African Crop Science Journal, Vol. 32, No. 1, pp. 29 - 50
pISSN 1021-9730/2024
eISSN 2072-6589/2024

DOI: https://dx.doi.org/10.4314/acsj.v32i1.3

GENETIC DIVERSITY AND HERITABILITY OF TOMATO PARENTAL LINES ASSEMBLED FOR *Ralstonia solanacearum* RESISTANCE

G. MUKAMANASASIRA[1,2], E.A. ADJEI[1,2,4], P. RUBAIHAYO[1], G. DDAMULIRA[3] and R. EDEMA[1,2]

[1]Department of Agricultural Production, College of Agricultural and Environmental Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda
[2]Makerere University Regional Centre for Crop Improvement, Makerere University, P. O. Box 7062, Kampala, Uganda
[3]National Crops Resources Research Institute, P. O. Box 7084, Kampala, Uganda
[4]Council for Scientific and Industrial Research - Savanna Agricultural Research Institute, P. O. Box TL 52 Tamale, Ghana
Corresponding author: godman.mukamanasasira@santannapisa.it

(*Received 3 July 2023; accepted 20 February 2024*)

ABSTRACT

Bacterial wilt is a disease caused by *Ralstonia solanacearum*, which affects over 450 plant species and causes significant reduction in crop yields including of tomato (*Solanum lycopersicum*) worldwide. Developing and identifying tomato genotypes with *Ralstonia solanacearum* tolerance and high yield potential, presents an opportunity for improvement of crop productivity. The objective of this study was to explore the heritability of tomato resistance against bacterial wilt and the genetic variation within the population for breeding purposes. A breeding population was created by crossing two bacterial wilt-resistant (MT56 and BL333) and three commercially desirable susceptible (Assila, Rambo and Heinz) tomato varieties, using North Carolina Design II, in a screen house at the Makerere University Agricultural Research Institute, Kabanyolo (MUARIK). Results showed that the Area Under Disease Progress Curve was significant ($P<0.001$), indicating that cumulative disease progress was less in resistant genotypes of segregating generations. Disease severity increased with days after inoculation (DAI), with Heinz showing the highest level of susceptibility. The General Combining Ability for male parent (GCA_m) was significant ($P< 0.01$), and Specific Combining Ability (SCA_{fxm}) and GCA_f were significant ($P<0.01$) for the F_2 generation. Broad-sense heritability was higher than the narrow-sense heritability in both F_1 and F_2 generations, suggesting that non-additive gene action predominately controlled tomato resistance to bacterial wilt infestation. The genetic diversity ranged from 0.5 to 0.6759, with a mean value of 0.5787. Polymorphism Information Content (PIC) varied from 0.375 to 0.6357, with a mean of 0.4888, indicating a high degree of variation. SLM 12-2 was the most polymorphic marker, with a PIC of 0.6357. The Unweighted Pair-Group Method with Arithmetic Average (UPGMA) classified all tomato genotypes into six clusters, namely Clusters 1 and 2 (Susceptible parents), Cluster 3 (Resistant parents), Clusters 4 and 5 (New source of resistance), and Cluster 6 (F_1P1×P5).

Key Words: General combining ability, *Solanum lycopersicum,* SSR marker

RÉSUMÉ

Le flétrissement bactérien est une maladie causée par *Ralstonia solanacearum*, qui affecte plus de 450 espèces de plantes et entraîne une réduction significative des productions de cultures telles que la tomate (*Solanum lycopersicum*) dans le monde. Le développement et l'identification de génotypes de tomates présentant une tolérance au *Ralstonia solanacearum* et un potentiel de rendement élevé présentent une opportunité d'amélioration de la productivité des cultures. L'objectif de cette étude était d'explorer l'héritabilité de la résistance de la tomate au flétrissement bactérien et la variation génétique au sein de la population à des fins de sélection. Une population reproductrice a été créée en croisant deux variétés de tomates résistantes au flétrissement bactérien (MT56 et BL333) et trois variétés de tomates sensibles commercialement souhaitables (Assila, Rambo et Heinz) en utilisant North Carolina Design II, dans une serre de l'institut de recherche agricole de Makerere University, Kabanyolo. L'étude a révélé que la courbe de progression de la zone sous maladie était significative (P <0,001), ce qui indique que la progression cumulative de la maladie était moindre chez les génotypes résistants des générations en ségrégation. La gravité de la maladie augmentait avec les jours suivant l'inoculation (DAI), Heinz présentant le niveau de susceptibilité le plus élevé. La capacité générale de combinaison du parent mâle (GCAm) était significative (P <0,01) et la capacité spécifique de combinaison (SCAf×m) et GCAf étaient significatives (P<0,01) pour la génération F2. L'héritabilité au sens large était supérieure à l'héritabilité au sens étroit dans les générations F1 et F2, ce qui suggère que l'action non additive des gènes contrôlait de manière prédominante la résistance des tomates à l'infestation par le flétrissement bactérien. La diversité génétique variait de 0,5 à 0,6759, avec une valeur moyenne de 0,5787. Le contenu des informations sur le polymorphisme (PIC) variait de 0,375 à 0,6357, avec une moyenne de 0,4888, indiquant un degré élevé de variation. SLM 12-2 était le marqueur le plus polymorphe, avec un PIC de 0,6357. La méthode non pondérée de groupes de paires avec moyenne arithmétique (UPGMA) a classé tous les génotypes de tomates en six groupes, à savoir les groupes 1 et 2 (parents sensibles), le groupe 3 (parents résistants), les groupes 4 et 5 (nouvelle source de résistance) et le groupe 6 (F_1P1×P5).

