC	PS	DI
Cycle	1	1.33***	0.56*	0.45	0.79***	0.51***	0.56***	0.27***	0.74***	0.97***
Rep(Cropping cycle)	1	0.01	0.01	0.00	0.02	0.00	0.00	0.01	0.00	0.00
Genotype	35	1.59***	0.53***	0.38***	2.17***	0.76***	0.74***	2.50***	1.19***	1.21***
Cycle ×Genotype	35	0.08***	0.05	0.04	0.04***	0.03***	0.03**	0.04***	0.03	0.04
Residual	71	0.01	0.07	0.04	0.01	0.01	0.01	0.01	0.03	0.04
CV %		0.70	1.10	1.10	0.60	0.10	0.50	0.50	0.60	0.40

*, **, *** Significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), CV Coefficient of variation, AUDPC Area Under Disease Progressive Curve, PS Plant Survival, DI Disease Incidence

Source of variation	df	AFW	FY

Table 3.5. Mean squares for days to maturity, average fruit weight and fruit yield of tomato genotypes evaluated in the greenhouse in cycle 1 at Egerton University, Njoro in 2020

Replication	1	11.28	7.05
Genotype	35	1577.62***	3.71***
Residual	35	8.64	0.99
CV %		2.90	18.40

*, **, ***Significant at ($p \leq 0.001$), CV Coefficient of variation, AFW Average Fruit Weight, FY Fruit YieldTable 3.6. Range and mean values of AUDPC, plant survival, disease incidence, average fruit weight and fruit yield for thirty-six tomato genotypes evaluated in the greenhouse at Egerton University, Njoro 2020

	45 days after inoculation					
Cycle		AUDPC		PS		DI
	Range	Mean± SE	Range	Mean± SE	Range	Mean± SE
1st cycle	0-1138	535±12.12	0-100	50±0.87	0-100	45±0.83
2nd cycle	0-1192	642±11.87	0-100	38±0.87	0-100	57±0.87

AUDPC Area Under Disease Progress Curve, DI Diseases Incidence, PS Plant Survival, SE Standard Error

Table 3.57. Contd…

60 days after inoculation										
	AUDPC		PS		DI		AFW		FY	
Cycle	Range	Mean± SE	Range	Mean± SE	Range	Mean± SE	Range	Mean± SE	Range	Mean± SE
1st cycle	0-1575	714±16.97	0-100	28±0.98	0-100	69±1.00	3-88.50	19.46±0.78	0.82-6.68	2.40±0.04
2nd cycle	0-1539	734±15.22	0-100	20±1.03	0-100	78±1.04	-	-	-	-

Data missing for second cycle as a result of plant mortality for >50 % before maturity due to high disease pressure, AUDPC Area Under Disease Progress Curve, DI Diseases Incidence, PS Plant Survival, SE Standard Error

Table 3.6. Mean values of top 13 wild tomato accessions evaluated for bacterial wilt resistance in the greenhouse and having AUDPC values ≤ 500 along with eight cultivated varieties

	AUDPC		PS		DI		AUDPC		PS		DI		AFW	FY
	45 DAI						60 DAI							
Genotypes	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC1
Wild														
KK acc 1	0	0	100	100	0	0	0	0	100	100	0	0	5.80	2.50
NRB	0	0	100	100	0	0	0	0	100	100	0	0	3.30	4.87
KK acc II	0	0	100	100	0	0	0	0	100	100	0	0	5.80	2.61
KISII	0	0	100	100	0	0	0	0	100	100	0	0	4.60	3.17
BRNG acc II	0	0	100	100	0	0	0	0	100	100	0	0	6.00	2.65
KLF acc III	0	0	100	100	0	0	0	0	100	100	0	0	3.00	2.47
NKR acc III	46	299	71	50	29	50	40	219	20	0	0	100	5.20	2.15
KITUI	52	122	80	50	20	29	49	41	40	29	40	50	4.60	2.13
MK	68	214	71	39	20	50	59	156	40	20	40	71	5.00	2.09
BRNG acc V	75	247	80	39	20	50	69	185	29	5	50	95	5.00	2.03
MCK acc II	75	230	60	50	29	39	66	168	29	11	61	82	6.00	2.02
KLF acc II	90	337	60	29	29	50	85	253	40	20	60	71	4.60	2.02
BRNG acc IV	97	253	60	61	29	29	107	209	29	29	61	50	5.00	1.94
Cultivated														
MARGLOBE	607	822	50	20	50	80	766	923	5	0	89	100	88.50	6.68
Table 3.6 Contd…														
RIONEX	651	636	50	39	50	50	822	515	5	5	95	95	85.50	5.77

M-82	945	1013	29	20	50	71	1308	1165	5	0	95	100	72.50	4.46
OXYLY	945	1086	20	0	80	100	1278	1278	0	0	100	100	69.00	2.66
MONEY MAKER	967	990	20	20	71	80	1339	1220	5	0	95	100	78.50	5.44
RIOGRANDE	967	1013	50	20	50	80	1339	1220	5	0	95	100	63.50	2.46
CAL-J	1112	1112	0	5	100	95	1504	1370	0	0	100	100	46.00	1.68
RED DIAMOND	1112	1112	0	0	100	100	1539	1403	0	0	100	100	55.00	2.02
Means	714	641	50	38	45	57	714	730	28	20	69	78	19.50	2.40
LSD[a]	0.16	0.16	0.24	0.24	0.23	0.23	0.17	0.17	0.34	0.34	0.39	0.39	5.97	2.02

CC- Cropping Cycle, AUDPC Area Under Disease Progress Curve, PS Plant Survival, DI Disease Incidence, AFW Average Fruit Weight, FY Fruit Yield, CC Cropping Cycle, KK Kakamega, NRB Nairobi, BRNG Baringo, KLF Kilifi, NKR Nakuru, MK Makueni, MCK Machakos

LSD[a] values based on transformed data

3.3.2 Phenotypic correlations

The Pearson correlation coefficient analysis revealed a significant positive correlation between the average fruit weight and disease incidence (r=0.30, $p \leq 0.05$) and fruit yield (r=0.62, $p \leq 0.01$) (Table 3.7). Average fruit weight displayed significant negative correlation with days to maturity (r=-0.34, $p \leq 0.01$). The remaining correlations were not significant.

Table 3.8. Correlation coefficients among average fruit weight, disease incidence, days to maturity and fruit yield

	AFW	DI	DM	FY
AFW		0.30*	-0.34**	0.62***
DI			0.03	-0.17
DM				-0.20
FY				

*, **, ***, significant at ($p \leq 0.05$), ($p \leq 0.01$) and ($p \leq 0.01$) respectively. AFW Average Fruit Weight, DI Disease Incidence, DM Days to Maturity, FY Fruit Yield

3.3.3 Genotypic superiority and stability of resistance for bacterial wilt disease

The superiority measure of (P_i) and the (GC) interaction MS for each genotype are shown in Table 3.8. Genotypes KK Acc I, NRB, KK Acc II, KISII, BRNG Acc II, and KLF Acc III showed the lowest mean values of AUDPC and P_i respectively. These genotypes displayed superior and stable performance across cropping cycles at 60 days after inoculation. Genotypes MK. NKR acc III, MCK acc II, KLF acc II, BRNG acc IV, NKR acc IV, NKR acc I, KRC, RIONEX, MARGLOBE, KSM and NYM had values above the cut-off point for (GC) interaction MS. These genotypes were significantly higher than the minimum response and may possibly require further investigations.

Table 3.8. Superiority measure (*P*i), genotype by cropping cycle interaction mean square and regression coefficient (*b*) of AUDPC of 36 genotypes arranged in the order of *P*i

Ranking	Genotype	Mean(AUDPC)	*P*i 1000	MS (GC)	*B*
1	KK acc I	3.23	0.00	0.00	-0.18
2	NRB	3.41	0.00	0.05	0.46
3	KK acc II	3.25	0.00	0.12	0.38
4	KISII	3.13	0.00	0.04	-0.48
5	BRNG acc II	3.44	0.00	0.05	-0.52
6	KLF acc III	3.10	0.00	0.00	-0.15
7	KITUI	7.51	0.03	0.01	-0.02
8	MK	10.57	0.05	8.65*	4.47
9	NKR acc III	11.28	0.06	25.96*	7.86
10	BRNG acc V	11.35	0.06	10.76*	5.00
11	MCK acc II	11.01	0.06	8.46*	4.42
12	KLF acc II	13.14	0.09	15.32*	6.00
13	BRNG acc IV	12.91	0.10	5.92*	3.67
14	NKR acc IV	17.98	0.20	34.61*	9.11
15	NKR acc I	20.38	0.31	12.46*	5.40
16	NKR acc II	22.49	0.46	0.53	0.98
17	KRC	28.82	0.73	14.12*	5.76
18	RIONEX	25.79	0.75	11.26*	-5.45
19	MARGLOBE	29.22	0.81	3.33*	2.71
20	KSM	31.40	0.95	3.02*	2.58
21	BRNG acc III	31.42	1.02	0.10	-0.66
22	NYM	33.74	1.11	4.60*	3.22
23	KLF acc I	33.71	1.20	0.33	-1.08
24	MCK acc I	34.68	1.26	0.01	-0.31
25	OXYLY	35.86	1.34	0.14	0.43
26	TVT	36.48	1.35	1.54	1.79
27	M-82	35.35	1.35	1.11	-1.83
28	BRNG acc1	35.68	1.37	0.59	-1.38
29	MONEY MAKER	35.98	1.39	0.63	-1.41

Table 3.8 Contd…

30	RIOGRANDE	35.90	1.40	1.15	-1.85
31	NYR	36.72	1.49	2.36	-2.58
32	TRNZ	36.94	1.51	2.41	-2.61
33	UAG	37.26	1.51	0.81	-1.58
34	CAL-J	38.05	1.59	1.36	-2.00
35	RED DIAMOND	38.21	1.60	0.97	-1.72
36	KLF acc IV	38.47	1.63	1.63	-2.18

AUDPC Area Under Disease Progress Curve, *Pi* Performance index, MS(GC) Means Square Genotype by Cropping Cycle, *b* regression coefficient, KK Kakamega, NRB Nairobi, BRNG Baringo, KLF Kilifi, MK Makueni, NKR Nakuru, MCK Machakos, KRC Kericho, KSM Kisumu, NYM Nyamira TVT Taita Taveta, NYR Nyeri, TRNZ Trans Nzoia, UAG Uasin Gishu.

