IDENTIFICATION OF MICROSATELLITE MARKERS LINKED TO BACTERIAL WILT RESISTANCE IN AFRICAN EGGPLANT

T. Nikuze[1*], E. M. Miinda[1*], J. Ambuko[2] and O. W. Owino[1*]
[1]Department of Horticulture and Food Security and [1]Department of Food Science and Technology, School of Agriculture, Jomo Kenyatta university of Agriculture and Technology, P. O. Box 62000-00100, Nairobi
[2]Department of Plant Science and Crop Protection, University of Nairobi, P.O Box29053-Kangemi

*Corresponding author. willis@agr.jkuat.ac.ke

Abstract

Bacterial wilt (BW) is distributed throughout the world and cause up to 100% yield loss of many solanaceous crops. Management of this soil borne bacterium mainly rely on chemical use which is ineffective and eco-unfriendly. However, use of resistant eggplants and non-chemical strategies can be a good alternative for BW management. The objectives of the study are to determine phenotypic reaction of eggplant to BW and identify eggplant bacterial wilt resistant markers. The African eggplants seeds were supplied by AVRDC-RCA, Arusha. They were raised in cocopeat in trays and transplanted at five true leaves (July–September 2016 and February–June 2019). Symptom severity was scored using a 1-5 scale for each accession. A total of 15 primers were screened for bacterial wilt resistance. In susceptible accessions, symptoms appeared 14 days after field transplanting and 21 days' post inoculation. After the assay, eggplant stem sections were sampled and put under sterile water for 2 hours to allow BW to ooze out. Further, 50ul of the suspension was streaked on TZC media, incubated at 28^{o}C for 48 hours. Bacterial wilt symptoms varied from one leaf wilt to whole plant death. The symptoms were first observed on upper leaves and progressed to other parts of the plant resulting to wilting. Brown disclouration in vascular systems of transversely cut stem sections was observed. Four accessions, i.e., RV100438, RV100246, RV100242 and RV100455 recorded a disease severity of ≤ 1.2. Twelve accessions, i.e., RV100386, RV100234, RV100201, RV100245, RV100331, RV100250, RV100447, RV100161, RV100247, RV100240, RV100271, RV100458 2.7-3.4. Accessions from *Solanum aethiopicum* and *S. anguivi* showed resistance at 1.1 – 1.3 severity and none showed full resistant. Of the 15 markers used, only 5, i.e., ecm009, mk03O04, ecm001, SOL5036 and emiO4P17 showed polymorphism between the R and S eggplants. Resistant eggplant accessions are therefore recommended for cultivation under integrated systems and used in breeding resistant eggplants.

Key words: Bacterial wilt, African eggplant accessions, Resistant, SSR markers.

Introduction

Eggplant *(Solanum sp)* is a member of the *Solanaceae* family, which includes other vegetable crops such as tomato, potato and capsicum (Gopalakrishnan, 2014). Eggplant is of importance in warm areas of Far East, being grown extensively in India, Bangladesh, Pakistan, China and the Philippines (FAO, 2018). It is also popular in Egypt, France, Italy and United States (FAO, 2018). In Italy, farmers had been utilizing the scarlet eggplant for many years and is being safegurded to provide useful genes to breed against the soil borne pathogen Fussarium wilt (Sunseri, *et al.,* 2010). In Africa, Egypt and Algeria are the leading eggplant producers. A total of 9.5 million MT were harvested from 501,002 ha in Africa (FAO, 2019). Cultivation of the African eggplant has been decreasing due to the effect of various soil borne diseases such as *Fusarium* (Mwaniki, 2016), *B. wilt* (Salgon, 2017;

Lebeau,, 2013; Lebeau *et al,* 2011; and Sunseri, *et al.,* 2010) and *Verticillium* wilt (*Verticillium dahliae*) (Mwaniki, 2016).

Bacterial wilt caused by *Ralstonia solanacearum* is a worldwide pathogen found in tropical, subtropical and warm regions causing lethal wilting in many important crops (Engelbrecht and Hattingh, 1989; Wickera, *et al.,* 2009; Tahat and Sijam 2010; Yadessa, *et al.,* 2010; Xue, *et al,.* 2011; Meng, 2013) as well as in temperate regions (Swanson, 2007). Bacterial wilt is considered as one of the most important bacterial disease of solanaceous plants. Over 200 plant species from 50 botanical families, have been reported as host plants to bacterial wilt, which include most economically important crops such as potato, tomato, eggplant, pepper, tobacco and banana and weeds (; Xue, *et. al.,* 2011; Meng, 2013; Ha, *et al.,* 2012; Hasabi, 2015). The bacterium, *Ralstonia solanacearum* invades its host through roots creating barriers to upward movement of water and other nutrients from the soil, thus causing irreversible foliar wilting leading to plant death (Kumar, 2016). Ornamental plants including anthurium, pothos, ornamental ginger, gerbera, zinnia, salvia, verbena, heliconia, sunflower, and geranium have been reported to host the pathogen (Norman, *et al.,* 2009).

Bacterial wilt is considered the single most destructive plant bacterial disease causing up to 100% loss. If uncontrolled, it causes crop losses ranging from 15% - 97% (Mwaniki, 2016). For instance, in Kenya it causes up to 80 % yield loss in potato (Mwaniki, 2016). Bacterial wilt prevalence range from 35% - 100% and reduces with an increase in altitude (Mwaniki, 2016). It is distributed in all solanaceous producing areas and the incidence stand at 0.78 – 1.47% (Gildermacher, 2009). The high prevalence is aggravated by spread from one region to another and borrowing of infested planting materials (Gildermacher, 2009; Mwaniki, 2016). *R. solanacearum* being a soil borne pathogen, with wide host range, long survival in the soil, and wide biological variation has made it difficult to be effectively controlled (Perez, *et al.,* 2008; Bi-hao, *et al,* 2009; Tahat and Sijam 2010; Muthoni, *et al.,* 2012).

Several methods exist which include chemical, biological, physical, cultural and use of resistant varieties (Yuliar, *et al.,* 2015). Continuous use of chemical control has a negative drawback on cost effectiveness, human, environment, and onto the pathogen itself (Reddy, *et al.,* 2015). In addition to chemical control, the soil borne pathogen cannot be completely eradicated due to deeper penetration into the soil and in plant xylem vessels (Huet, 2014). Thus, breeding for resistance to the soil borne pathogen is an essential alternative strategy for controlling bacterial wilt in infested areas (Huet, 2014; Salgon, 2017). Many plants such as tomato, pepper and eggplant exhibit some tolerance, immunity and resistance to bacterial wilt (Mohan, 1997; Lebeau, 2011; Lebeau, 2013; Truong, 2015; Salgon S, 2017). However, the variation in *R. solanacearum* phylotype and strains makes it difficult for multi-locational control (Huet, 2014).