*Mots Clé*s : Capacité générale de combinaison, *Solanum lycopersicum*, marqueur SSR

INTRODUCTION

Bacterial wilt disease, caused by a soil-borne plant pathogen, *Ralstonia solanacearum*, is a severe disease, especially on tomato (*Solanum lycopersicum* L.*)* in tropical and subtropical areas. The pathogen obstructs plant tissues, arteries or ducts (Bradbury, 1986); yet tomato is one of the world's most popular fruit vegetables (Kumar *et al*., 2020). Muhammad (2017) concluded that in endemic tropical and subtropical areas across the world, the disease causes up to 90.6% yield losses. The fruit can be consumed fresh, boiled, or processed into a paste or powder and canned (Zohoungbogbo *et al*., 2021).

Ralstonia solanacearum has acquired prominence as a result of its destructive behavior, wide host range, and geographical spread (Vanitha *et al*., 2009). The bacterial pathogen infects around 400 plant species, mostly in the *solanaceae* family (Choi *et al*., 2020); and the pathogen is a soil-borne, aerobic, non-sporulating, gram-negative bacteria that colonises the xylem and causes wilt in many host plants (Bradbury, 1986).

Ralstonia solanacearum is also exceedingly diverse, having been classified into five races based on host ranges and six biovars based on the capacity to metabolise sugar, alcohols and disaccharides, with at least five pathogenic races and biovars faced discrimination (Denny, 2006). Fegan and Prior (2005) proposed a new hierarchical classification of *R. solanacearum* into four phylotypes, according to their geographical origins; namely I (Asia), II

(America), III (Africa and the Indian Ocean), and IV (Indonesia, Australia, and Japan) based on analyses of sequence data derived from the internal transcribed spacer (ITS) region between 16S and 23S.

Ralstonia solanacearum can infect crops as a soil-borne, water-borne, or seed/tuber-borne organism, making it challenging to implement integrated management strategies (Huet, 2014). Since *R. solanacearum* persists in the soil for many years, with a vast host range and being xylem-borne, it is challenging and costly to manage using antagonistic and cultural practices (Garæia *et al*., 2019).

Although resistance to bacterial wilt in tomatoes is a quantitative trait, as highlighted by Wang *et al*. (2000), complexity arises when considering the genetic diversity among tomato varieties. In the absence of a thorough understanding of this genetic diversity and the heritability of resistance within different tomato genotypes, the task of breeding for genetic resistance in tomato cultivars becomes inherently intricate. Addressing this complexity by unravelling the genetic intricacies of resistance would undoubtedly enhance the efficacy of breeding efforts.

Genetic diversity is the variation in a population's heritable traits, and evolution, mutation, migration, domestication, plant breeding and selection are some of the processes that lead to genetic variation (Osawaru *et al*., 2015). Knowledge about genetic diversity and relationships among plants are an invaluable aid in plant breeding and classification (Osawaru *et al*., 2015). Genetic diversity in quantitative traits can be divided into many parts based on genes' additive dominance, and interaction effects. Majority of the link between relatives and the possibility for genetic change through natural or artificial selection by the additive genetic variance is the most significant (Mathai *et al*., 2022).

Genes may possess an additive effect on a trait's quantitative characteristics, known as additive genetic variance (Mathai *et al*., 2022). As a result of a specific allele being inherited and its relative impact on the phenotype, there is a phenotypic deviation from the mean. This measures how well the additive effects of allelic changes can predict the variations in individual phenotypes. The non-additive gene effect occurs when the influence of a recessive allele at a specific locus is masked by a dominant gene (Huang and Mackay, 2016).

Establishing a successful breeding programme is aided by knowledge of combining abilities. Combining ability analysis is crucial for making it easier to select acceptable parents for hybridisation (Suvi *et al*., 2021). However, combining ability analyses and genetic predictions may depend on the test populations and the environment (Suvi *et al*., 2021).

More substantial studies on combining abilities have been conducted on other diseases of tomatoes (Mathai *et al*., 2022), but not on bacterial wilt. A case in point is a half-diallel mating design used to identify three tomato lines as prospective donors for resistance to tomato yellow leaf curl virus disease (Mathai *et al*., 2022). While breeding for resistance to the rice yellow mottle virus disease, parental lines with negative general combining ability (GCA) values and families with negative specific combing ability (SCA) values; were chosen to harness specific alleles or traits that may not be apparent in the individual average performance (Suvi *et al*., 2021). Using the North Carolina II mating design in parental hybridisation, breeding populations examining the heritability of important characteristics in diverse crops have been produced by plant breeders (Mathai *et al*., 2022). The design makes it possible to estimate the General and Specific Combining abilities (Fasahat *et al*., 2016). GCA is the average performance of a genotype in a series of hybrid pairings. Simultaneously, SCA is defined as when certain hybrid combinations outperform or underperform their inbred parental lines on average (Fasahat *et al*., 2016). The method of

action of a gene may be deduced from observations of how different cross patterns function using SCA. The significant SCA effects observed in crossings where both parents are competent, general combiners may explain additive gene activity (Temesgen, 2021).