3.4 Discussion

Introgression of bacterial wilt resistance genes in adapted tomato cultivars is a key breeding strategy in control of the disease. Germplasm screening to find novel sources of resistance is a key step towards attaining this objective. In this study, the significant main effects due to genotypes for area under disease progressive curve (AUDPC), plant survival and disease incidence observed at 30, 45 and 60 days after inoculation (DAI) in analysis of variance (ANOVA) suggests the existence of genotypic variation for bacterial wilt resistance among wild tomato genotypes evaluated (Table 3.3). Asiimwe *et al.* (2013) reported the existence of genotypic variation for bacterial wilt resistance in tomato germplasm. The significant effects due to genotype for average fruit weight and yield signifies the existence of considerable genotypic variation (Table 3.4). Earlier studies also show evidence of genotypic variation for average fruit weight and fruit yield among tomato genotypes (Meena *et al*., 2015). The significant mean squares due to cropping cycles for AUDPC and plant survival at 30 DAI and AUDPC, plant survival and disease incidence at 45 and 60 DAI suggested the significance of cropping cycle in phenotypic expression for bacterial wilt resistance. Factors such as, temperatures soil pH and soil moisture are key environmental components known to contribute to the expression of the disease symptoms among genotypes across the cropping cycles in the greenhouse or across seasons in the field in different locations (Hailu *et al*., 2017; Velásquez *et al*., 2018). Effect of cropping cycle contributed to the differential performance of tomato genotypes under greenhouse conditions for bacterial wilt resistance

based on AUDPC, disease incidence and plant survival in earlier studies (Onduso, 2014; Sarfo, 2018). Significant genotype-by-cropping cycle (GC) interaction for AUDPC suggests inconsistent pattern of performance among genotypes depending on the growth period. There is therefore need to understand the change of disease patterns occasioned by fluctuations in plant growth conditions for more effective genetic control.

Higher AUDPC mean values of 642 and 734 and disease incidence of 57% and 78% at 45 and 60 DAI observed in the second cropping cycle compared to first cropping cycle indicated increased disease pressure among the genotypes (Table 3.5). High temperatures in the greenhouse could have attributed to increased disease presence thereby affecting the resistance of genotypes to bacterial wilt disease which resulted in low plant survival. High temperatures of between 20^0C to 35^0C cause increased disease pressure (Asare *et al*., 2017; Bittner *et al*., 2016; Santhosha *et al*., 2015). Reduction in plant survival across seasons was associated with high disease pressure in eggplant (*Solanum melongena* L.) (Manickam *et al*., 2021)

Wild resistant genotypes were more resistant bacterial wilt compared to cultivated varieties as revealed by low mean values for AUDPC, disease incidence and plant survival (Table 3.6). Superior genetic resistance of the wild genotypes could also be associated with presence of higher concentrations of secondary metabolites comprising polyphenols which hinder the movement of the bacteria in the plant system (Vasse *et al*., 2005). Wild genotypes KK acc I, NRB, KK acc II, KISII, BRNG acc II and KLF acc III expressed high resistance while NKR acc III, KITUI, MK, BRNG acc V, MCK acc II, KLF acc II and BRNG acc IV exhibited very low, low and moderately resistant reaction based on AUDPC, disease incidence and plant survival. Reaction of wild genotypes to bacterial wilt may vary from to moderate resistance or tolerance to high levels of resistance (Timila & Joshi, 2007).

Significant variations observed for average fruit weight and fruit yield among all the genotypes indicated that there exists a genetic variation among the wild and cultivated genotypes (Table 3.6). Differences were reported among wild and cultivated tomato genotypes for average fruit weight and fruit yield (Kouam *et al*., 2016). Cultivated tomato genotypes had better average fruit weight relative to the wild types. A trend of improved fruit weight and fruit yield in cultivated tomato varieties compared to wild genotypes had been previously reported (Kouam *et al*., 2018). The low mean values of fruit yield observed in cultivated genotypes (Cal-J, Red Diamond, Riogrande and Oxyly) was attributed to the susceptibility of these genotypes to bacterial wilt. Bacterial wilt generally had a negative impact on the highly susceptible genotypes resulting to low yields. Low fruit yields occur in

tomato varieties when infected by bacterial wilt (Ambang *et al*., 2016). However, low mean values of fruit yield observed in wild genotypes was due to their inherent characteristic of small fruit size (Kouam *et al*., 2018).

Inverse relationship was present between disease incidence and fruit yield and this implies that bacterial wilt disease incidence directly affects the fruit quality of tomatoes (Table 3.7). The results are consistent with those of Guji *et al*. (2019) who found a negative correlation between yield and disease incidence of bacterial wilt in ginger varieties. Significant negative correlation of days to maturity and average fruit weight and a negative non-significant correlation with fruit yield are in agreement with those of Reddy *et al*. (2013). Positive significant correlation of average fruit weight with fruit yield are also in tandem with the findings of Hasan (2016).

Based on cultivar superiority index, six genotypes KK Acc I, NRB, KK Acc II, KISII, BRNG Acc II and KLF Acc III had the lowest P_i value of 0.00 (Table 3.8). These genotypes were the most stable for bacterial wilt resistance across the two cropping cycles (De Oliveira *et al*., 2013).The performances of the six stable genotypes were near the minimum genotype across both cropping cycles (Lin & Binns, 1988). The identification of resistant genotypes is a major step towards breeding and deployment of resistant tomato genotypes against bacterial wilt.

3.5 Conclusion

This study identified potential sources of resistance mainly KK acc I, NRB, KK acc II, KISII, BRNG acc II and KLF acc III against bacterial wilt disease. The identified genotypes will be useful parental breeding stock for developing tomato genotypes with high levels of resistance and minimal yield losses. A backcrossing breeding scheme can be deployed in order to introgress useful sources of resistance into adapted but susceptible genetic backgrounds to circumvent linkage drag that could introduce unwanted genetic background from wild genotypes. Preliminary yield trials and subsequently advance yield trials of the materials that would be developed will potentially culminate in to rolling out of improved varieties with resistance to bacterial wilt.

CHAPTER FOUR

COMBINING ABILITY ANALYSIS AND GENE ACTION FOR BACTERIAL WILT DISEASE RESISTANCE IN WILD TOMATO (*Solanum pimpinellifolium*) AND CULTIVATED TOMATO (*Solanum lycopersicum*) GENOTYPES

Abstract

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most destructive and widespread diseases of tomato in Kenya. The objective of this study was to determine the combining ability effects and gene action conditioning bacterial wilt disease resistance in tomato. Eight parents were crossed in North Carolina II mating design scheme to estimate the General Combining Ability (GCA) of the parents and the Specific Combining Ability (SCA) of the F_1s hybrids. The F_1s hybrids and the parental genotypes were evaluated for bacterial wilt in an *alpha lattice* of four blocks with six experimental units within the blocks in two replications for two cropping cycles. Genotype main effects were significant ($p \leq 0.001$) for area under disease progress curve (AUDPC), disease incidence and plant survival. Cropping cycle effects were significant ($p \leq 0.001$) for disease incidence and plant survival. Genotype × cropping cycle interaction was significant ($p \leq 0.05$), ($p \leq 0.01$) and ($p \leq 0.001$) for area under disease progress curve and plant survival. Testers main effects were significant ($p \leq 0.01$) and ($p \leq 0.001$) for AUDPC, disease incidence and plant survival. Lines × Testers interaction were significant ($p \leq 0.001$) for area under disease progress curve and disease incidence. Among the parents, KLF acc III was the best general combiner for AUDPC, disease incidence and plant survival across the two cropping cycles with GCA values of -1.20, -0.52 and 0.72 respectively. Red Diamond × KLF acc III, Money Maker× KK acc I, Oxyly× KLF acc III and Money Maker× KK acc II best specific combiners for AUDPC, disease incidence and plant survival. Low narrow sense heritability values of 0.14, 0.16 and 0.20 were obtained for AUDPC, disease incidence and plant survival. Relative weights of additive versus non-additive gene action obtained for AUDPC, disease incidence and plant survival were 0.19, 0.20 and 0.50. General predictability ratios (GPR) values of 0.27, 0.29 and 0.50 were obtained for AUDPC, disease incidence and plant survival. These results indicated the predominance of non-additive gene action in governing the traits.

4.1 Introduction

Tomato (*Solanum lycopersicon* L.) is one of the most widely cultivated vegetables worldwide. The area under production of this vegetable in Kenya has been on the rise due to the increase in demand (FAOSTAT, 2018; Ochilo, *et al*., 2019). The consumption outstrips the demand and this results from low production that cannot meet the need of the population. Further, there is a gap between the actual and potential yield arising from limiting factors such as lack of suitable varieties coupled with inadequate crop management strategies for control of pests and diseases. Bacterial wilt caused by *Ralstonia solanacearum* has been identified as a major biotic constraint affecting tomato production in Kenya (Laeshita & Arwivanto, 2017). The disease causes wilting of young leaves during the hottest part of the day which later leads to stunting and eventual death of the plant (Elnaggar *et al*., 2018). Yield losses of 64%-100% both in the field and in the greenhouse have been documented, however the level of loss may depend on the susceptibility of the cultivar and the development of infection (Ireri *et al*., 2018). Resistance to bacterial wilt has been reported in tomato varieties from a number of countries (Aslam, 2017; Scott *et al*., 2009; Sharma *et al*., 2006). However, most farmer-preferred tomato varieties in Kenya are susceptible. Therefore, to enhance tomato production, it is plausible to develop locally adapted productive tomato varieties with bacterial wilt resistance alongside farmer-preferred traits. Such a strategy has the potential to reduce bacterial wilt disease pressure, reduce the yield gap and enhance adoption of tomato varieties.