Studies have shown that eggplants carry resistance genes to bacterial wilt (Salgon , 2017; Gopalakrishnan, 2014). However, a lot has been done on *S. melongena* and its crosses with wild relatives (Collonnier, 2001; Lebeau, 2011; Lebeau, 2013; Meng, 2013; Gopalakrishnan, 2014). Markers assisted selection have been used to construct eggplant genetic maps (Nunome, 2009). Thus, breeding for resistance by identifying markers tightly linked to resistance to plant pathogens through marker assisted selection has improved the breeding process (Mutlu, 2008; Bi-hao, 2009; Lebeau, 2013; Huet, 2014; Salgon, 2017). Resistant and susceptible crosses were evaluated for resistance to bacterial wilt under a collection of bacterial strains (Lebeau, 2011; Lebeau 2013; Huet, 2014; Salgon, 2017). Although these crosses were successful, but their progeny were not fully resistant to the bacterial wilt pathogen (Salgon, 2017).

In identification of resistance to *Fusarium oxysporum* Schelcht. F. sp. *melongenae* (FOM),

16 sets of markers yielded polymorphism. These markers included; 4-SRAPs, 4-RGAs, 6-SRAP-RGAs and 2-RAPDs, which were subsequently tested on resistant and susceptible plants (Mutlu, 2008). Further, these markers were detected in all resistant plants and tightly linked to *Fusarium* wilt resistance (Mutlu, 2008). Salgon (2017) phenotyped a population of recombinant inbred line (RILS) from crosses between susceptible parent (line MM738) and a resistant parent (line AG91-25) against 4 *Ralstonia solanacearun* species complex (RSSC) strains. In this study, 3 QTL conferring bacterial wilt resistance were identified with different markers. In another study, a major locus on LG2 mapped by AFLP marker carrying a QTL (*ERsl*) was found to be tightly linked with resistance gene (Lebeau, 2013). The present study shows that resistance to bacterial wilt exists in these eggplant accessions and can be tapped for management strategies.

Materials and Methods

Study Site and Experimental Materials

Seeds of African eggplant accessions (S*olanaum aethiopicum*, S*olanum sp* and *Solanum anguivi*) were collected from the Asian Vegetable Research and development centre- regional centre for Africa (AVRDC- RCA) in Arusha Tanzania (Table 1). The study was conducted at Jomo Kenyatta University of Agriculture and Technology.

Seedling Propagation and Experimental Design

The African eggplant seeds, 20 per accession were sown in germination trays containing washed cocopeat and allowed to germinate. This was done by sorting clean and whole seeds without defects or broken. Prior to sowing the seeds were soaked in warm water 45^{o}C for 24 hours in order to have a uniform germination. At 5 true leaves, African eggplants seedlings were transplanted into the field and in the greenhouse for evaluation of disease reaction. Disease screening on African eggplants for bacterial wilt (*Ralstonia solanacearum*) response was carried out in naturally infected field and under controlled artificial inoculation. The experiment was conducted in a randomized complete block design (RCBD) for the field trials and completely randomized design (CRD) for the greenhouse experiment.

Field and Greenhouse Experiments

Prior to transplanting of eggplant seedlings into the field, the bacterial load was determined. This was done by identifying six points in the field by using a zigzag method of soil sampling. The soil was collected by use of a soil auger at a depth of 30 cm. 1 g of the soil sample from each point was dissolved in 10ml of sterile water. The soil solution was diluted to six folds (10^{-6}), each in 10ml of sterile water. Serological test of the soil was carried out on CPG media to determine the extent at which the field was contaminated with bacterial wilt. In the greenhouse, eggplants were transplanted into poly bags of 21inch x 14inch x 14-inch. Each poly bag was planted with one seedling and spaced at 30cm and twelve plants per accession were planted in three replicates. The bags were filled with sterilised soil media and later inoculated with *R. solanacearum*. For the field experiment, all plants were transplanted into three 45m long and 3m wide beds spaced at 90cm by 50cm and separated by 150cm path. Here the plants were let to be naturally inoculated while in the greenhouse, artificial inoculation was carried as described below. Watering and pest management was carried out when required.

Bacterial Wilt Disease Assessment

The bacterium, *R. solanacearum* was isolated from diseased eggplants obtained from the field showing typical BW wilting symptoms. Bacterial isolate was grown at 28^{o}C on 2, 3, 5- triphenyl tetrazolium chloride (TTC) (Kelman, 1954). Actively growing colonies were harvested from 48 hours culture by use of a sterile wire loop. Colonies were then transferred to CPG (Casamino hydrolysate 1 gPeptone 10 g and Glucose 5 g pH 7) nutrient broth and incubated on an orbital shaker (Orbital Shaker-incubator ES-20 Grant-Bio) for 24 hours at 28^{o}C. The *R. solanacearum* inoculum was prepared by adjusting the

concentration to 10^7 CFU/ml (OD=0.0977) at 600 nm optical density (OD) using spectrophotometer. Plants were inoculated at seven days after transplanting (DAT) upon preliminary puncturing of roots 2 cm away from the stem using sterile knife. The bacterial suspension was drenched immediately after the roots were damaged. Each plant received 30 ml of bacterial suspension. Bacterial wilt severity was determined by visual assessment on the symptom development once a week for six weeks according to a disease score scales; 1: asymptomatic plant/healthy, 2: one wilting leaf, 3: less than 50 % wilted leaves, 4: more than 50 % wilted leaves, and 5: completely wilted leaves (dead plant) with modification from (Lebeau, *et al.,* 2013). At each scoring date the disease incidence was calculated for each accession as;

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

The area under the disease progression curve (AUDPC) was determined as according to Lebeau (2013),

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

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

Table 1: African eggplant accessions, lines and origin in the present study

S/no	Accession Number	Origin	Family
1	RV100246	Unknown	*Solanum aethiopicum*
2	RV100245	Mali	*Solanum aethiopicum*
3	RV100334	Mali	*Solanum aethiopicum*
4	RV100352	Uganda	*Solanum aethiopicum*
5	RV100328	Mali	*Solanum aethiopicum*
6	RV100330	Mali	*Solanum aethiopicum*
7	RV100264	Mali	*Solanum aethiopicum*
8	RV100432	Unknown	Solanum spp
9	RV100445	Unknown	Solanum spp
10	RV100333	Mali	*Solanum aethiopicum*
11	RV100185	Gabon	*Solanum aethiopicum*
12	RV100259	Senegal	*Solanum aethiopicum*
13	RV100250	Mali	*Solanum aethiopicum*
14	RV100453	Unknown	*Solanum spp*
15	RV100342	Cameroon	*Solanum aethiopicum*
16	GBK50591	Unknown	*Solanum spp*
17	RV100452	Unknown	*Solanum spp*
18	RV100270	Mali	*Solanum aethiopicum*
19	RV100201	Malawi	*Solanum aethiopicum*
20	RV100455	Unknown	*Solanum spp*
21	RV100332	Bukina Faso	*Solanum aethiopicum*
22	RV100247	Mali	*Solanum aethiopicum*
23	RV100447	Mali	*Solanum spp*
24	RV100335	Cameroon	*Solanum anguivi*
25	RV100161	Tanzania	*Solanum aethiopicum*
26	RV100242	Mali	*Solanum aethiopicum*
27	RV100234	Mali	*Solanum aethiopicum*
28	RV100438	Unknown	*Solanum aethiopicum*
29	RV100240	Mali	*Solanum aethiopicum*
30	RV100218	Unknown	*Solanum aethiopicum*
31	RV100364	Uganda	*Solanum anguivi*
32	RV100263	Mali	*Solanum aethiopicum*