Despite studies showing the importance of both GCA and SCA in critical traits of a variety of crops, such as quality traits, disease resistance and yield, there is limited data on the determination of GCA and SCA from crosses between commercially cultivated tomato varieties susceptible to bacterial wilt and resistant tomato genotypes that are not farmer-preferred (Mathai *et al*., 2022). The objective of this study was to explore the heritability of tomato resistance against bacterial wilt and the genetic variation within the population for breeding purposes.

MATERIALS AND METHODS

Study site and materials. This study was carried out at the Biotechnology Laboratory and Screen house at the Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) in Uganda. Five parents were used in this study, comprising of two resistant genotypes (MT 56 and BL333) and three susceptible varieties (Assila, Rambo and Heinz) (Table 1).

Experimental procedures. A five-parent NCD2 mating scheme was established in a screen house to produce 6 F_1 families. All these six populations were advanced to F_2 by selfing. For crossing, each parent was planted in two buckets using soil that had been sterilised and fertilised.

Planting was staggered to allow for synchronisation of flowering; while crossing was done by manual pollination of hand-emasculated flowers. Seedlings were raised in sterilised soil in a tray, up to the hardening stage and when they were transplanted to the pots of size 5-litre. They were spaced at 0.5 m within rows and 1 m between blocks; in an RCBD, replicated three times. The experiment was repeated twice.

Seedlings were mechanically inoculated with bacterial wilt, according to Singh *et al.* (2018). Dithane M-45 (mancozeb 80%WP), a fungicide, was used as a blanket treatment to protect the plants against late blight (*P. infestans*) and other fungal diseases. Dimethoate (4EC) was also sprayed as a blanket treatment to manage insect pests on plants. The experiment was conducted under screen house conditions and was replicated thrice.

Phenotyping of tomato genotypes. The five parents, F_1 and F_2 populations were planted in a screen-house in plastic buckets, with one plant in each bucket containing *R. solanacearum* inoculum. Each replication/ block consisted of one row of each of the resistant (AVTO1219) and resistant (AVTO9802) checks; for a total of 7 rows per

TABLE 1. Tomato parents used in the North Carolina Design 2 mating scheme to produce F_1s

Varieties	Genealogy	Reaction to BW	Origin
MT56	Multiline	R	Wooster Breeding Program, Ohio, USA
BL333	Multiline	R	Netherlands
Assila	Commercial	S	AVRDC
Rambo	Commercial	S	AVRDC
Heinz	Commercial	S	AVRDC

BW = Bacterial Wilt, R = Resistant to bacterial wilt, S = susceptible to bacterial wilt, AVRDC = Asian Vegetable Research and Development Centre

block. Each of the seven rows contained 20 plants of P1, P2, and F_1 generations and 30 plants of the F_2 population per replication.

Populations were grouped according to parental combinations, forming 6 unique groups for 20 plastic buckets per row per replication. The groupings were done to ensure that treatments belonging to the same group (e.g., F_1, F_2, sharing the same parents) were planted in homogeneous blocks/trays to be compared with a high degree of precision (minimising tray-to-tray variability).

The parental genotypes and F_1 and F_2 populations were phenotyped after artificial inoculation with *R. solanacearum*, using the soil drench method. Phenotypic data were collected on bacterial wilt intensity, at 3-day intervals for 30 days, after inoculation (Table 2), using the disease scale suggested by Hussain *et al*. (2005). The treatments were laid out in a randomised complete block design, with three replications (Gomez, 1984).

Sample collection. *Ralstonia solanacearum* diseased plants were used to collect samples in fields. The presence of the pathogen was determined by putting longitudinal sections of the collar area containing vascular tissues, from sick plants in a test tube containing clean water (Yabuuchi *et al*., 1995). Fine milky threads made of large amounts of bacteria could be seen in the affected tissues, which soon began to ooze out from the sliced portion's edge.

Bacterial strains preparations. Three samples were isolated from bacterial wilt-affected tomato plants, collected from three different areas on the MUARIK farm, during the rainy 2019 season (September to November). Stem pieces of (8-10 cm long), of wilted tomato plants were collected from each field; washed thoroughly using 70% ethanol, air-dried and delivered to the Biotechnology laboratory for further investigations. The samples were surface disinfected with 70% ethanol, peeled, subsampled and macerated in sterile distilled water. Macerates were streaked on Kelman's triphenyl tetrazolium chloride (TZC) agar medium (Peptone, 10 g; glucose, 5 g; Casamino acid, 1 g; agar, 17 g; TZC, 50 mg L^{-1}; at pH = 6.5) (Kelman, 1954). Plates were incubated at 28 °C for up to 72 hr.