Studies carried out on the inheritance of resistance to bacterial wilt in tomatoes reported the significance of both major and minor genes in regulating the resistance. A single gene was important for control of bacterial wilt resistance in tomato (Grimault *et al*., 1995; Thakur *et al*., 2004). In contrast, the resistance of tomato to bacterial wilt was reported to be under the control of quantitative trait loci (QTLs) (Ishihara *et al*., 2012). The difference in the results has been attributed to the use of different sources of resistance, variations in environmental conditions and different isolates of *Ralstonia solanacearum* species (Da-Silvia *et al*., 2018). Understanding gene action involved in bacterial wilt resistance in tomato would provide a basis for planning a breeding strategy for developing breeding populations that would lead to identification of superior lines through selection. Non-additive gene action was predominant over additive gene action for the control of resistance to bacterial wilt (Singh *et al*., 2014). On the other hand, Oliveira *et al*. (1999) found additive gene action to be responsible in modulating bacterial wilt resistance.

Information on combining ability can help to establish an effective breeding programme. Combining ability analyses are important in facilitating the choice of suitable parents for hybridisation (Suvi *et al*., 2021). However, combining ability analyses and genetic predictions may depend on the test populations as well as the environment (Suvi *et al*., 2021). Ideally, when GCA mean square is significant, it implies that additive gene action is predominant and therefore hybridisation followed by selfing and selection would be effective in identification of superior recombinants (Kenga *et al*., 2004). On the other hand, the predominance of SCA effects suggests the significance of non-additive gene action which would be key in development of hybrids (Kenga *et al*., 2004). GCA is described as the average performance of a line in a series of cross combinations while SCA is described as the deviation in the performance of hybrids from the expected productivity in relation to the average performance of lines involved in the hybrid combination (Fasahat *et al*., 2016). The SCA effects are associated with non-additive gene actions such as dominance and epistasis (Suvi *et al*., 2021) Both the GCA and SCA estimates are important in understanding the genetic architecture of quantitative traits which is useful in the establishment of an efficient breeding programme (Muthoni *et al*., 2015).The North Carolina II mating design has been widely employed in parental hybridisation for population development and investigating the inheritance of important traits of various crops (Acquaah, 2009; Makanda *et al*., 2010; Oppong-Sekyere *et al*., 2019).The design, allows a breeder to estimate the GCA and SCA (Borrell *et a*l., 2012). Although studies have revealed the significance of both GCA and SCA in key traits of a number crops including quality traits, disease resistance and yield, limited information exists in the estimation of GCA and SCA from crosses between cultivated and with wild species of tomato (Tyagi *et al*., 2018). Hence, the study focused on understanding the gene action involved in the control of bacterial wilt and its inheritance. Knowledge of inheritance will be handy in developing a breeding strategy for developing bacterial wilt resistant tomato for both greenhouse and field production.

4.2 Materials and methods

4.2.1 Experimental site

The experiment was carried out in the greenhouse at Egerton University, Njoro Campus in the Department of Crops, Horticulture and Soils. The site lies approximately at 35°55'58.0''E and 0°22'11.0''S and an altitude of 2238 m above the sea level. The area is situated in the lower highland agro-ecological zone 3 (LH 3) (Jaetzold & Schmidt, 2012). It has a tropical climate and the temperature ranges between 15°C- 21°C. The area receives a

mean annual rainfall of 1200 mm. The distribution of the rain is bimodal with long rains between April and August and short rains between October and December, yearly. The soils at the experimental site are well drained, sandy loams which are dark brown in colour characterized by a pH of 6 (Jaetzold & Schmidt, 2012).

4.2.2 Genotypes

Eight parental genotypes included four commercial susceptible varieties and four wild tomato genotypes with resistance to bacterial wilt were used in the study. Detailed description of these parental materials is provided in Table 4.1.

Table 4.1. Description of parental genotypes used to generate F_1s hybrids

Genotype	Source	Bacterial wilt response	Cultivation status	Role in crosses
Cal-J	Kenya Seed Company	Susceptible	Cultivated	Female
Money Maker	Kenya Seed Company	Susceptible	Cultivated	Female
Red Diamond	Continental Seed Company	Susceptible	Cultivated	Female
Oxyly	Royal Seed Company	Susceptible	Cultivated	Female
KK acc II	Kakamega County	Resistant	Wild	Male
KK acc I	Kakamega County	Resistant	Wild	Male
KISII	Kisii County	Resistant	Wild	Male
KLF acc II	Kilifi County	Resistant	Wild	Male

KK Kakamega, KLF Kilifi

4.2.3 Mating parental genotypes

Crossing blocks having eight parents were planted in the greenhouse. Four male parents were crossed to four female parents in North Carolina Design II mating scheme. A total of 16 F_1 progenies were obtained. The planting of the parental material was done by staggering to eliminate the possibility of differential flowering time in order to ensure a synchronized flowering period to allow successful crossing. This was achieved by planting the late flowering parents first followed by the early flowering.

4.2.4 Evaluation of parents and F_1s for bacterial wilt resistance

Sixteen F1 alongside eight parents were sowed in a nursery for a period of about 5 weeks before transplanting. Four hundred kilogrammes of forest soil were sterilized by autoclaving in an autoclave at 121ºC for one hour. The soil was placed in plastic pots measuring 25 cm in diameter and 30 cm in height. The seedlings were transplanted at three-leaf stage in the pots which were spaced at 40 cm between the rows and 30 cm plant to plant distance within the rows. Five pots were used for each entry per experimental unit giving a total of five plants per plot. The experimental design was an *alpha*-lattice design of 4 blocks and 6 units within the blocks, in two replicates. During transplanting Di-Ammonium Phosphate (DAP) was applied at the rate of 5 g per pot during transplanting to supply 62.5 kg N/ha and 95 kg P_5/ha. Calcium Ammonium Nitrate (CAN) was applied at the rate of 5 g per pot to provide 250 kg Ca^2 /ha. Magnesium was applied to each pot at a rate of 5 g per pot to supply 60 kg Mg/ha. Three weeks later after applying CAN, Nitrogen, Phosphate, Potassium (NPK) compound fertilizer was applied at the rate of 5 g per pot to supply 100kg N/ha, 60 kg P/ha and 60 kg K/ha respectively. In addition to the main fertilizer, foliar fertilizers were also supplemented. Evisect (Thiocyclam hydrogen oxalate, water soluble powder, Arysta Life Science, BP Noguères France) was administered at the rate of 500kg/ha to safeguard against white flies. Confidor (Imidacloprid 700kg/ha; Bayer Crop Science AG, Kalser-Wilhelm-Allee 1, Germany) was applied at the rate of 100 mL ha-1 for defence against aphids, thrips and leaf miners. Ridomil Gold (Metalaxyl-M 40g/kg+Mancozeb-640g/kg, United States) was applied at the rate of 2.5kg/ha to combat downy mildew, early and late blight fungal diseases. Token (Azoxystrobin 259g/l + Difeconazole 125g/l, Osho Chemicals Industries Limited, Nairobi Osho Compl) was applied at the rate of 2kg/ha) to manage early and late blight. No control measures were carried out against bacterial wilt disease. Manual weeding was used to maintain the crop clean from weeds. Pruning was done on a regular basis by physically pruning the emerging branches. Stakes were used to train the plants. Irrigation was done once a day under gloomy weather and twice a day during periods of bright sunlight.

4.2.5 Plant inoculation with bacterial wilt pathogen

The 16 F_1s with 8 parental genotypes were inoculated with the cultured pathogen 14 days after transplanting. Before inoculation, incisions were made using a sterile scalpel on either side of the main stem to a depth of 5-6 cm each to cause injury to the secondary roots (Mwangi *et al*., 2008; Onduso, 2014). Thirty millimetres of the standardized bacterial suspension containing 1×10^9 colony forming units per ml inoculation of *R. solanacearum* was

poured over the roots (Singh *et al*., 2018). Thereafter, the plants were watered at alternative days to maintain a high soil moisture for the development of the disease.

4.2.6 Data collection

All plants in each experimental unit were used for data collection. The disease symptoms were observed daily from 30, 45 and 60 days after inoculation (DAI). The percent disease severity in plants was evaluated using a scale of 0-5 as described by Kempe and Sequeira (1983) (Table 4.2).

Table 4.2. Disease rating scale for bacterial wilt

Rating scale	Description	Disease reaction
0	No symptoms	Highly resistant
1	1 to 25% leaves wilted	Resistant
2	26 to 50% leaves wilted	Moderately resistant
3	51 to 75% leaves wilted	Moderately susceptible
4	75% but less than 100% of leaves wilted	Susceptible
5	All leaves wilted and plant dead	Highly susceptible

Source: Moussa *et al.* (2017).

The disease evaluation data were summarized using the percent disease severity (PDS) formula as described by Pranamika and Saikia (2013) and expressed as the AUDPC. AUDPC was estimated following Wilcoxson *et al.* (1975) as;

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

Where, y_i is the % disease severity on the i^{th} scoring; t_i is the number of days from sowing to i^{th} scoring; n is the total number of scores.

Disease incidence was calculated using the formula described by Gashaw *et al*. (2014) as;

$$\text{DI} = \frac{\text{NIP}}{\text{NPA}} \times 100 \quad (2)$$

Where NIP is the number of infected plants, NPA is the total number of plants assessed.

Data on plant survival was calculated using the formula described by Jyoti *et al.* (2015) as;

$$\text{PS} = \frac{\text{NHP}}{\text{NPE}} \times 100 \quad (3)$$

Where NHP is the number of healthy plants, NPE is the number of plants established.

4.2.7 Data analyses

Data for AUDPC were log transformed while data for disease incidence and plant survival were arcsine square root transformed to obtain a normal frequency distribution. Data were subjected to analysis of variance using the computer software programme GenStat 15th edition (VSN International, Hemel Hempstead, UK). The statistical model for the analysis was;

$$Y_{ijklm} = \mu + C_j + R_l + B_{k(l)} + G_i + GC_{ij} + \varepsilon_{ijklm} \quad (4)$$

Where; Y_{ijkl} is the observed performance from each experimental unit; C_j is the effect due to j^{th} cropping cycle; R_l is the effect due to l^{th} replicate; $B_{k(l)}$ is the effect due to k^{th} block within the l^{th} replicate; G_i is the effect due to i^{th} genotype; GC_{ij} is the effect due to interaction between the genotype and the cycle; ε_{ijklm} is the random error component.