S/no	Accession Number	Origin	Family
33	RV100239	Mali	*Solanum aethiopicum*
34	RV100271	Mali	*Solanum aethiopicum*
35	RV100169	Tanzania	*Solanum aethiopicum*
36	RV100268	Mali	*Solanum aethiopicum*
37	RV100386	Ivory Coast	*Solanum aethiopicum*
38	RV100377	Uganda	*Solanum aethiopicum*
39	RV100265	Mali	*Solanum aethiopicum*
40	RV100261	Mali	*Solanum aethiopicum*
41	RV100217	Mali	*Solanum aethiopicum*
42	RV100327	Mali	*Solanum aethiopicum*
43	RV100511	Tanzania	*Solanum aethiopicum*
44	RV100360	Uganda	*Solanum anguivi*
45	RV100190	Tanzania	*Solanum anguivi*
46	RV100458	Unknown	
47	RV100 331	Unknown	

African eggplant accession seeds were sourced from Asian Vegetable Research and development centre- regional centre for Africa (AVRDC- ARC) Arusha Tanzania

***Ralstonia solanacearum* confirmatory test**

At the end of the assay, latent infection tests for bacterial wilt was carried out by taking asymptomatic and symptomatic plants for bacterial isolation according to Lebeau (2013, 2011) with slight modifications. The plants were washed off the soil in running tap water, rinsed with distilled water and finally sterilised with 70% ethanol. Eggplant stem of about two centimetres in length picked from the stem base were placed in clear glass test tubes filled with 10 ml sterile water (Plate 1E). Stem sections were allowed to stand for 1–2 hours at room temperature ((23^{0}C)) for bacteria to stream out of the xylem vessels (Plate 1E). The presence of milky oozing exudates (Plate 1E) from the cut stem section was proof that the pathogen was *R. solanacearum.* Serial dilution (10^{-6}) from the original streaming was done, where an aliquot of 50 µL from each sample was streaked onto Kelman's media triphenyl tetrazolium chloride (TTC) (Kelman, 1954) agar and incubated at 28^{o}C for 48 hours in orbital shaker-incubator ES-20 (Grant-bio). Stem sections from which characteristic *R. solanacearum* colonies were isolated, were scored as positive for the presence of bacterial wilt (Plate 1F).

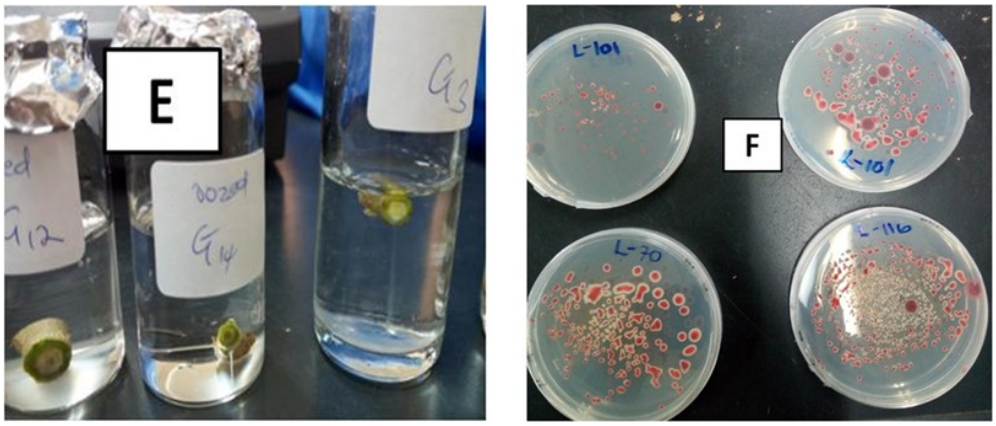

Plate 1: Diagnosis and morphological characteristic of BW; (E) Oozing plant sections and (F) *Ralstonia solanacearum* colonies

DNA extraction

DNA of symptomatic and asymptomatic plants of each accession was extracted from 0.5 gm of the youngest leaves using modified CTAB procedure (Doyle, 1987). The DNA quality was assessed using 2% (w/v) agarose gel electrophoresis (Lebeau *et al.,* 2013).

SSR-Based Diversity Analysis

A polymorphism survey was conducted using 15 SSR primers on all the accessions, with equal amounts of genomic DNA (Nunome, 2009) (Table 2). Only five primers were polymorphic whereas the other ten did not yield quality bands, hence discarded (Plate 2). Markers with polymorphic bands were then considered for subsequently screening for BW resistance of eggplant. The polymorphic SSR bands for each accession was individually scored for the presence (1) or absence (0) of the expected bands sizes (Lebeau, 2013).

All the PCR markers were amplified in a 25 µl reaction volume with 2 µl of genomic DNA, 0.5 µl of each forward and reverse primer, 9.5 µl of free nuclease water and 12.5 µl of X2 master mix taq (Biolabs, New England). (Nunome, 2009) A touchdown PCR protocol was applied, of one cycle of 94^{o}C for 3 min of denaturation; 10 cycles of 94^{o}C for 30 s, 65–55^{o}C decreasing by 1^{o}C per cycle for 1 min; one cycle of 72^{o}C for 1 min; 30 cycles of 94^{o}C for 30 s; one cycle of 55^{o}C for 1 min; one cycle of 72^{o}C for 1 min; and a final cycle of 72^{o}C for 5 min. Amplification was carried out using the GeneAmp PCR system 2720 thermal cycler (Applied Biosystems, USA). The PCR products (100 base pair molecular-weight ladder) were separated through electrophoresis in 2.0% agarose gel run in 5x Tris-Borate-EDTA (TBE) at 80V after staining with ethidium bromide and then photographed under UV light using benchtop 2UV transilluminator (UVP). The data was analyzed using GenAIEx 6.503. The bands were scored as 1 for presence and 0 for absence (Lebeau, 2013).

Statistical Analysis of Phenotypic Data

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

Genetic Diversity Analysis

The genetic diversity was analysed by computing allele frequency-based analyses including heterozygosity, Nei's genetic distance and information index by use of Gen AIEx 6.503 and DARwin 6.0.010 version software.