Bacterial colonies developing typical irregular mucoid colonies, were again streaked onto fresh TZC medium for further purification (Fig. 1).

DNA extraction. The bacterial cultures were grown on nutrient broth and incubated at 28±1 °C at 200 rpm for 48 hr. The bacterial cells were harvested as a pellet, in a tube, by centrifugation for 3 minutes at 13,000 rpm, and DNA extracted using the CTAB method (Kumar *et al*., 2019).

TABLE 2. Disease rating scale used to phenotype parental, F_1 and F_2 generations

Severity score	Disease reaction	Range
1	Highly resistant (HR)	Zero leaves wilted
2	Resistant (R)	1- 20% of leaves wilted
3	Moderately resistant (MR)	21- 40% of leaves wilted
4	Moderately susceptible (MS)	41 – 60% of leaves wilted
5	Susceptible (S)	61 – 80% of leaves wilted
6	Highly susceptible (HS)	More than 80% of leaves wilted, and plant dead

Source: Hussain *et al*. (2005)

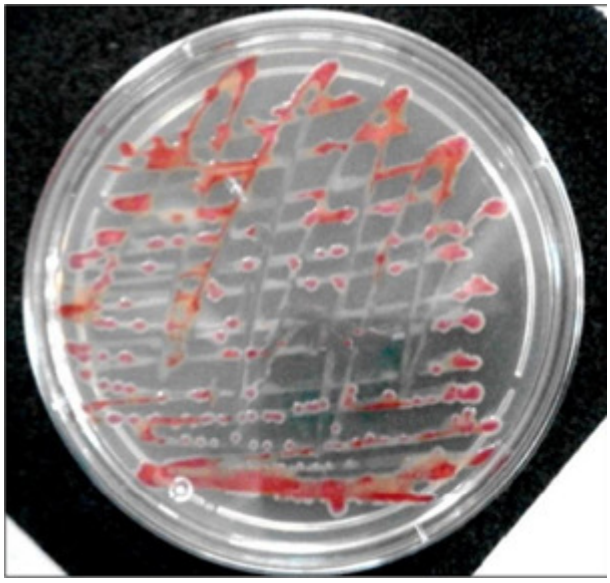

Figure 1. *Ralstonia solanacearum* virulent colonies on triphenyl tetrazolium chloride (TZC) media.

Phylotype analysis. The phylotype identification of the strain was conducted according to the protocol outlined by Fegan and Prior (2005). Subsequently, phylotype-specific multiplex PCR (Pmx-PCR) was performed in a 20 µl final volume reaction mixture. This mixture comprised of 1 µl of template DNA, 2 µl of 2 mM dNTP, 0.5 µM of specific primers (Nmult: 22: RR, Nmult: 21: 1F, Nmult:21:2F, Nmult:22:InF, Nmult:23: AF) along with 0.5 µM of primers 759 and 760 (Opina *et al*., 1997), 2 µl of 10x PCR buffer, 1 unit of Taq polymerase, and autoclaved double distilled water to reach the 20 µl volume. The PCR program consisted of an initial denaturation at 96 °C for 5 minutes, followed by 35 cycles of 94 °C for 5 sec, 59 °C for 30 sec, and synthesis at 72 °C for 30 sec (Kumar *et al*., 2019). Finally, the amplification was completed with a final extension step, allowing the reaction to proceed for 10 minutes at 72 °C in a thermocycler (EP Gradient S, Eppendorf).

The PCR product was resolved in 1% agarose gel to analyse the amplification. This Pmx-PCR amplifies the 280-bp "universal" *R. solanacearum* specific reference band, plus the following phylotype-specific PCR products, such as a 144-bp amplicon from phylotype I strain; a 372- bp amplicon from phylotype II strains, a 91-bp amplicon from phylotype III strains; and a 213-bp amplicon from phylotype IV strains (Kumar *et al*., 2019).

Isolation and inoculation of *R. solanacearum*. The bacterial wilt-confirmed that the tissue was used for isolation. The outer parts of the infected material were removed with a sterilised scalpel, and the small pieces were placed in sterilised distilled water for 15 minutes (Chaudhry and Rashid, 2011). After being dipped in the ooze, the inoculation loop was streaked on TZC media. For one litre of distilled water, the TZC medium contained 1 g of casein hydrolysate, 5 g of dextrose, 17 g of agar, and 5 ml of 1% TZC. Thereafter, the streaked plates underwent a 36-hour incubation period at 31±1 °C.

Virulent colonies were separated, and suspended in sterile distilled water in screw-capped vials. Thereafter, they were then kept at room temperature (Kumar *et al*., 2019). The *R. solanacearum* culture (OD_{600}= 0.3) was then inoculated to the seedlings, using the soil

drench method, a day after transplanting (Xian-Qui *et al*., 2006). By this method, plant roots were wounded by making a 5 cm deep cut on either side of the plant's main stem to create a trench, and then 10 ml of 10^8 cfu ml^{-1} inoculum was poured into the wounded root zone (Kumar *et al*., 2019).