Genotypes, cycles and replications were considered as fixed effect while blocks were considered as random effects. Mean separation was performed using Least Significant Difference (LSD) test at $p \leq 0.05$ given as:

$$\text{LSD} = \frac{t(s\sqrt{2})}{\sqrt{n}} \quad (5)$$

Where t is tabulated t value, s is standard deviation of all the plots and n is number of observations.

Standard error of the mean was calculated using the following formula:

$$\text{Standard error of the mean} = \frac{sd}{\sqrt{n}} \quad (6)$$

Where sd is the standard deviation and n is the number of samples

Combining ability analysis was done using line × tester procedure developed by Kempthorne (1957) and implemented in R software package version 4.0.4 in R Studio 1.4.1106 (Lüdecke *et al.*, 2020).

The linear model for genetic analysis was as follows:

$$Y_{ijk} = \mu + g_i + g_j + S_{ij} + \varepsilon_{ijk} \quad (7)$$

Where; Y_{ijk} is the value of the ijk^{th} observation of the cross involving i^{th} cross, and j^{th} tester in the *kth* replication. μ is the general mean. g_i is the GCA effect of the i^{th} line. g_j is the

GCA effect of the j^{th} tester. S_{ij} is the SCA effect of the cross involving i^{th} line and j^{th} tester. ε_{ijk} is the error associated with the ijk^{th} observation.

Narrow sense heritability was estimated, after derivation of the variance components using the following formula:

$$h^2 = \frac{\sigma^2 GCA}{\sigma^2 GCA + \sigma^2 SCA + \sigma^2 e} \quad \text{..... (8)}$$

Where h^2 heritability in narrow sense, σ^2 GCA is the variance of General Combining Ability, σ^2 SCA is the variance of Specific Combining Ability.

Relative weight of additive and non-additive gene action was estimated according to (Verma & Srivastava, 2004) which is given as:

$$\frac{\sigma^2 GCA}{\sigma^2 SCA} \quad \text{..... (9)}$$

Where σ^2 GCA is the variance of general combining ability, σ^2 SCA is the variance of specific combining ability.

Baker's ratios were also computed to estimate the relative importance of additive and non-additive gene action in the expression of disease traits using Baker's general predicted ratio (GPR) as follows:

$$GPR = \frac{2\,\sigma^2\,\text{GCA}}{2\,\sigma^2\,\text{GCA} + \sigma^2\,\text{SCA}} \quad \text{..... 10)}$$

Where σ^2 GCA is the variance of general combining ability, σ^2 SCA is the variance of specific combining ability

4.3 Results

4.3.1 Analysis of variance and phenotypic performance

Significant ($p \leq 0.001$) variation among the genotypes was observed across the cropping cycles for AUDPC and plant survival at 30 days and for AUDPC, disease incidence and plant survival at 45 and 60 DAI (Table 4.3). Cropping cycles effects were significant ($p \leq 0.001$) for plant survival at 30 DAI, disease incidence and plant survival at 45 DAI and AUDPC, disease incidence and plant survival at 60 DAI. Effects due to interaction between genotypes and cropping cycles were significant ($p \leq 0.05$) for plant survival at 60 DAI, ($p \leq 0.01$) for plant survival at 30 and 45 DAI and ($p \leq 0.001$) for AUDPC at 60 DAI. AUDPC

values of 0-150, 151-300, 301-500 and > 500 were considered to represent very low, low, moderate and high levels of resistance, respectively.

Genotypes expressed variation for AUDPC, disease incidence and plant survival in the two cropping cycles. There was a trend of high disease pressure in the first cropping cycle with mean AUDPC of 543 and 940 at 45 and 60 DAI compared to the second cropping cycle with mean AUDPC of 543 and at 45 and 563 at 60 DAI. In contrast, the plant survival was higher in the second cropping cycle at 45 and 60 DAI with 72% and 58% of the plants surviving compared to the first cropping cycle when only 56% and 38% of the plants survived at 45 and 60 DAI (Table 4.4). Genotypic variation was displayed among the parents and the crosses for AUDPC, disease incidence and plant survival. In general, the crosses had lower values for AUDPC and disease incidence and high values of plant survival as compared to the parents. Three crosses Cal-J × KLF acc III, Oxyly × KLF acc III and Red Diamond × KLF acc III were highly resistant to bacterial wilt as compared to the thirteen crosses which displayed a susceptible reaction cropping cycles. These crosses had AUDPC and disease incidence values of 0 and 100% plant survival. Apparently all the resistant F_1s were progenies of KLF acc III parent (Table 4.5). Four wild parental genotypes KK acc II, KK acc I, KISII and KLF acc III were highly resistant as compared to the four commercial varieties which were susceptible to bacterial wilt across cropping cycles. These four wild genotypes had AUDPC and disease incidence values of 0 and 100 % plant survival (Table 4.6)

Table 4.3. Mean squares for AUDPC, disease incidence and plant survival of tomato genotypes at 30, 45 and 60 days after inoculation evaluated for two cropping cycles in the greenhouse at Egerton University, Njoro in 2020

Source of variation	df	30 days after inoculation			45 days after inoculation			60 days after inoculation		
		AUDPC	DI	PS	AUDPC	DI	PS	AUDPC	DI	PS
Cycle	1	0.00	0.18	1.70***	0.00	0.19***	0.65***	0.06***	0.45***	1.60***
Rep(Cropping cycle)	1	0.01	0.03	0.02	0.00	0.02	0.01	0.00	0.30	0.00
Genotype	23	1.72***	0.14***	0.39***	3.04***	0.35***	0.62***	2.77***	0.73***	1.20***
Cycle ×Genotype	23	0.00	0.02	0.07**	0.00	0.01	0.02**	0.01***	0.04	0.06*
Residual	47	0.00	0.02	0.02	0.00	0.01	0.01	0.00	0.03	0.03
CV %		0.70	23.20	1.60	0.20	4.50	1.60	0.00	12.90	0.50

*, **, *** Significant at, ($p \leq 0.05$), ($p \leq 0.01$), ($p \leq 0.001$) respectively AUDPC Area Under Disease Progress Curve, PS Plant Survival, DI Disease Incidence, CV Coefficient of variation.

Table 4.4. Range and mean values of AUDPC, Disease incidence and Plant survival at 45 and 60 days after inoculation for thirty-six tomato

	45 days after inoculation						60 days after inoculations					
Cycle	AUDPC		Disease incidence		Plant survival		AUDPC		Disease incidence		Plant survival	
	Range	Mean± SE	Range	Mean± SE	Range	Mean± SE	Range	Mean± SE	Range	Mean± SE	Range	Mean± SE
1st cycle	0-945	543.±15.25	0-71	27±1.00	20-100	56±1.38	0-1575	940±26.23	0-93	48±1.38	0-100	38±1.76
2nd cycle	0-906	534±15.50	0-50	19±0.61	29-100	72±0.95	0-1352	564±23.48	0-79	39 ±1.17	0-100	58±1.38

AUDPC Area Under Disease Progress Curve, SE Standard Error.

Table 4.5. Mean values of AUDPC, disease incidence and plant survival at 30, 45 and 60 days after inoculation for 8 parents evaluated for bacterial wilt resistance in the greenhouse for two cropping cycles in the greenhouse at Egerton University, Njoro in 2020

	AUDPC		DI		PS		AUDPC		DI		PS		AUDPC		DI		PS	
	30 DAI						45 DAI						60 DAI					
Genotypes	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2
KK acc II	0	0	0	0	100	100	0	0	0	0	100	100	0	0	0	0	100	100
KK acc I	0	0	0	0	100	100	0	0	0	0	100	100	0	0	0	0	100	100
KISII	0	0	0	0	100	100	0	0	0	0	100	100	0	0	0	0	100	100
KLF acc III	0	0	0	0	100	100	0	0	0	0	100	100	0	0	0	0	100	100
Money Maker	219	235	20	20	40	80	698	784	40	20	20	60	1220	1192	71	51	0	51
Oxyly	272	259	20	20	40	71	841	841	60	40	20	40	1469	1278	79	61	0	23
Red Diamond	299	306	10	5	40	80	902	902	39	29	20	50	1504	1339	71	61	0	9
Cal-J	314	278	50	50	20	50	945	861	71	50	20	29	1575	1278	93	79	0	23
CV %	3.10	1.2	22.30	21.1	0.7	2.4	1.0	0.0	5.5	1.2	1.4	1.7	1.50	0.2	11.2	14.9	1.2	1.7
LSD(0.05)	0.32	0.32	0.30	0.30	0.31	0.31	0.08	0.08	0.19	0.19	0.19	0.19	0.06	0.06	0.32	0.32	0.35	0.35

AUDPC Area Under Disease Progress Curve, DI Disease Incidence, PS Plant Survival, DAI Days After Inoculation CC Cropping Cycle, KLF Kilifi, KK Kakamega, CV Coefficient of Variation, LSD Least Significant Difference.