Results

Disease incidence (DI)

African eggplant accessions (Table 1) were planted in sick plots of the bacterial load 1 x10^{-6} CFU and in greenhouse and inoculated with (1 x 10^{-7} CFU) *R. solanacerum* suspension. The control plants were subsequently treated with sterile water. Temperatures during the experiment ranged from 18^{0}C to 36^{0}C. Accessions; RV100386, RV100234, RV100201, RV100245, RV100331, RV100250, RV100447, RV100161, and RV100240 showed bacterial wilt symptoms 14 days after inoculation (DAI). Majority of these accessions had disease incidence and severity of >46% and >2.4 respectively. Symptoms ranged from one leaf wilt to whole plant wilt and even complete plant death. The symptoms were first observed on upper leaves and progressed to other parts of the plant resulting to wilting (Plate 3C). Brown disclouration in vascular systems of transversely cut stem sections was observed (Plate 3B). On the other hand, symptoms appeared on leaves followed by chlorosis and no wilting while other plants had a stunted growth.

Variation difference in bacterial wilt response among the accessions was observed (Table 3). Some accessions showed mild to severe response to bacterial wilt, whereas severe wilt was observed during early hours of the day and recovered in the evening. None of the eggplant accessions showed total resistance to the bacterium. However, there was a mixture of reactions to the bacterium from all the accessions (Figure 1).

Table 2: SSR markers used in the study

SSR/SNPs	Repeat motif of the SSR/SNP	Size range of the PCR product
ecmoo9	(TAT)13	245
emi04p17	(AC)14A(TA)11	226
emk03O04	(AC)23A(TA)9CA(TA)12	179
est_ae507f01	*	582
SOL7229	*	289
SOL8240	*	228
est_rbw03m09	*	198
SOL5036	*	134
Est_rbw01106	*	135
SOL8269	*	70
SOL7124	*	412
SOL8253	*	132
ecmoo1	(TC)17	229
SOL7274	*	185
SOL5085	*	212

*Indicate that, the primer lacks repeat motif

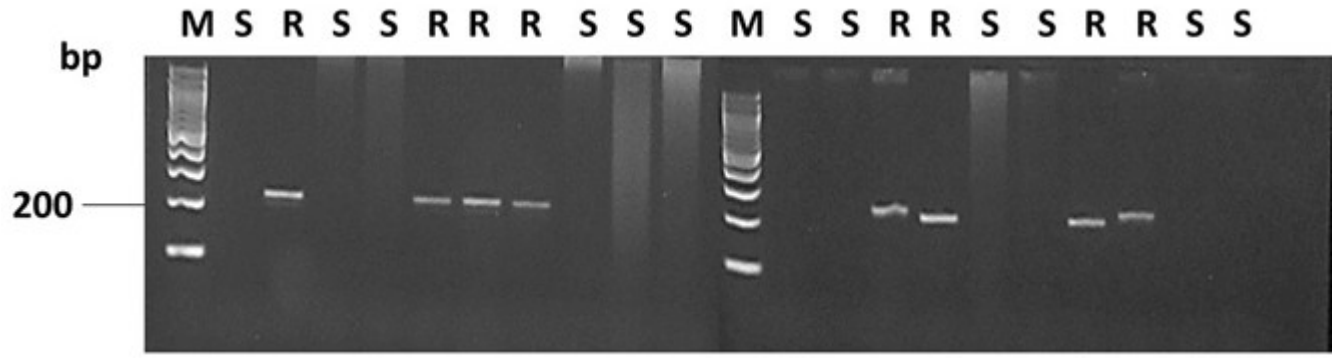

Plate 2; Agarose gel electrophoresis of 15 SSR primers on pooled showed polymorphism between susceptible (S) and resistant (R) eggplants DNA. Lanes M 100 bp ladders

Plate 3: Bacterial wilt symptoms on various parts of eggplant plants; (B) cross section of infected stem, (C) wilted plant in the field and (D) an oozing stem

A high percentage of accessions from the *Solanum aethiopicum* were highly susceptible to the pathogen having disease incidence of 60-66.8% (table 3). Area under disease progress curve (AUDPC) was also calculated (table 3). White smoky oozes were observed streaming out of symptomatic plant sections (Plate 1E) Diagnostic test showed characteristic colonies upon culture of the bacterial suspension on growth media (plate 1 F).

Table 3: Reaction of African eggplant accessions to bacterial wilt resistance

Accessions	Severity (1-5)	% DI ± SD	% DI ± SE	AUDPC	Reaction
RV100386	3.4	66.8±1.38	66.8±0.16	472.5	HS
RV100234	3.1	60.4±1.37	60.4±0.16	422.5	HS
RV100201	3	59.4±1.42	59.4±0.17	414.5	HS
RV100245	3	59.4±1.26	59.4±0.15	415	HS
RV100331	3	58.4±1.42	58.4±0.17	404.5	HS
RV100250	2.7	53.7±1.42	53.7±0.17	372	HS
RV100447	2.7	53.6±1.50	53.6±0.18	374.5	HS
RV100161	2.7	53.6±1.26	53.6±0.15	370	HS
RV100247	2.6	52.2±1.26	52.2±0.15	361.5	HS
RV100240	2.6	51.6±1.33	51.6±0.16	358	HS
RV100271	2.4	48.8±1.28	48.8±0.15	334	HS
RV100458	2.5	48.4±1.23	48.4±0.15	331	HS
RV100342	2.4	46.5±1.27	46.5±0.15	327	HS
RV100328	2.3	44.9±1.35	44.9±0.16	316	S
RV100432	2.3	44.6±1.50	44.6±0.18	314.5	S
RV100431	2.2	43.1±1.38	43.1±0.16	298	S
RV100330	2.1	41.5±1.53	41.5±0.18	293.5	S
RV100335	2.2	41.0±1.60	41.0±0.19	311	S
RV100259	1.8	33.1±1.15	33.1±0.14	244.5	S
RV100327	1.8	32.4±1.08	32.4±0,13	230.5	S
RV100169	1.7	32.1±1.23	32.1±0.15	248.5	S
RV100270	1.6	29.5±0.77	29.5±0.10	223.5	S
RV100511	1.5	28.3±0.96	28.3±0.11	215.5	S
RV100217	1.5	26.9±0.86	26.9±0.10	195	S
GBK050572	1.5	26.4±1.16	26.4±0.14	192	S
RV100377	1.3	24.6±0.57	24.6±0.07	191	MS
RV100185	1.3	24.0±0.84	24.0±0.10	178	MS
RV100452	1.4	23.7±0.59	23.7±0.07	177	MS
RV100364	1.5	23.4±0.74	23.4±0.09	187	MS
RV100261	1.3	23.1±0.74	23.1±0.09	162.5	MS
RV100190	1.3	21.4±0.66	21.4±0.08	168	MS
RV100334	1.3	20.9±0.86	20.9±0.10	157	MS
RV100333	1.3	19.7±0.92	19.7±0.11	139	MS
RV100268	1.3	17.3±1.10	17.3±0.13	140.5	MS
RV100352	1.2	16.2±0.57	16.2±0.07	138	MR
RV100263	1.2	15.6±0.98	15.6±0.11	130	MR
RV100218	1.2	15.3±0.46	15.3±0.05	148	MR
RV100360	1.2	15.3±0.36	15.3±0.04	108.5	MR
RV100264	1.2	14.4±0.58	14.4±0.07	112	MR
RV100332	1.1	12.8±0.60	12.8±0.07	118	MR
RV100265	1.1	11.6±0.50	11.6±0.06	95	MR
RV100445	1.1	11.5±0.49	11.5±0.06	97	MR
RV100453	1.1	10.9±0.43	10.9±0.05	112.5	MR
RV100239	1.1	10.8±0.59	10.8±0.07	119.5	MR
RV100438	1.1	8.2±0.59	8.2±0.07	73.5	R
RV100246	1.1	7.7±0.45	7.7±0.05	88	R
RV100242	1.1	6.8±0.90	6.8±0.11	85	R
RV100455	1.1	2.7±0.42	2.7±0.05	38.5	R