DNA isolation. Total genomic DNA was collected from young trifoliate leaves, two weeks after transplanting; following the cetyl trimethyl ammonium bromide (CTAB) method of Maughan *et al*. (1995). DNA quality and concentration were determined using a NanoDrop ND-1000 spectrophotometer. For amplification use in PCR analysis, the final concentration was adjusted to 50 ng μl^{-1}, as described by Bisen *et al*. (2015) and then stored at -20 °C.

Simple Sequence Repeats (SSR) analysis. A total of 14 SSR markers were selected for initial screening (Table 3). For PCR, a 20 µl total volume containing 2 µl of genomic DNA (50 ng μl^{-1}), 10 µl of liquid premix, 7 µl of distilled water, and 0.5 µl each of forwarding and reverse primers (10 nmol), was prepared following the protocal of Bioneer, Inc, Republic of Korea. Six random tomato DNA samples were used in gradient PCR for each primer, to standardise the annealing temperature for the final amplification. Fourteen SSR primers showed good amplification and were used for further study.

The amplification process was carried out in a thermocycler (G Storm, UK), with the following conditions: Initial denaturation took place at 95 °C for 5 minutes, then there were 35 cycles of 95 °C for 2 minutes, annealing temperature at 53 to 58 °C for 30 seconds, extension at 72 °C for 40 seconds, and final extension at 72 °C for 5 minutes (Clever 2019).

Gel electrophoresis was used to separate the PCR products on 2% metaphor agarose gel (Lonza Bioscience, Singapore), stained with ethidium bromide stain (10 µl /100 µl of 1X TAE buffer) with a constant supply of 100

TABLE 3. SSR primers used for genotyping 19 tomato genotypes

Sl. No.	Primer name	Marker type	Forward sequence (5'-3')	Reverse sequence (5'-3')	Annealing temperature (°C)
1	SLM12-2	SCAR	ATCTCATTCAACGCACACCA	AACGGTGGAAACTATTGAAA GG	57
2	SCU176-534	SCAR	TTGAACCAAGAATCTATTCG	GAACTTGAATGCCTACCAAA	53
3	SCU176-1190F2R2	SCAR	TCACTCGGTGAGTCAATAGAT	TTTGCCGATGTTATCATGT	53
4	SCU176-1190F1R1	SCAR	TGCGGATACTATCGGAAATA	CAACTCATTTCAGTCCGATT	53
5	SLM12-10	SCAR	ACCGCCCTAGCCATAAAGAC	TGCGTCGAAAATAGTTGCAT	55
6	TSCARAAG/CAT	SCAR	AGAAGGTCACGGCGAGA	TGAGTCCTGAGTAACTGG	53
7	TSCARAAT/CGA	SCAR	TAGATGGAATCCAATATCAGG	AACCACAGTGAAGGAATATACA	53
8	TG564	SSR	TGAGGTGCAAATGGGGTAGTG	GCAATGAAGGCCTACAGATGAC	56
9	TG230	SSR	TTGCAGAAGCAACCCTTGAC	TACTTCTCCCCATTCCATGC	55
10	LEaat002	SSR	GCGAAGAAGATGAGTCTAGA GCATAG	CTCTCTCCCATG AGTTCT CCTCTTC	58
11	LEat006	SSR	CATAATCACAAGCTTCTTTCGCC A	CATATCCGCTCGTTTCGTTA TGTAAT	59
12	SSR 20	SSR	GAGGACGACAACAACAACGA	GACATGCCACTTAGATCCACCA	58
13	SSR128	SSR	GGTCCAGTTCAATCAACCGA	TGAAGTCGTCTCATGGTTCG	55
14	SSR306	SSR	ACATGAGCCCAATGAACCTC	AACCATTCCGCACGTACATA	57

volts for 1 hour with a size standard of 100 bp for the ladder (Clever, 2019). Using a Bio-Doc-ItTM Imaging System, gel pictures were captured (Biotium, USA).

Data collection and analysis

Disease severity. Bacterial Wilt disease severity (DS) was recorded on individual plant basis, taken as the number of days from transplanting to complete wilting date; to assess the degree of resistance. Mean severity scores were calculated using the disease score rating obtained from the plants at three days intervals in Microsoft Excel. The means obtained were then used to generate the AUDPC value using the formula suggested by Campbell and Laurence (1991), *viz*:

$$Y = \sum_{i=1}^{n} [(X_i + X_{i+1})/2](t_{i+1} - t_i)$$

..... Equation 1

Where:

Y is the AUDPC; X_i is the disease severity of the i^{th} evaluation; X_{i+1} is the disease severity of the $i + 1^{st}$ evaluation; and $t_{i+1} - t_i$ is the number of days between two evaluations.

Combining ability. Combining ability and gene action analyses were conducted using a randomised complete block design with three replications, encompassing parents and crosses. To estimate error variance, Dabholkar's method (1992) was employed. The resulting error variance was converted to an entry mean basis and utilised to calculate variance components. Significance tests were performed for general combining ability (GCA) in both male and female parents, specific combining ability (SCA), and the effects of GCA and SCA among the F_2 generation in response to bacterial wilt. The mathematical Linear Model for analysis of combining ability in NCD-11 crosses was adopted from (Singh and Chaudhary, 1985).