[a]LSD values based on transformed data

Table 4.6. Mean values of AUDPC, disease incidence and plant survival at 30, 45 and 60 days after inoculation for 16 F_1 hybrids evaluated for bacterial wilt resistance in the greenhouse for two cropping cycles in the greenhouse at Egerton University, Njoro in 2020

	AUDPC		DI		PS		AUDPC		DI		PS		AUDPC		DI		PS	
	30 DAI						45 DAI						60 DAI					
	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2
Cal-J × KLF acc III	0	0	0	0	100	100	0	0	0	0	100	100	0	0	0	0	100	100
Oxyly × KLF acc III	0	0	0	0	100	100	0	0	0	0	100	100	0	0	0	0	100	100
Red Diamond × KLF acc III	0	0	0	0	100	100	0	0	0	0	100	100	0	0	0	0	100	100
Cal-J × KK acc II	172	199	0	0	100	100	579	651	29	20	80	80	1037	967	61	23	42	79
Money Maker × KK acc II	190	122	0	0	50	95	636	259	40	20	29	60	1138	427	79	51	4	51
Money Maker× KLF acc III	199	224	0	0	80	100	636	714	29	20	60	80	1165	1086	61	32	32	42
Oxyly × KISII	230	214	0	0	60	100	714	698	20	29	40	60	1249	1220	42	79	4	23
Cal-J × KK acc I	235	247	0	0	95	100	749	803	20	20	71	80	1308	1220	51	23	32	79
Cal-J × KISII	235	259	0	0	61	95	714	822	29	29	39	71	1192	1220	51	51	9	23
Oxyly × KK acc II	241	253	0	0	60	100	766	731	29	20	40	80	1308	1112	71	42	23	61

Table 4.6 Contd…

Money Maker × KK acc I	247	285	5	0	50	95	766	881	40	29	29	60	1308	1308	71	51	4	51
Oxyly × KK acc I	247	224	0	0	100	95	749	651	29	20	71	71	1435	990	61	32	32	42
Red Diamond × KK acc II	253	292	29	5	39	71	749	861	50	29	29	40	1278	1278	79	61	4	42
Money Maker × KSII	265	230	5	5	50	95	803	714	29	20	29	71	1370	1086	51	32	4	42
Red Diamond × KK acc I	292	285	5.	0	61	95	881	841	40	20	39	60	1469	1278	71	42	32	51
Red Diamond × KISII	306	272	29	5	40	60	902	822	60	40	20	40	1539	1220	79	79	0	0
Cv %	3.10	0.90	22.30	21.1	0.7	2.4	1.0	0.0	5.5	1.2	1.4	1.7	1.30	0.2	11.2	14.9	1.2	1.7
LSD(0.05)	0.32	0.32	0.30	0.30	0.31	0.31	0.08	0.08	0.19	0.19	0.19	0.19	0.06	0.06	0.32	0.32	0.35	0.35

AUDPC Area Under Disease Progress Curve, DI Disease Incidence, PS Plant Survival, DAI Days After Inoculation CC Cropping Cycle, KLF Kilifi, KK Kakamega, Cv Coefficient of variation, LSD Least Significant Difference.

[a]LSD values based on transformed data

4.3.2. Combining ability analyses

Means squares due to parents and crosses were significant ($p \leq 0.000$) for AUDPC, disease incidence and plant survival. Means squares of Parents× Crosses was significant ($p \leq 0.000$) for AUDPC and disease incidence (Table 4.7). Means squares due to Crosses were significant ($p \leq 0.000$) for AUDPC, disease incidence and plant survival. Means squares due to Lines× Testers interaction were significant ($p \leq 0.000$) for AUDPC and disease incidence. Means squares due to Testers was significant for ($p \leq 0.01$) for AUDPC and disease incidence and ($p \leq 0.001$) for plant survival. Among the eight parents, KLF acc III had the lowest negative GCA value of -1.20 for AUDPC and -0.52 for disease incidence and high GCA value of 0.72 of plant survival as compared to the seven parents (Table 4.8). Among the F_1s, Red Diamond × KLF acc III, Oxyly× KLF acc III, Money Maker× KK acc II and Money Maker× KISII had the lowest negative SCA values of -0.41, -0.40, -0.39 and 0.37 for AUDPC, -0.28. -0.12.-0.12 and -0.23 for disease incidence and 0.30, 0.14, 0.12 and 0.32 for plant survival respectively (Table 4.9).

Relative weight of additive and non-additive gene action obtained for AUDPC, disease incidence and plant survival were 0.19, 0.20 and 0.50 respectively. Narrow sense heritability values of 0.14, 0.16 and 0.20 were obtained for AUDPC, disease incidence and plant survival. General Predictability Ratios (GPR) values of 0.27, 0.29 and 0.50 were obtained for AUDPC, disease incidence and plant survival. The proportional contribution to the total variation of the testers was higher for all the disease measurements as compared to the lines and the lines by testers interaction (Table 4.10).

Table 4.7. Combining ability mean squares for AUDPC, disease incidence and plant survival for two cropping cycles in the greenhouse at Egerton University, Njoro in 2020

Source of variation	Df	AUDPC	DI	PS
Replications	1	0.00	0.17	0.00
Treatments	23	1.96***	0.44***	0.79 ***
Parents	7	2.69***	0.73***	1.41***
Parents vs. Crosses	1	4.28***	0.39***	0.36
Crosses	15	1.46***	0.31***	0.53***
Lines	3	0.51	0.21	0.34
Testers	3	5.14*	1.04*	1.95**
Lines× Testers	9	0.54***	0.10***	0.11

Table 4.7 Contd…

Error	23	0.00	0.01	0.04

*, **, ***, Significant at ($p \leq 0.01$), ($p \leq 0.001$) and ($p \leq 0.000$) respectively, AUDPC Area Under Disease Progress Curve, DI Disease Incidence, PS Plant Survival.

Table 4.8. General combining ability (GCA) effects of eight parents for AUDPC, disease incidence, plant survival for two cropping cycles in the greenhouse at Egerton University, Njoro in 2020

GCA	AUDPC	DI	PS
Lines			
Cal-J	-0.16	-0.16	0.19
Money Maker	0.38	0.20	-0.29
Oxyly	-0.12	-0.10	1.06
Red Diamond	-0.10	0.06	-0.01
SE	0.10	0.04	0.07
Testers			
KK acc II	0.36	0.28	-0.20
KK acc I	0.43	0.17	-0.11
KISII	0.42	0.07	0.40
KLF acc III	-1.20	-0.52	0.72
SE	0.10	0.04	0.07

AUDPC Area Under Disease Progress Curve, DI Disease Incidence, PS Plant Survival, KK Kakamega, KLF Kilifi, SE Standard Error.

Table 4.9. Specific combining ability (SCA) effects of 16 F1s for AUDPC, disease incidence, Plant survival for two cropping cycles in the greenhouse at Egerton University, Njoro in 2020

Genotype	AUDPC	DI	PS
Cal-J× KK acc II	0.01	0.02	0.09
Cal-J× KK acc I	0.13	0.02	-0.10
Cal-J× KISII	0.13	0.02	-0.05
Cal-J× KLF acc III	-0.36	-0.07	0.06
Money Maker× KK acc II	-0.39	-0.12	0.12
Money Maker× KK acc I	-0.40	-0.12	0.03
Money Maker× KISII	-0.37	-0.23	0.32
Money Maker× KLF acc III	1.17	0.47	-0.46
Oxyly× KK acc II	0.17	0.08	-0.05
Oxyly× KK acc I	0.14	0.08	-0.02
Oxyly× KISII	0.08	-0.03	-0.08
Oxyly× KLF acc III	-0.39	-0.12	0.14
Red Diamond× KK acc II	0.13	0.02	-0.16
Red Diamond × KK acc I	0.13	0.02	0.09
Red Diamond × KISII	0.15	0.24	-0.19
Red Diamond × KLF acc III	-0.41	-0.28	0.30
SE	0.02	0.08	0.14

AUDPC Area Under Disease Progress Curve, DI Disease Incidence, PS Plant Survival, KK Kakamega, KLF Kilifi, SE Standard Error.

Table 4.10. Estimates of genetic variance components and percentage contribution of the lines, testers and their interaction to the total variation for AUDPC, disease incidence and plant survival

Parameter	AUDPC	DI	PS
GCA	0.05	0.01	0.02
SCA	0.27	0.05	0.04
GCA/SCA	0.19	0.20	0.50
(h^2)	0.16	0.14	0.20
GPR	0.27	0.29	0.50

Table 4.10 Contd…

% contribution			
Lines	7.08	13.41	13.01
Testers	70.61	66.54	73.82
Lines × testers	22.31	20.04	13.17

AUDPC Area Under Disease Progress Curve, DI Disease Incidence, PS Plant Survival, GCA General Combining Ability, SCA Specific Combining Ability, h^2 Narrow sense heritability, GPR General Predictability Ratio

4.4 Discussion

Bacterial wilt resistance is a major breeding objective for tomato improvement. This is because of the magnitude of yield loss inflicted by the disease which impacts negatively on tomato grown either in the field or under greenhouse conditions. Screening for bacterial wilt resistance has in the past resulted in identification of resistant cultivars (Acharya *et al*., 2018; Oussou *et al*., 2020). Despite the existing reports on resistance to bacterial wilt in tomato, local varieties in Kenya are largely susceptible. Introgression of novel sources of resistance from diverse sources including cultivated species and wild relatives is a necessity towards deployment of bacterial wilt resistant tomato cultivars (Kim *et al*., 2016). Such genetic improvement not only results in reduced yield gap but also helps to reduce production costs and limits the environmental hazards caused by overuse of bactericides.

To determine differential performance among tomato germplasm, AUDPC, disease incidence and plant survival were measured. The results from the analysis of variance revealed the importance of cropping cycle on the performance of tomato against bacterial wilt (Table 4.3). Significant genotype-by-cropping cycle (GC) interaction for plant survival at 30 and 45 DAI and AUDPC and plant survival at 60 DAI suggested that the genotypic performance was not independent of the difference among the cropping cycles. These findings agree with earlier reports (Ganiyu, 2017; Guji *et al*., 2019; Sarfo, 2018) and implicate the screening conditions to be key in determining the outcome of disease screening experiment. The variation arising from effects of cropping cycle may result from inconsistent temperature and humidity within the greenhouse. High temperature coupled with high relative humidity accelerate disease development (Velásquez *et al*., 2018; Grace *et al.*, 2019).

Significant main effects due to genotypes for AUDPC, disease incidence and plant survival at 30, 45 and 60 DAI explained the presence of genetic differences among the evaluated genotypes. The trend of higher mean values for AUDPC and disease incidence and

lower plant survival at 45 and 60 DAI, observed in the first cropping cycle as opposed to the second cropping cycle suggested higher disease pressure in the second cycle among the genotypes (Table 4.4). The differential performance may be explained by an increase in temperature during the first cropping cycle. Namisy *et al*. (2019) found that high temperatures of between 28 C to 36^0C triggered increased disease pressure.