R resistant, *MR* moderately resistant, *MS* moderately susceptible, *S* susceptible and *HS* highly susceptible

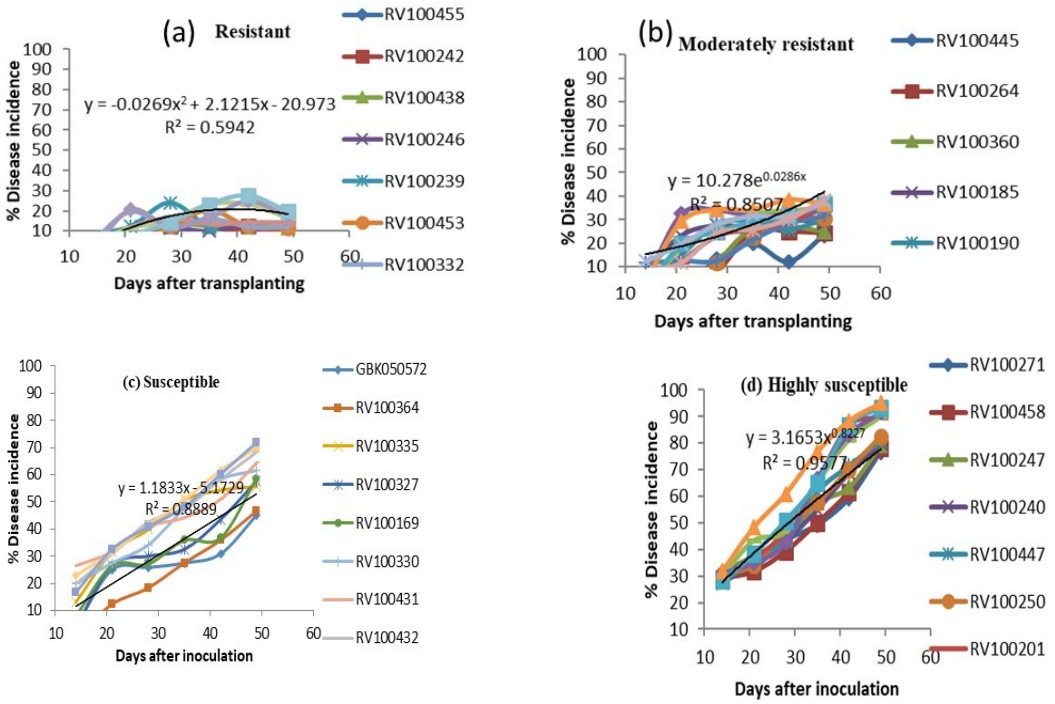

Figure 1: Response of African eggplants to bacterial wilt (a) Resistant, (b) Moderately resistant, (c) Susceptible and (d) highly susceptible

Bacterial Wilt Resistant Eggplant Accessions Using SSR Markers

African eggplant accessions were divided into two populations with GBK (Gene Bank Kenya) as the reference and rest as tested varieties. A total of 15 primers were screened for BW resistance in all the accessions. The genomic DNA was pooled for all the accessions that showed wilting symptoms as well as the asymptomatic plants. Of the 15 total SSR markers, five produced quality bands and were considered for identifying bacterial wilt resistance in African eggplant accessions. Resistant SSR markers were present in 27 out of the 47 tested. A total of 172 alleles were observed in the African eggplant accessions, with 1.56 average Eigen value. Ecm009 carried a total of 56 alleles, while emiO4P17 had the lowest number of alleles in all the amplified genotypes. Not all the primers were present in the same accessions, indicating diversity in eggplants. In some instances, susceptible eggplants were amplified by the resistant markers. The previously reported markers, ecm009 and emiO4P17, were also detected in symptomatic accessions (plate 4 and 5). The effective number of alleles ranged from 1 to 1.63 with an average of 1.1 and > 5% band frequency. The primers ecm009 and emk03O04 were present in 14 accessions, ecm001 and SOL5036 were present in 21 genotypes, and emiO4P17 was present in 16 (data not shown). However, 20 accessions were not amplified. The accessions originating from Mali, *S. aethiopicum* and *S. anguivi* carried resistant markers while closely followed by those from Uganda and Tanzania.

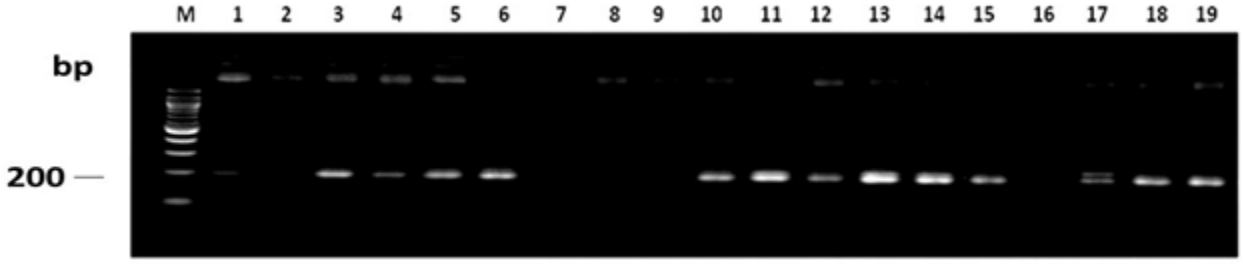

Plate 4: Representation of the DNA band pattern of the eggplant accessions using SSR marker emi04P17. Lane M represents the 100 bp molecular ladder and 1–19 amplified samples.

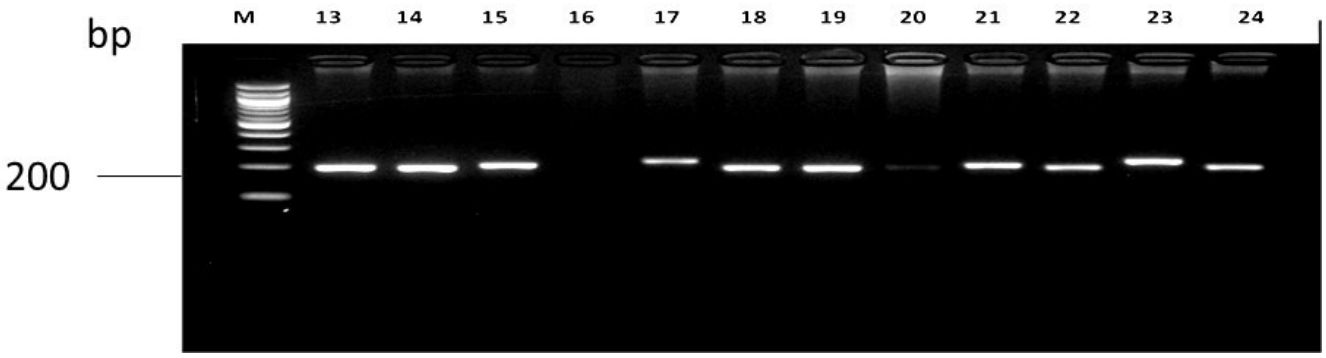

Plate 5: Representation of the DNA band pattern of the eggplant accessions using SSR marker ecm009. Lane M represents the 100 bp molecular ladder and 13–24 amplified samples.