$Y = U + f_j + m_k + (fxm)_{jk} + e_{ijk}$ ..... Equation 2

Where:

Y_{ijk} = effects observed due to, r^{th} replications; j^{th} = female; k^{th} = male; U = Overall mean of the experiment; r_i = Observed effects due to i^{th} replication; f_j = GCA effects due to the j^{th} female parent; m_k = GCA effects due to the k^{th} male parent; $(fxm)_{jk}$ = Interaction between k^{th} and j^{th}; and e_{ijk} = Random error of the experiments.

The GCA effect, which reflects additive gene action and the SCA effect, and reflects non-additive gene action, were calculated using the ratio of variance components from the formula (Equation 3) as recommended by Baker (1978):

$\delta^2GCA_f + \delta^2GCA_m / (\delta^2GCA_f + \delta^2GCA_m + SCA_{fxm})$ ..... Equation 3

Where:

GCA_m is the general combining ability of the male parents; GCA_f is the general combining ability of the female parents; and SCA_{fxm} is the specific combining ability of the interaction between male and female parents.

Heritability of bacterial wilt resistance. The Variance Components' Approach, from analysis of North Carolina II, where the parents were used in the crosses, were considered fixed effects, and therefore, heritability estimates were not regarded as appropriate. Instead, the analogous broad-sense and narrow-sense coefficients of genetic determination were estimated by the formulas suggested by Yu *et al*. (2004) as follows:

Broad-sense heritability (H) =

$$\frac{\delta^2GCA_f + \delta^2GCA_m + SCA_{fxm}}{\delta^2GCA_f + \delta^2GCA_m + SCA_{fxm+}\ \delta^2e}$$

..... Equation 4

Narrow-sense coefficient genetic was determned as:

$$(h^2) = \frac{\delta^2GCA_f + \delta^2GCA_m}{(\delta^2GCA_f + \delta^2GCA_m + SCA_{fxm} + \delta^2e)}$$

..... Equation 5

SSR marker polymorphism and genetic diversity. Allele sizes for each of the 14 polymorphic primers, were used to score the presence or absence of a band in the PCR products. The SSR primer band that appeared without ambiguity was given a score of 1 (present) or 0 (absent). To estimate genetic diversity, several effective alleles, heterozygosity, fixation index and Shannon's Information Index, GenAIEx V6.51 software (Peakall and Smouse, 2012) was used. DARwin V6.0.21 software (Perrier and Jacquemoud-Collet, 2006) was used in a pairwise comparison, to estimate the genetic similarity between genotypes by calculating dissimilarity coefficients. With the unweighted pair-group method arithmetic average (UPGMA) clustering algorithm, a hierarchical cluster analysis was carried out utilising dissimilarity coefficients. (Tantasawat *et al*., 2011).

The effectiveness of cluster analysis was evaluated using 1000 bootstrapped replicates (Tantasawat *et al*., 2011). Each locus' allelic diversity was calculated based on its polymorphic information content (PIC), using the equation of Anderson *et al*. (1993), *viz.*

$PIC = 1 - \Sigma P^2i$ ..... Equation 6

Where:

Pi is the frequency of the i^{th} allele in the set of genotypes analysed; and calculated for each SSR locus.

DARwin 6.0.21 software was used to perform factorial component analysis (FCA). In a scatter plot, several aspects of the distribution of released and elite genotypes are, thus shown (Hipparagi *et al*., 2017) to supplement the data from the hierarchical cluster analysis (Tantasawat *et al*., 2011).

RESULTS

Molecular confirmation of *R. solanacearum.* In multiplex PCR, the two infected samples showed two bands of 280 bp and 91 bp, with all five specific primers. The result confirmed that only the two infected samples were *R. pseudosolanacearum.* All isolates produced fragments at 280 bp, characteristic of Rs genomic DNA amplified with universal oligonucleotides 759/760 primers with 91bp confirming phylotype 3 (Fig. 2).

Severity assessment. Days to complete wilting was significantly ($P < 0.001$) influenced by genotype, and the emergence of the bacterial wilt; and disease severity differed significantly ($F_{20,40} = 3.14$, $P < 0.001$) beyond 15 DAI (Table 4).

Varying degrees of Bacterial Wilt (BW) disease severity were observed in 21 genotypes under six cross-combinations (Table 5). The range of disease severity in different genotypes, under different cross combinations and generations, varied greatly between resistant and susceptible groups. Results of progressive wilting over three generations of six crosses, indicated that inoculum distribution was uniform and high in experimental pots (Table 5).

The means of the crosses at F_1 were relatively higher in severity than those of F_2 (Table 6). The lowest mean severity score observed with F_1 was between the resistant parent BL333, and the susceptible parents (Assila, Heinz and Rambo), which was 1.167; while in F_2, the cross between BL333 and Assila had the lowest observed severity value of 1 (Table 6).