The observed genetic variation and mean performance of parents and their progenies was based on AUDPC, disease incidence and plant survival which revealed mixed levels of resistance and susceptibility (Table 4.5 and Table 4.6). Parents with low mean values for AUDPC and disease incidence and high mean values for plant survival indicated the presence of genes for resistance and the possible potential of transmitting these genes to their progenies (Fellahi *et al*., 2013). The difference in performance among the parents and the crosses for AUDPC, disease incidence and plant survival indicated the existence of genotypic variation among the parents and the crosses. Suvi *et al.* (2021) reported genotypic variation for rice yellow mottle virus mottle disease among parents and crosses in rice.

Significant mean squares due to testers for the diseases variates suggested the prevalence of additive genetic variance among the male parents in conferring resistance to bacterial wilt (Table 4.7). These results concur with the earlier findings (Ajjappalavara *et al*., 2010; Kargbo *et al*., 2019; Mosa *et al*., 2017) and therefore indicate that the genetic advance for the disease traits can be realised through hybridisation and selection. Significant mean squares for line × tester interaction for all the traits measured demonstrated the existence of non-additive genetic variance in bacterial wilt resistance. Presence of non-additive genetic variance in the current breeding populations presents the possibility of implementing a hybrid breeding programme that would exploit heterosis in addition to additive gene action to develop new varieties. Tomato hybrids are high yielding and widely cultivated in Kenya and therefore pyramiding genes for resistance in inbred lines for deployment of resistant hybrid varieties would greatly improve yield (Ashkani *et al*., 2015; Dormatey *et al*., 2020; Fuchs, 2017) Negative and lower GCA effects for AUDPC and disease incidence observed for KLF acc III parent indicated that it was the best general combiner for resistance to bacterial wilt disease (Table 4.8). Similar findings were reported by Odogwu (2016) on bean rust resistance in common bean (*Phaseolus vulgaris*). The crosses Money Maker× KK acc II, Oxyly× KLF acc III and Red Diamond × KLF acc III had negative and lower SCA effects for AUDPC which showed that these crosses were good specific combiners for resistance to bacterial wilt (Table 4.9). Bokmeyer *et al*. (2009) reported that negative SCA effects are desirable for disease resistance.

Heritability is possibly the most important statistic that can be obtained from variance components (Kearsey & Pooni, 1996). Narrow sense heritability measures the proportion of phenotypic variation which arises from additive effects of genes in a given population. Low narrow sense heritability estimates of 0.14, 0.16 and 0.20 obtained for disease traits (Table 4.10) indicated that dominance gene action was critical in expression of disease resistance for the traits. Low heritability estimates imply that prediction of progeny performance would be difficult because of prevalence of non-heritable variation (Schmidt *et al*., 2019). Therefore, a selection procedure that could accumulate positive of resistance genes should be adopted. Nsabiyera *et al*. (2013) reported similar low narrow sense heritability value of 0.16 for bacterial spot. In contrast, Da- Silva Costa *et al.* (2018) reported narrow sense heritability values of 0.26 and 0.53 for bacterial wilt.

Relative weights of additive and dominance gene action of 0.19, 0.20 and 0.50 respectively for disease traits indicated the superiority of non-additive gene action in their expression (Table 4.10). Verma and Srivastava (2004) reported the preponderance of non-additive gene action in the expression of traits. General predictability ratio of 0.27, 0.29 and 0.50 for disease traits further revealed the predominance of non-additive gene action over additive gene action. This implies that the selection will not be effective and therefore the traits can be improved through use of hybrid vigour. The results are in agreement with Nsabiyera *et al.* (2013) who reported the predominance of non-additive gene action in the expression of resistance to disease traits. In contrast, the inheritance of bacterial wilt has been reported to be controlled by a single dominant gene (Grimault *et al*., 1997; Thakur *et al*., 2004). Oliveira *et al*. (1999) reported additive gene action for resistance to bacterial wilt. Monma *et al.* (1993) reported the inheritance of bacterial wilt to be partially recessive. Sharma and Sharma (2015) reported the genetic control of bacterial wilt to be oligogenic. In addition, Da- Silva Costa *et al.* (2018) reported the predominance of additive gene action in the expression of resistance to bacterial wilt. The proportional contribution of lines, testers and their interaction for the disease traits was an indication that testers played an important role in inheritance of disease resistance. The testers contributed more positive alleles for the disease traits (Kargbo *et al*.., 2019). Although both the gene action and both general and specific combining ability effects were evidenced, the predominance of non-additive gene action showed the presence of heterozygosity among the genotypes.

4.5 Conclusion

This study revealed the significance of non-additive gene action in conferring resistance to bacterial wilt. Low narrow sense heritability estimates revealed the

predominance of dominance gene action in the expression of AUDPC, disease incidence and plant survival. The parental genotype KLF acc III was identified as the best general combiner for bacterial wilt. The cross combinations Money Maker× KK acc II, Oxyly× KLF acc III and Red Diamond × KLF acc III and Money Maker× KISII were identified having good specific combining ability for resistance to bacterial wilt. From these findings, a good breeding strategy should concentrate resistance genes in inbred lines with good genetic background through a backcrossing scheme followed by testing for general and specific combing ability for development of hybrids and potential future deployment of genetic resistance in tomato production in Kenya. In addition, a selection technique that allows for the accumulation of positive resistance genes should be used.

CHAPTER FIVE
GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussion

There are several management strategies used to control bacterial wilt on tomatoes which include: soil solarization, hot water treatment, use of copper-based fungicides, crop rotation, and grafting. Breeding for resistance to bacterial wilt is the most effective way to safe guard tomatoes against the disease. Although the method is time consuming it remains to be the most effective approach for the disease management (Costa *et al*., 2019). Lack of sources of resistance and the changes in tomato crop management systems has slowed down the breeding efforts for resistance to bacterial wilt (Lu *et al*., 2016). Therefore, it is necessary to identify sources of resistance and understand the genetics of resistance to bacterial wilt. This will help in developing new varieties with resistance to bacterial wilt (Da- Silva Costa, 2018). The use of resistant tomato genotypes against bacterial wilt is the most cost-effective and environmentally sustainable approach of controlling bacterial wilt disease (Kunwar *et al*., 2018). In addition, the strategy lowers the cost of production incurred by the farmers.

In Kenya, bacterial wilt recently emerged as the major biotic constraint on tomato production. Most of the commercial varieties grown by farmers such as Riogrande, Cal-J, Marglobe and M-82 are susceptible to the disease and this has led to reduction in yield of tomatoes. Breeding of tomato genotypes with durable resistance to bacterial wilt is a prime concern. Two experiments were carried out to contribute to improved tomato production in Kenya through breeding tomato varieties that are resistant to bacterial wilt. The first experiment involved identifying new sources of resistance to bacterial wilt among wild tomato genotypes from different agro-ecological zones in Kenya. Thirty-six tomato genotypes comprising of twenty eight wild genotypes and eight cultivated varieties were screened for resistance to bacterial wilt in two cropping cycles. From the results, six wild genotypes KK acc I, NRB, KK acc II, KISII, BRNG acc II and KLF acc III were identified as resistant genotypes to bacterial wilt across the two cropping cycles. These identified resistant tomato genotypes will be useful in tomato breeding programmes in Kenya for the development of tomato genotypes with high resistance to bacterial wilt and low yield losses.

The second experiment involved understanding the mode of gene action involved in the control of bacterial wilt resistance and its inheritance. In this experiment, four male parents were crossed to four female parents in a North Carolina Mating Design II to generate 16 F_1s. The 16 F_1s and the eight parents were evaluated for bacterial wilt resistance in two

cropping cycles. KK acc II, KK acc I, KISII and KLF acc III were identified as resistant parents as compared to the cultivated varieties. The AUDPC and disease incidence values for these genotypes was 0 and the plant survival was 100%. Cal-J × KLF acc III, Oxyly × KLF acc III and Red Diamond × KLF acc III crosses were identified as highly resistant to bacterial wilt. The AUDPC and disease incidence values for these crosses was 0 and plant survival was 100%. KLF acc III was identified as the best general combiner for resistance to bacterial wilt. Four crosses Money Maker× KK acc II, Oxyly× KLF acc III and Red Diamond × KLF acc III and Money Maker× KISII were also identified as good specific combiners for bacterial wilt resistance. The parent and the crosses identified would be would be useful sources of resistance for future breeding of bacterial wilt. In this experiment, general predictability ration revealed the prevalence of non- additive gene action over additive gene action in conferring resistance to bacterial wilt.

5.2 Conclusion

i. There was variation in resistance to bacterial wilt among wild and cultivated tomato genotypes. Six wild genotypes KK acc I, NRB, KK acc II, KISII, BRNG acc II, KLF acc III exhibited resistance against bacterial wilt.

ii. Combining ability analyses revealed the importance of non-additive gene action in conferring resistance to bacterial wilt. KLF acc III was identified as the best general combiner for bacterial wilt resistance while Money Maker× KK acc II, Oxyly× KLF acc III and Red Diamond × KLF acc III crosses were identified as good specific combiners for resistance to bacterial wilt.