On the other hand, accessions with unknown origins carried the tested markers. RV100453, RV100246, RV100360, RV100242, RV100445, RV100217 and RV100268 were amplified by the entire set of markers and were distinct. Accessions obtained from Mali and Uganda had a high number of resistant markers which is a clear proof that they are resistant. Tanzanian accessions had expressions of three markers (data not shown).

Genetic Diversity of African Eggplants

The SSRs used in this study revealed different levels of polymorphism across all the accessions. The screening of resistant traits in the 47 genotypes using different SSRs revealed varying alleles among the accessions. The reliability of the SSRs in identifying the resistant traits was revealed by the presence of markers in the accessions with bacterial wilt symptoms (results not shown). The genetic variability of each population was studied in terms of the number of different alleles (Na), number of effective alleles (Ne), Shannon's information index (I), expected heterozygosity (He), unbiased expected heterozygosity (uHe) and standard error (SE) (Table 4). The Kenyan population carried two different alleles while the international population carried four. The number of effective alleles was high in the international accessions and least in the Kenyan ones, indicating that a large number of alleles were present in this international population. The overall population's mean expected heterozygosity was 0.162, ranging from 0.277 in ecm009 to 0.387 in SOL5036. The binary (diploid) data showed five bands for the international accessions and one band for the gene bank accessions, and the band frequency was greater than 5%. Four principal coordinates were obtained in the PCoA plot. Generally, the accessions grouped themselves in all the quadrants, indicating genetic variations (Figure 2). A total of five alleles were detected in all the accessions with 100% polymorphism, and the primers showed an average heterozygosity of 0.33.

Table 4: Genetic diversity of Eggplant

Pop	Locus	Band Freq.	p	q	N	Na	Ne	I	He	uHe
Kenya	S27C2/**C3**	0.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000	0.000
	S27C6/**C7**	0.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000	0.000
	S27DA/**DB**	0.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000	0.000
	S27C4/**C5**	1.000	1.000	0.000	1.000	1.000	1.000	0.000	0.000	0.000
	S27D0/**D1**	0.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000	0.000
International	S27C2/**C3**	0.304	0.166	0.834	46.000	2.000	1.383	0.449	0.277	0.280
	S27C6/**C7**	0.304	0.166	0.834	46.000	2.000	1.383	0.449	0.277	0.280
	S27DA/**DB**	0.457	0.263	0.737	46.000	2.000	1.633	0.576	0.387	0.392
	S27C4/**C5**	0.326	0.179	0.821	46.000	2.000	1.416	0.470	0.294	0.297
	S27D0/**D1**	0.457	0.263	0.737	46.000	2.000	1.633	0.576	0.387	0.392

Estimated allele frequencies (p & q), sample sizes (N), no. of alleles (Na), no. of effective alleles (Ne), information index (He) and expected and unbiased expected heterozygosity (uHe).

Discussion

Bacterial wilt disease incidence was evaluated using disease symptoms. It was assumed that all plants produced wilt symptoms, however some may not have shown wilting symptoms in spite of pathogen pressure (Miller, 1953). However, the results showed that none of the accessions was resistant to bacterial wilt. In recent times, SSR markers have been used to characterize genotypes for resistance to plant pathogens (Mutlu, 2008; Lebeau, 2010; Lebeau, 2013; Salgon, 2017). *Ralstonia solanacearum* severity and death varied in between the tested varieties with all reacting to BW indiscriminatively with the highest scoring a disease incidence (DI) of 66.8%. Confirmatory tests for *Ralstonia solanacearum* by streaming out ooze from diseased stem sections and on casein hydrolisate-peptone-glucose (CPG) culture media showed a relatively similar incidence of all wilted samples collected from the field and in the greenhouse. This not only confirmed the causal organism as was reported by Chaudhry and Rashid (2011), Marques *et al*. (2012) and Muradashvil *et al*. (2015), but distinguished the bacterial wilt symptom from the other wilts.

The extent of reaction was dependent on the accession and not origin where the *S. aethiopicum* showed relatively higher levels of severity. The current study also showed that all susceptible accessions showed wilt symptoms 14 -21 days after transplanting (DAT). At this time, majority of the plants had wilted. Aslam (2017), reported that it only took 4 days for susceptible tomato to show wilt symptoms and complete wilting 14 days later. According to Swanson (2007), there was variation in mortality rate in African eggplants due to effect of the BW and none of the accessions was immune (Kumar, 2016; Aslam, 2017), while the delayed effect could be due to root cortical cells and a high level of phenolics content that prevent the entry and continued multiplication of bacterium (Bi-hao, 2009). Similarly, we report a delay in symptom development which was observed 14 days after inoculation with 50% of leaves wilted which could be due to formation of cortical cells in the roots. Also we noted that, the disease incidence during the second season was high (February – June, 2017) compared to season 1 (July – November, 2017. This difference could be due to favorable temperature (22^0C to 27^0C) and Li, (1988) reported such findings. The time difference in wilt symptoms development, could have been due to weather conditions at the time of inoculation, where during the first season fell in the month of July to November which are typically colder than the rest of the year (Kumar, 2016; Li, 1988) and survival of the inoculum after administered into plants would have been affected.

The variation in symptom development between the 2 seasons was in consistent with Kumar (2016) and Miller (1953) in his findings reported that soil borne diseases such as *Ralstonia*

solanacearum cannot survive in low temperatures for long. Our results showed that in some accessions, symptoms were observed at the first days of inoculation/transplanting and later disappeared which could be attributed to development of immunity/ or escape mechanism. Such findings were similar to those reported by Kumar (2016). However, there is a marked genetic advance in the level of resistance of plants to bacterial wilt reported resistant difference in tomato, eggplant and pepper accessions where eggplant displayed broadest resistance though overcame by an aggressive variant of *R. soalancearum* species compared to tomato and pepper (Collonnier, 2001; Mutlu, 2008; Siri, 2009; Lebeau, 2013; Aslam, 2017; Salgon, 2017).