The disease severity values increased with increase in DAI (Fig. 3), with Heinz variety having the highest curve, indicating the highest

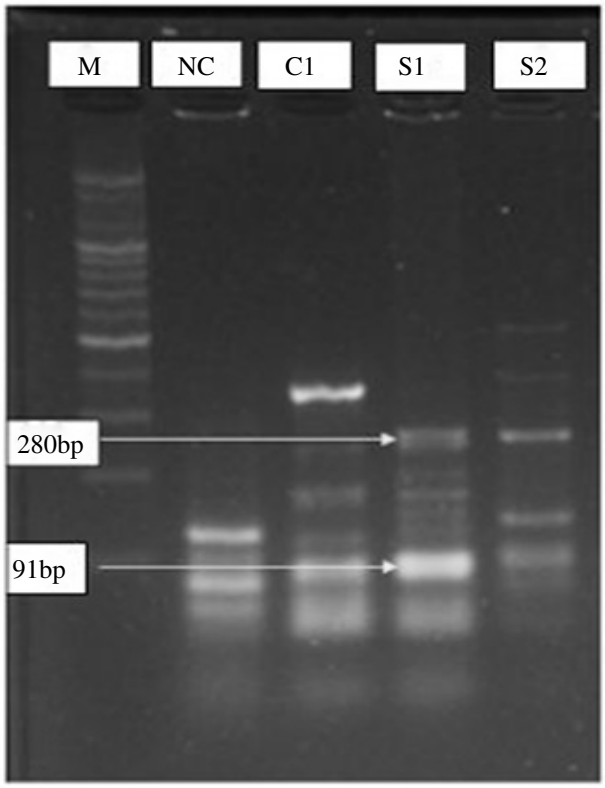

Figure 2. Amplification pattern generated with *Ralstonia solanaecerum* diagonistic primers in multiplex. M = Marker ladder, NC = Negative control that lacked the 759/760 primers; C1 = Control 1; S1 = Sample 1, and S2 = Sample 2.

TABLE 4. AUDPC ANOVA for 19 tomato genotypes from 12-30 days after inoculation with *R. pseudosolanacearum* at Kabanyolo in Uganda

Source of variation	Degrees of freedom	Mean square	F prob
Rep	2	23.80	
Genotype	18	211.94 ***	<.001
Residual	36	14.88	

*** = significant at 0.001 probability level

level of susceptibility to BW. In contrast, variety AVTO 1219 had the flattest curve, showing the highest resistance level to BW.

Combining ability. The General Combining Ability for the female parent (GCA_f) and specific combining ability for the male parents (SCA_{fxm}) were not significant (P>0.05). In contrast, the general combining ability for male parents (GCA_m) was significant (P<0.01) at F_1.

Specific combining abilities (SCA_{fxm}) and GCA_f were significant for the F_2 generation (P<0.01); while GCA_m was not significant (P>0.05) (Table 7). The Broad Sense Coefficient of Genetic Diversity was higher

TABLE 5. AUDPC scores for 21 tomato genotypes from 12 – 30 days after inoculation

Genotype	AUDPC
P1 (MT56)	23.33
P2 (BL333)	22.33
P3 (Assila)	43.25
P4 (Rambo)	30.75
P5 (Heinz)	43.75
P6 (AVTO1219)	21.50
P7 (AVTO9802)	24.71
F1 (P1xP3)	30.50
F1 (P1xP4)	26.50
F1 (P1xP5)	39.00
F1 (P2xP3)	25.00
F1 (P2xP4)	25.00
F1 (P2xP5)	25.00
F2 (P1xP3)	32.00
F2 (P1xP4)	28.00
F2 (P1xP5)	35.50
F2 (P2xP3)	21.50
F2 (P2xP4)	30.50
F2 (P2xP5)	36.00
LSD (0.05)	6.39
CV%	3.70

AUDPC= desease severity scores expressed as Area under deisease Progress curve, C.V = Coefficient of Variation, and LSD = Least Significant Differences

than the Narrow sense Coefficient of Genetic Diversity, in both F_1 and F_2 generations, and Bakers ratio decreased from 0.778 in F_1 to 0.453 in F_2.

Marker diversity. Table 8 shows the results of estimated genetic diversity parameters across 19 tomato genotypes, at each locus. The genotypes had 47 alleles, with an average of 3.43 per locus. The number of alleles varied from 2 (SCU176-534, SCU176-1190F1R, TSCARAAG/CAT, TG230, SSR128, and SSR306), to 5 (SLM12-2 and SCU176-1190F2R2); and the primary alleles' frequency varied from 0.45 on TSCARAAT/CGA to 0.5 on SLM12-2, with an average of 0.4887. The PIC value ranged from 0.375 on M10, to 0.6357 on M1, with an average of 0.4888. The observed heterozygosity varied from 0.09474 to 1, with an average of 0.9962.