5.3 Recommendations

i. Six wild genotypes KK acc I, NRB, KK acc II, KISII, BRNG acc II and KLF acc III resistant to bacterial wilt are potential sources of resistance which can be exploited in tomato breeding programmes for development of high yielding and bacterial wilt resistant tomato genotypes.

ii. Parent KLF acc III, Money Maker× KK acc II, Oxyly× KLF acc III and Red Diamond × KLF acc III crosses would be useful genetic resources of bacterial wilt resistance for further breeding

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APPENDICES

Appendix A. Analysis of variance for five parameters for bacterial wilt resistance in the greenhouse in experiment one at Egerton University, Njoro in 2020

i) Variate: Area Under Disease Progress Curve (AUDPC) at 30 days after inoculation (DAI)

Source of variation	Df	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0156	0.0156	1.74	
Genotype	35	55.5307	1.5866	176.81	≤ .001
Cycle	1	1.3272	1.3272	147.90	≤ .001
Genotype. Cycle	35	2.8479	0.0814	9.07	≤ .001
Residual	71	0.6371	0.0089		
Total	143	60.3586			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	72	2
d.f.	71	71	71
s.e.d.	0.067	0.016	0.095

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	72	2
d.f.	71	71	71
l.s.d.	0.134	0.031	0.189

ii)Variate: Area Under Disease Progress Curve (AUDPC) at 45 days after inoculation (DAI)

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0181	0.0181	2.87	≤ .001
Genotype	35	75.8305	2.1667	344.50	≤ .001
Cycle	1	0.7869	0.7869	125.13	≤ .001
Genotype.cycle	35	1.5083	0.0431	6.85	
Residual	71	0.4465	0.0063		
Total	143	78.5904			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	72	2
d.f.	71	71	71
s.e.d.	0.056	0.013	0.079

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype.Cycle
rep	4	72	2
d.f.	71	71	71
l.s.d.	0.112	0.026	0.158

iii)Variate: Area Under Disease Progress Curve (AUDPC) at 60 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0126	0.0126	1.68	
Genotype	35	87.4023	2.4972	331.56	≤ .001
Cycle	1	0.2734	0.2734	36.31	≤ .001
Genotype.Cycle	35	1.3681	0.0391	5.19	≤ .001
Residual	71	0.5347	0.0075		
Total	143	89.5912			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	72	2
d.f.	71	71	71
s.e.d.	0.061	0.014	0.087

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	72	2
d.f.	71	71	71
l.s.d.	0.122	0.029	0.173

iv)Variate: Plant survival (PS) at 30 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0092	0.0092	0.13	
Genotype	35	18.6303	0.5323	7.35	≤ .001
Cycle	1	0.5624	0.5624	7.77	0.007
Genotype.Cycle	35	1.8105	0.0517	0.71	0.862
Residual	71	5.1396	0.0723		
Total	143	26.1519			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	72	2
d.f.	71	71	71
s.e.d.	0.190	0.045	0.269

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	72	2
d.f.	71	71	71
l.s.d.	0.379	0.089	0.536

v)Variate: Plant survival (PS) at 45 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0000	0.0000	0.00	
Genotype	35	26.5435	0.7584	53.36	≤ .001
Cycle	1	0.5052	0.5052	35.55	≤ .001
Genotype.Cycle	35	1.1985	0.0342	2.41	≤ .001
Residual	71	1.0090	0.0142		
Total	143	29.2562			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	72	2
d.f.	71	71	71
s.e.d.	0.084	0.020	0.119

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	72	2
d.f.	71	71	71
l.s.d.	0.168	0.040	0.238

vi)Variate: Plant survival (PS) at 60 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0004	0.0004	0.01	
Genotype	35	41.7722	1.1935	40.34	≤ .001
Cycle	1	0.7449	0.7449	25.18	≤ .001
Genotype.Cycle	35	0.9943	0.0284	0.36	0.542
Residual	71	2.1004	0.0296		
Total	143	45.6123			

vii)Variate: Disease incidence (DI) at 30 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0019	0.0019	0.04	
Genotype	35	13.3819	0.3823	8.71	≤ .001
Cycle	1	0.4458	0.4458	10.15	0.002
Genotype.Cycle	35	1.3703	0.0391	0.89	0.639
Residual	71	3.1173	0.0439		
Total	143	18.3173			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	72	2
d.f.	71	71	71
s.e.d.	0.148	0.035	0.210

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype.Cycle
rep.	2	72	2
d.f.	71	71	71
l.s.d.	0.295	0.070	0.418

viii)Variate: Disease incidence (DI) at 45 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0011	0.0011	0.09	
Genotype	35	25.7284	0.7351	56.27	≤ .001
Cycle	1	0.5589	0.5589	42.78	≤ .001
Genotype.Cycle	35	0.9761	0.0279	2.13	0.003
Residual	71	0.9275	0.0131		
Total	143	28.1921			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	72	2
d.f.	71	71	71
s.e.d.	0.081	0.019	0.114

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	72	2
d.f.	71	71	71
s.e.d.	0.161	0.038	0.228

ix)Variate: Disease incidence (DI) at 60 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0012	0.0012	0.03	
Genotype	35	42.3944	1.2113	31.54	≤ .001
Cycle	1	0.9711	0.9711	25.29	≤ .001
Genotype.Cycle	35	1.4869	0.0425	1.11	0.352
Residual	71	2.7264	0.0384		
Total	143	47.5801			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	72	2
d.f.	71	71	71
s.e.d.	0.139	0.033	0.196

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	72	2
d.f.	71	71	71
l.s.d.	0.276	0.065	0.391

x)Variate: Average fruit weight (AFW) at 60 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	11.281	11.281	1.31	
Genotype	35	55216.552	1577.616	182.53	≤ .001
Residual	35	302.504	8.643		
Total	71	55530.337			

Standard errors of differences of means

Table	Genotype
rep.	2
d.f.	35
s.e.d.	2.940

Least significant differences of means (5% level)

Table	Genotype
Rep.	2
d.f.	35
l.s.d.	5.968

xi)Variate: Fruit Yield (FY) at 60 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	7.0500	7.0500	7.12	
Genotype	35	129.8684	3.7105	3.75	≤ .001
Residual	35	34.6742	0.9907		
Total	71	171.5927			

Standard errors of differences of means

Table	Genotype
rep.	2
d.f.	35
s.e.d.	0.995

Least significant differences of means (5% level)

Table	Genotype
rep.	2
d.f.	35
s.e.d.	2.021

Appendix B. Analyses of variance for three parameters for bacterial wilt resistance in the greenhouse in experiment two Egerton University, Njoro in 2020

i) Variate: Area Under Disease Progress Curve (AUDPC) at 30 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0088	0.0088	2.30	
Genotype	23	39.4544	1.7154	450.26	≤ .001
Cycle	1	0.0002	0.0002	0.06	0.801
Genotype.Cycle	23	0.0601	0.0026	0.69	0.835
Residual	47	0.1791	0.0038		
Total	95	39.7025			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	48	2
d.f.	47	47	47
s.e.d.	0.044	0.013	0.062

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	48	2
d.f.	47	47	47
l.s.d.	0.088	0.025	0.124

ii) Variate: Area Under Disease Progress Curve (AUDPC) at 45 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0008	0.0008	0.59	
Genotype	23	69.8366	3.0363	2137.00	≤ .001
Cycle	1	0.0035	0.0035	2.47	0.123
Genotype.Cycle	23	0.0466	0.0020	1.43	0.150
Residual	47	0.0668	0.0014		
Total	95	69.9543			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	48	2
d.f.	47	47	47
s.e.d.	0.027	0.008	0.038

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	48	2
d.f.	47	47	47
l.s.d.	0.054	0.015	0.076

iii) Variate: Area Under Disease Progress Curve (AUDPC) at 60 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0000	0.0000	0.00	
Genotype	23	86.8196	3.7748	4265.25	≤ .001
Cycle	1	0.0610	0.0610	68.97	≤ .001
Genotype.Cycle	23	0.1867	0.0081	9.17	≤ .001
Residual	47	0.0416	0.0009		
Total	95	87.1089			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	48	2
d.f.	47	47	47
s.e.d.	0.021	0.006	0.030

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	48	2
d.f.	47	47	47
l.s.d.	0.042	0.012	0.060

iv) Variate: Disease incidence(DI) at 30 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0343	0.0343	1.57	
Genotype	23	3.1134	0.1358	6.20	≤ .001
Cycle	1	0.1759	0.1759	8.06	0.007
Genotype.Cycle	23	0.4793	0.0208	0.95	0.535
Residual	47	1.0267	0.0218		
Total	95	4.8297			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	48	2
d.f.	47	47	47
s.e.d.	0.105	0.030	0.148

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	48	2
d.f.	47	47	47
l.s.d.	0.210	0.061	0.297

v) Variate: Disease incidence (DI) at 45 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0178	0.0178	2.06	
Genotype	23	8.1243	0.3532	40.97	≤ .001
Cycle	1	0.1911	0.1911	22.17	≤ .001
Genotype.Cycle	23	0.2215	0.0096	1.12	0.364
Residual	47	0.4052	0.0086		
Total	95	8.9599			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	48	2
d.f.	47	47	47
s.e.d.	0.068	0.019	0.093

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	48	2
d.f.	47	47	47
l.s.d.	0.132	0.038	0.187

vi) Variate: Disease incidence (DI) at 45 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0178	0.0178	2.06	
Genotype	23	8.1243	0.3532	40.97	≤ .001
Cycle	1	0.1911	0.1911	22.17	≤ .001
Genotype.Cycle	23	0.2215	0.0096	1.12	0.364
Residual	47	0.4052	0.0086		
Total	95	8.9599			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	48	2
d.f.	47	47	47
s.e.d.	0.068	0.019	0.093

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	48	2
d.f.	47	47	47
l.s.d.	0.132	0.038	0.187

vii) Variate: Disease incidence(DI) at 60 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.3031	0.3031	11.92	
Genotype	23	16.7435	0.7279	28.64	≤ .001
Cycle	1	0.4531	0.4531	17.83	≤ .001
Genotype. Cycle	23	0.8097	0.0352	1.39	0.170
Residual	47	1.1946	0.0254		
Total	95	19.5040			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	48	2
d.f.	47	47	47
s.e.d.	0.11	0.03	0.16

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	48	2
d.f.	47	47	47
l.s.d.	0.23	0.07	0.32

viii) Variate: Plant survival (PS) at 30 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0195	0.0196	0.81	
Genotype	23	8.9855	0.3907	16.24	≤ .001
Cycle	1	1.6968	1.6968	70.55	≤ .001
Genotype.Cycle	23	1.5770	0.0686	2.85	0.001
Residual	47	1.1304	0.0241		
Total	95	13.4092			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	48	2
d.f.	47	47	47
s.e.d.	0.110	0.032	0.155

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	48	2
d.f.	47	47	47
l.s.d.	0.221	0.064	0.312

ix) Variate: Plant survival (PS) at 45 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0123	0.0123	1.34	
Genotype	23	14.3731	0.6249	68.18	≤ .001
Cycle	1	0.6475	0.6475	70.64	≤ .001
Genotype.Cycle	23	0.5579	0.0243	2.65	0.002
Residual	47	0.4308	0.0092		
Total	95	16.0216			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	48	2
d.f.	47	47	47
s.e.d.	0.068	0.020	0.096