In a study by Mwaniki, (2016), *S. anguivi* was susceptible to the three *Fusarium* wilt species while *S. aethipicum* showed considerable resistance to *Fusarium* wilt tested which is contrary of bacterial wilt on the same species. Sources of genetic resistance to the bacterial wilt of various crop plants tomato, eggplant, and pepper has been previously studied (Bi-hao, 2009; Lebeau, 2011; Lebeau, 2013; Aslam, 2017). The identification of resistant traits in African eggplant accessions using SSR markers is important for marker-assisted breeding and the selection of genotypes that resist plant pathogens (Mutlu, 2008; Siri, 2009).

SSR markers have been used to characterize genotypes for resistance to BW (Lebeau, 2010; Lebeau, 2013). In the present study, 47 accessions were tested for response to bacterial wilt and later 15 SSRs were used to screen the genotypes, and only five showed a considerable level of polymorphism. Of all the used primers, only ecm009 and emiO4P17 have previously been reported as resistant markers for bacterial wilt (Lebeau, 2013). In this study, markers ecm009 and emiO4P17 were present in the accessions from Mali and Uganda. *S. aetiopicum* and *S. anguivi* also carried resistant markers to BW. Some eggplants could collapse during the day and in the evening have recovered from wilt symptoms, which is characteristic in BW infestation. However, some accessions that manifested bacterial wilt symptoms were also proven to carry tested markers; this indicates that the cause of wilting was due to other factors such as high temperatures and not bacterial wilt.

The amplification of the markers was not uniform across all the accessions, which was indicative of accession diversity. Earlier studies compared resistant and susceptible varieties of eggplants, tomatoes and pepper to bacterial wilt, but none of the accessions were reported as resistant to *R. solanacearum* in these studies (Lebeau, 2013; Bainsla, 2016; Salgon, 2017; Dheemanth, 2018). Marker ecm009 (Lebeau, 2013) and emi04P17 (not published) were reported to be associated with bacterial wilt resistance and were present in 10 accessions belonging to different eggplant species. Dheemanth (2018) reported resistant SSR markers in tomato produced polymorphic bands, while the susceptible varieties gave various amplification patterns. The same was in tandem to their findings in all the sets during the study. In a study by Truong (2015), RAPD markers were only associated with resistant parents and amplified the polymorphic fragments alone. With the same markers (Truong, 2015), 92 tomato lines were evaluated for bacterial wilt resistance and none of them, including the asymptomatic line, carried the markers. Siri (2009), reported the presence of resistant markers in *Solanum commersonii* originating from different locations across Uruguay. Similarly, we found markers of resistance to bacterial wilt in accessions from Mali, Uganda and Tanzania. This indicates that there is instability in the resistant to bacterial wilt as it was reported by earlier researchers (Hayward, 1991).

Conclusion

This study showed that use of molecular markers to classify individual plants as resistant is more elaborate than use of phenotypic reactions. Farmers are advised to use the identified resistant eggplants to maximize on the production. Use of

resistant eggplant accessions is in the long run the best way in the management of BW.

Recommendations

The SSRs identified in this study can be utilized for marker-assisted selection of eggplants resistant to bacterial wilt. To increase the breeding utility of eggplants, additional efforts are required to identify allele-specific markers and validate the reported markers which can be used across breeding populations.

Acknowledgement

The support for this research work was provided by U.S. Agency for International Development through the Partnerships for Enhanced Engagement in Research (PEER) program Sub-Grant Number: PGA-2000003426 to W.O. The authors would like to thank the AVRDC- World Vegetable Center in Arusha, Tanzania for the provision of the African eggplant seeds.

References

Álvarez, B., Biosca, E. G. and López, M.M. 2010. On the life of *Ralstonia solanacearum*, a destructive bacterial plant pathogen. *Formatex* 267-279.

Aslam, M. N., Mukhtar, T., Hussain M. A., and Raheel, M. 2017. Assessment of Resistance to Bacterial Wilt incited by *Ralstonia solanacearum* in Tomato Germplasm. *Journal of Plant Diseases Protection* 585-590.

Bainsla, K. N., Singh, S., Singh, P. K., Kumar, K., Singh, K. A., and Gautam, R. K. 2016. Genetic behaiour of Bacterial wilt Resistance in Brinjal (*Solanum melongena* L.) in Tropics of Andaman and Nicobar Islands of India. *American Journal of Plant Science* 333-338.

Bi-hao, C. Jian-jun, L., Yong, W. and Guo-ju, C. 2009. Inheritance and identification of SCAR marker linked to bacterial wilt-resistance in eggplant. *African Journal of Biotechnology* **8** (20): 5201-5207.

Chaudhry, Z. and Rashid, H. 2011. Isolation and characterization of *Ralstonia solanacearum* from infected tomato plants of soan skesar valley of punjab. Pak. J. Bot. 2979 2085.

Collonnier, C. Mulya, K. Fock, I. Mariska, I. Servaes, A. Vedel, F. Siljak-Yakovlev, S. Souvannavong, V. Ducreux, G. and Sihachakr, D. 2001. Source of resistance against *Ralstonia solanacearum* in fertile somatic hybrids of eggplant (*Solanum melongena* L.) with *Solanum aethiopicum* L. *Plant Science* **160**: 301-313.

Dheemanth, T. L., Nazeem, P. A., Kumar, S. G. P., Mathew, K. S. and Reddy, A. M. 2018. Validation of SSR Markers for Imparting Disease Resistance in Tomato (*Solanum lycopersicum* L.). *International Journal of Current Microbiology Applied Science* 1513-1522.

Doyle, J. J. and Doyle, J. L. 1987. A rpid DNA isolation procedure for small quantities of fresh leaf tissues. Phytochemical Bulletin 11-15.

Engelbrecht, M. C. and Hattingh, M. J. 1989. Numerical analysis of phenotypic features of *Pseudomonas solanacearum* strains isolated from tobacco and other hosts from South Africa. *Plant disease* 73: 893-898.

FAO. 2019. FAOSTAT. Accessed 04 30, 2020. Fao.org/faostat/en/#data/QC.

Gildermacher, P. R., Demo, P., Barker, I., Kanguogo, W., Woldegiorgis, G., Wagoire, W. W., Wakahiu, M., Leeuwis, C. and Struik, P. C. 2009. A Distribution of Potato Seed Systems in Kenya, Uganda, and Ethiopia. *A. J. Pot Res* 373-382.

Gopalakrishnan, C., Singh, T.H. and Rashmi B. A. 2014. Evaluation of eggplant accessions for resistance to bacterial wilt caused by *Ralstonia solanacearum* (E.F. Smith) Yabuuchi et al. *Journal of Horticultural Science* **9** (2): 202-205.

Ha, Y., Kim, J.-S., Denny, T. P., and Schell, M. A. 2012. A rapid, sensitive assay for *Ralstonia solanacearum* race 3 biovar 2 in plant and soil samples using magnetic beads and real-time PCR. *American Phytopathological Society* **96** (2): 258-264.

Hasabi, V. 2015. Study on the Response of some *Solanaceous* Plants to *Ralstonia solanacearum* Biovars 2A and 2T. *Turkish Journal of*

Agriculture - Food Science and Technology, **3**(2): 80-83

Hayward, A. C. 1991. Biology and epidemiology of Bacterial wilt caused by *Pseudomonas solanacearum*. *Annual Reviews Phytopathology* 29: 65 - 87.