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	48	2
d.f.	47	47	47
l.s.d.	0.136	0.039	0.193

x) Variate: Plant survival (PS) at 60 days after inoculation (DAI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.0008	0.0008	0.03	
Genotype	23	27.5051	1.1959	38.71	≤ .001
Cycle	1	1.6025	1.6025	51.87	≤ .001
Genotype.Cycle	23	1.3501	0.0587	1.90	0.031
Residual	47	1.4520	0.0309		
Total	95	31.9105			

Standard errors of differences of means

Table	Genotype	Cycle	Genotype.Cycle
rep.	4	48	2
d.f.	47	47	47
s.e.d.	0.12	0.04	0.18

Least significant differences of means (5% level)

Table	Genotype	Cycle	Genotype. Cycle
rep.	4	48	2
d.f.	47	47	47
l.s.d.	0.25	0.07	0.35

	AUDPC		PS		DI		AUDPC		PS		DI		AFW	FY
	45 DAI						60 DAI							
Genotypes	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC2	CC1	CC1
KK acc 1	0	0	100	100	0	0	0	0	100	100	0	0	5.80	2.50
NRB	0	0	100	100	0	0	0	0	100	100	0	0	3.30	4.87
KK acc II	0	0	100	100	0	0	0	0	100	100	0	0	5.80	2.61
KISII	0	0	100	100	0	0	0	0	100	100	0	0	4.60	3.17
BRNG acc II	0	0	100	100	0	0	0	0	100	100	0	0	6.00	2.65
KLF acc III	0	0	100	100	0	0	0	0	100	100	0	0	3.00	2.47
NKR acc III	46	299	71	50	29	50	40	219	20	0	0	100	5.20	2.15
KITUI	52	122	80	50	20	29	49	41	40	29	40	50	4.60	2.13
MK	68	214	71	39	20	50	59	156	40	20	40	71	5.00	2.09
BRNG acc V	75	247	80	39	20	50	69	185	29	5	50	95	5.00	2.03
MCK acc II	75	230	60	50	29	39	66	168	29	11	61	82	6.00	2.02
KLF acc II	90	337	60	29	29	50	85	253	40	20	60	71	4.60	2.02
BRNG acc IV	97	253	60	61	29	29	107	209	29	29	61	50	5.00	1.94
NKR acc IV	138	552	29	40	50	50	128	491	5	5	89	95	5.60	2.01
NKR acc I	292	585	50	20	40	71	299	527	20	0	71	100	4.50	1.92
NKR acc II	437	491	60	20	40	71	480	527	29	0	71	100	6.00	1.84
KRC	540	861	50	20	40	71	651	990	20	0	80	100	5.75	1.27
MARGLOBE	607	822	50	20	50	80	766	923	5	0	89	100	88.50	6.68
RIONEX	651	636	50	39	50	50	822	515	5	5	95	95	85.50	5.77
KSM	682	902	60	20	40	71	881	1062	20	5	71	95	3.30	1.71
BRNG acc III	731	841	20	29	60	71	990	967	5	5	95	95	6.20	1.67
NYM	749	990	39	20	50	80	990	1249	5	0	95	100	5.30	1.66
MCK acc I	841	990	40	29	60	71	1192	1192	0	0	100	100	4.00	1.57

KLF acc I	851	967	29	29	71	71	1165	1112	5	0	95	100	5.80	1.57
M-82	945	1013	29	20	50	71	1308	1165	5	0	95	100	72.50	4.46
OXYLY	945	1086	20	0	80	100	1278	1278	0	0	100	100	69.00	2.66
BRNG acc I	967	1037	20	20	60	80	1339	1220	5	0	95	100	4.70	1.43
MONEY MAKER	967	990	20	20	71	80	1339	1220	5	0	95	100	78.50	5.44
RIOGRANDE	967	1013	50	20	50	80	1339	1220	5	0	95	100	63.50	2.46
TRNZ	1062	1037	20	20	80	71	1469	1249	0	0	100	100	4.40	1.20
NYR	1062	1037	20	20	80	71	1469	1249	0	0	100	100	5.40	1.21
UAG	1062	1086	20	29	71	71	1469	1308	0	0	100	100	6.50	1.19
CAL-J	1112	1112	0	5	100	95	1504	1370	0	0	100	100	46.00	1.68
RED DIAMOND	1112	1112	0	0	100	100	1539	1403	0	0	100	100	55.00	2.02
KLF acc IV	1138	1138	0	0	100	100	1575	1403	0	0	100	100	5.60	0.82

Appendix C. Means of wild (*Solanum pimpinellifolium*) and cultivated (*Solanum lycopersicum*) tomato genotypes evaluated for bacterial wilt resistance in the greenhouse for two cropping cycles at Egerton University, Njoro in 2020

Appendix D. Analyses of variance for combining ability for three parameters in the greenhouse in experiment two at Egerton University, Njoro in 2020

i)Variate Area Under Disease Progress Curve (AUDPC)

Source of variation	df	Sum Sq	Mean Sq	F value	Pr(>F)
Replications	1	6.0208e-04	0.0006	0.747	0.3964
Treatments	23	4.4968e+0	1.9551	2424.428	0.0000
Parents	7	1.8828e+01	2.6898	3335.497	0.0000
Parents vs. Crosses	1	4.2841e+00	4.2841	5312.481	0.0000
Crosses	15	2.1854e+01	1.4569	1806.725	0.0000
Lines	3	1.5470e+00	0.5156	0.952	0.4559
Testers	3	1.5431e+01	5.1437	9.493	0.0038
Lines× Testers	9	1.5431e+01	0.5418	671.933	0.0000
Error	23	4.8768e+00	0.0008		
Total	47	1.8547e-02			

Standard Errors for Combining Ability Effects

S.E. (gca for line):	0.0100
S.E. (gca for tester):	0.0100
S.E. (sca effect):	0.0201
S.E. (gi - gj) line	0.0142
S.E. (gi - gj)tester	0.0142
S.E. (sij - skl)tester	0.0284

ii) Variate: Disease incidence (DI)

Source of variation	df	Sum Sq	Mean Sq	F value	Pr(>F)
Replications	1	0.16803	0.1680	13.430	0.0013
Treatments	23	10.1647	0.4419	35.323	0.0000
Parents	7	5.1047	0.7292	58.286	0.0000
Parents vs. Crosses	1	0.3850	0.3850	30.777	0.0000
Crosses	15	4.6749	0.3116	24.910	0.0000
Lines	3	0.6269	0.2089	2.007	0.1836
Testers	3	3.1109	1.0369	9.960	0.0032
Lines× Testers	9	0.9370	0.1041	8.322	0.0000
Error	23	0.2877	0.0125		
Total	47	10.6205			

Standard Errors for Combining Ability Effects

S.E. (gca for line): 0.0395
S.E. (gca for tester): 0.0395
S.E. (sca effect): 0.0790
S.E. (gi - gj) line 0.0559
S.E. (gi - gj)tester 0.0559
S.E. (sij - skl)tester 0.1118

iii) Variate: Plant survival (PS)

Source of variation	df	Sum Sq	Mean Sq	F value	Pr(>F)
Replications	1	0.0014	0.0014	0.038	0.8472
Treatments	23	18.1485	0.7891	21.212	0.0000
Parents	7	9.8596	1.4085	37.864	0.0000
Parents vs. Crosses	1	0.3602	0.3601	9.682	0.0049
Crosses	15	7.9288	0.5285	14.209	0.0000
Lines	3	1.0314	0.3438	2.963	0.0900
Testers	3	5.8529	1.9509	16.813	0.0005
Lines× Testers	9	1.0444	0.1160	3.119	0.0133
Error	23	0.8556	0.0372		
Total	47	19.0055			

Standard Errors for Combining Ability Effects

S.E. (gca for line): 0.0681
S.E. (gca for tester): 0.0681
S.E. (sca effect): 0.1363
S.E. (gi - gj) line 0.0964
S.E. (gi - gj)tester 0.0964
S.E. (sij - skl)tester 0.1928

Appendix E. Research Permit

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Ref No: **431431**

Date of Issue: **03/April/2020**

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This is to Certify that Miss.. Faith WANGUI Wangui of Egerton University, has been licensed to conduct research in Nakuru on the topic: inheritance of Bacterial Wilt (Ralstonia solanacearum) Resistance among Wild and Cultivated tomato (Solanum lycopersicon L.) Germplasm in Kenya. for the period ending : 03/April/2021.

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Appendix F. Publication

Abstract

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Full Length Research Paper

Combining ability and gene action for bacterial wilt disease resistance in wild tomato (*Solanum pimpinellifolium*) and cultivated tomato (*Solanum lycopersicum*) genotypes

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Bacterial wilt caused by *Ralstonia solanacearum* is one of the most destructive and widespread diseases of tomato in Kenya. The objective of this study was to determine the combining ability effects and gene action conditioning bacterial wilt disease resistance in tomato. Eight parents were crossed in North Carolina II mating design scheme to produce sixteen F_1 hybrids. The F_1 hybrids and the parental genotypes were evaluated for bacterial wilt in an *alpha* lattice design. Among the parents, KLF acc III was the best general combiner for area under the disease progress curve (AUDPC) and disease incidence across the two cropping cycles. Red Diamond × KLF acc III, Money Maker× KK acc I, Oxyly× KLF acc III and Money Maker× KK acc II were the best specific combiners for AUDPC. Low narrow sense heritability values of 0.14, 0.16 and 0.20 were obtained for AUDPC, disease incidence and plant survival. Relative weights of additive versus non-additive gene action obtained for AUDPC, disease incidence and plant survival were 0.19, 0.20 and 0.50. General predictability ratios (GPR) values of 0.27, 0.29 and 0.50 were obtained for AUDPC, disease incidence and plant survival. These results indicated the predominance of non-additive gene action in governing the traits.

Key words: Disease resistance, bacterial wilt, combining ability, gene action, tomato.