Huet, G. 2014. Breeding for resistance to *Ralstonia solanacearum*. *Frontiers in Plant Science* **5**(715): 1 - 5.

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

Lebeau, A., Daunay, M.-C., Frary, A., Palloix, A., Wang, J.-F., Dintinger, J., Chiroleu, F.,Wicker, E., and Prior, P. 2011. Bacterial Wilt Resistance in Tomato, Pepper and Eggplant: Genetic Resources Response to Diverse Strains in *Ralstonia Solanacearum* Species Complex. *American Phytopathology Society* 154-165.

Lebeau, A., Gouy, M., Daunay, M. C., Wicker, E., Chiroleu, F., Prior, P., Frary, A. and Dintinger, J. 2013. Genetic mapping of a major dominant gene for resistance to *Ralstonia solanacearum* in eggplant. *Theor Appl Genet* 126: 143-158.

Li, P. H., Goth, R. W. and Barksdale T. H. 1988. Evaluation of resistance of bacterial wilt in eggplant. *Plant Disease* 72: 437-439.

21. Marques, E., Uesugi, C.H., Ferreira, M. S. A. V., and De Rezende, D.V. 2012. Characterization of isolates of *Ralstonia solanacearum* biovar 2, pathogenic to *Eucalyptus* "urograndis" hybrids. *Tropical Plant Pathology* 399 408.

Meng, F. 2013. *Ralstonia Solanacearum* Species Complex and Bacterial Wilt Disease. *Journal of Bacteriol Parasitol* 4 (2).

Mohan, M. Nair, S. Bhagwat, A. Krishna, T.G. Yano, M. Bhatia, C.R. and Sasaki, T. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding* 3: 87-103.

Muradashvili, M. T., Meparishvili, G. V., Tediashvili, M.I. and Sikharulidze, Z. V. 2015. Phenotypic properties of Georgian isolates of *Ralstonia solanacearum*. *Annals of Agrarian Science* **13**(3): 20-25.

Muthoni, J., Shimelis, H. and Melis, R. 2012. Management of Bacterial Wilt [*Ralstonia solanacearum* Yabuuchi et al., 1995] of Potatoes: Opportunity for Host Resistance in Kenya. *Journal of Agricultural Science* **4**(9): 64-78.

Mutlu, N., Boyaci, F. H., Gocmen, M. and Abak, K. 2008. Development of SRAP, SRAP-RGA, RAPD and SCAR markers linked with a Fusarium resistance gene in eggplant. *Theor Appl Genet* 117: 1303-1312.

Mwaniki, K. P., Abang, M. M., Wagara, N. I., Wolukau, N. J., and Hans-Josef, S. 2016. Response of African eggplant to *Fusarium* spp. and identification of sources of Resistance. *African Journal of Biotechnology* 394-400.

Mwaniki, P. K., Birech, R., Wagara, I. N., Kinyua, Z. M., Schulte-Geldermann, E. E. and Freyer, Bernhard. 2016. Distribution, Prevalence and Incidence of Potato Bacterial wilt in Nakuru County, Kenya. *International Journal of Innovative Research and Development* 435 - 442.

Norman, D. J., Zapata, M., Gabriel, D. W., Duan, Y. P., Yuen, J. M. F., Mangravita-Novo, A., and Donahoo, R. S. 2009. Genetic diversity and host range variation of *Ralstonia solanacearum* strains entering North America. *Phytopathology* 99: 1070-1077.

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

Perez, A. S., Mejia, L., Fegan, M. and Allen, C. 2008. Diversity and distribution of *Ralstonia solanacearum* strains in Guatemala and rare occurrence of tomato fruit infection. *Plant Pathology* 57: 320-331.

Reddy, A. C., Venkat, S., Aswath, C., Reddy, D. C. L., Singh, T.H., and Reddy, K.M. 2015. Isolation, characterization and evolution of NBS-LRR encoding disease-resistance gene

analogs in eggplant against bacterial wilt. Eur *J Plant Pathol* 143: 417-426.

Salgon S, Jourda C, Sauvage C, Daunay M-C, Reynaud B, Wicker E and Dintinger. 2017. "Eggplant Resistance to the *Ralstonia solanacearum* Species Complex Involves Both Broad-Spectrum and Strain-Specific Quantitative Trait Loci. *Fron. Frontiers in Plant science* 1-21.

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

Sunseri, F., Polignano, G.B., Alba, V., Lottj, C., Bisignano, V., Mennella, G., Alessandro, A., Bacchi, M., Riccardi, P., Fiore, M. C., and Ricciardi, L. 2010. Genetic diversity and characterization of African eggplant germplasm collection. *African Journal of Plant Science* 4(7): 231-241.

Swanson, J. K., Montes, L., Mejia, L., and Allen, C. 2007. Detection of Latent Infections of *Ralstonia solanacearum* Race 3 Biovar 2 in Geranium. *The American Phytopathological Society-Plant Disease* 828-834.

Tahat, M. M. and Sijam, K. 2010. *Ralstonia solanacearum*: The bacterial wilt causal agent. *Asian Journal of Plant Sciences* **9**(7): 385-393.

Truong, H. T. H., Kim, S., Tran, H. N., Nguyen, T. T. T., Nguyen, L. T. and Huang, T. K. 2015. Development of a SCAR Marker Linked to Bacterial Wilt (*Ralstonia solanacearum*) Resistance in Tomato Line Hawaii 7996 Using Bulked-Segregant Analysis. *Horticulture and Environmental Biotechenology* 506-515.

Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D., Guilbaud, C., Fegan, M., and Prior, P. 2007. *Ralstonia solanacearum* strains from Martinique (West French Indies) Exhibiting a new Pathogenic Potential. *Applied and Environmental Microbiology* 6790-6801.

Wickera, E., Grassart, L., Coranson-Beaudua, R., Mian, D. and Prior, P. 2009. Epidemiological evidence for the emergence of a new pathogenic variant of *Ralstonia solanacearumin* Martinique (French West Indies). *Plant Pathology* 58: 853-861.

Xue, Q., Yin, Y., Yang, W., Heuer, H., Prior, P., Guo, J. & Smalla, K. 2011. Genetic diversity of *Ralstonia solanacearum* strains from China assessed by PCR-based on fingerprints to unravel host plant- and site-dependent distribution patterns. *Federation of European Microbiological Societies* 75: 507-519.

Yadessa, G. B., Van Bruggen, A.H.C. and ocha F.L. 2010. Effects of different soil Amendments on Bacterial Wilt caused by *Ralstonia solanacearum* and on the yield of Tomatoes. *Journal of Plant Pathology* 92 (2): 439-450.

Yuliar, Nion, Y. A. and Toyota, K. 2015. Recent Trends in Control Methods for Bacterial Wilt Diseases Caused by *Ralstonia solanacearum*. *Microbes and Environments* 30 (1): 1-11.

Blank Page