Hierarchical cluster analysis. Figure 4 displays results of similarity estimations among the 19 evaluated germplasms, with values ranging from 0 to 0.5. Using 14 polymorphic markers, an UPGMA cluster analysis was conducted on the tomato varieties. The resulting dendrogram was a good match for the genetic similarity matrix (Clever, 2019). Truncating the dendrogram at genetic similarity

TABLE 6. Mean bacterial wilt severity scores for the tomato crosses at F_1 and F_2 generations

Female	Male			
	F_1		F_2	
	BL333	MT56	BL333	MT56
Assila	1.167	1.400	1.000	1.500
Heinz	1.167	1.667	1.733	1.633
Rambo	1.167	1.233	1.467	1.333
C.V%	6.800		4.400	
L.S.D	0.2573		0.2812	

C.V = Coefficient of Variation, LSD = Least Significant Differences

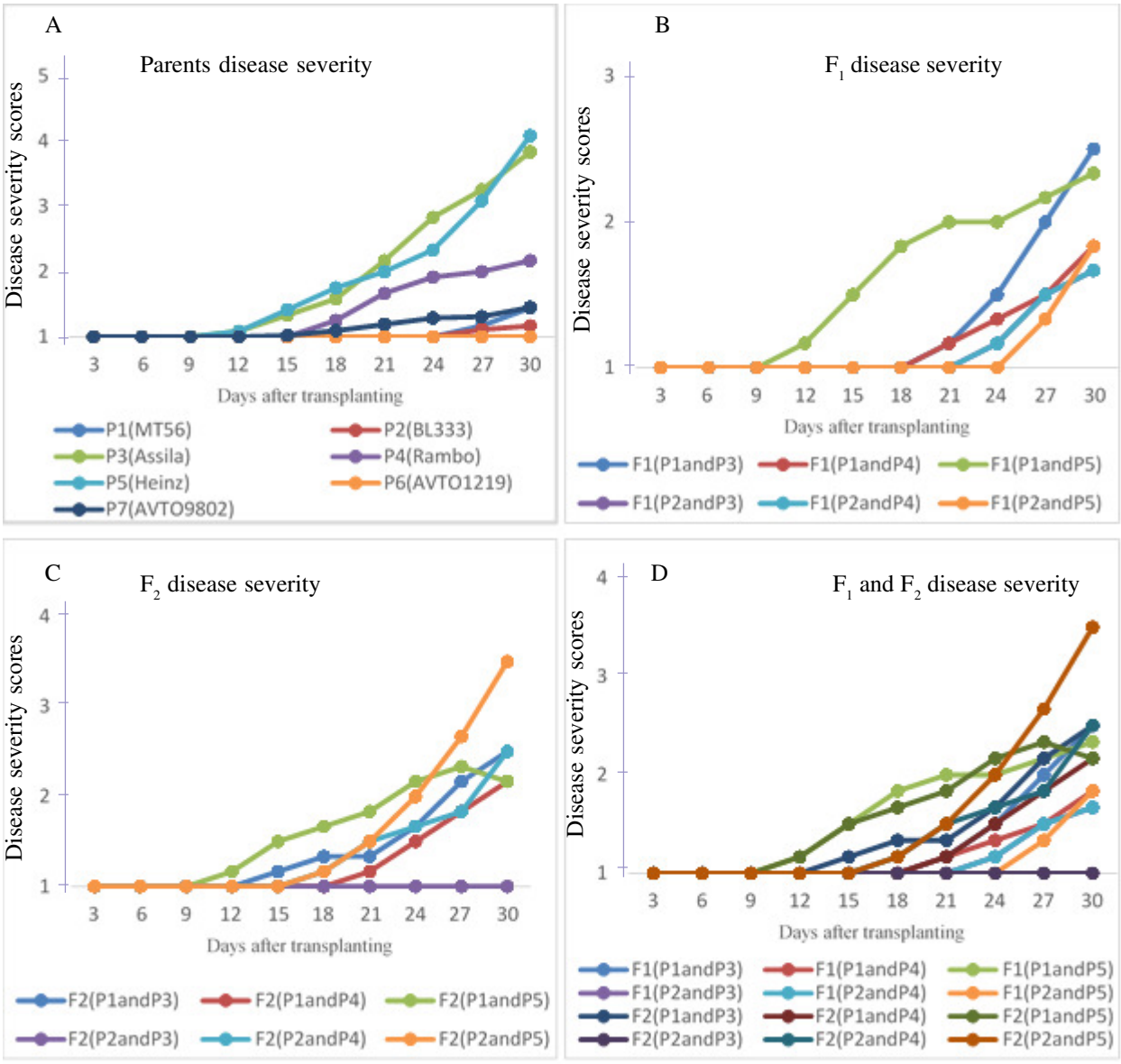

Figure 3. AUDPC for the different tomato genotypes: A: Parents disease severity; B: F_1 disease severity; C: F_2 Disease severity; and D: F_1 and F_2 disease severity.

values of 0.02, 0.01, 0.05, 0.1, 0.11 and 0 produced six distinct clusters, namely CI, CII, CIII, CIV, CV and CVI. All the 19 genotypes were grouped into these clusters, with nine genotypes belonging to cluster I (Fig. 4), which was inseparable.

Four genotypes (F_1P1×P3, P3Assila, P5Heinz, and P4Rambo) were placed in cluster II, two genotypes (P2BL333 and P1MT56) in cluster III, one genotype (F_1P1×P4) in cluster IV, two genotypes (AVTO1219 and AVTO9802) in cluster V, and one genotype (F_1P1×P5) in cluster VI. The majority of the genotypes were included in cluster II, which also included the susceptible varieties (Assila, Rambo, and Heinz). The NaCRRI and AVRDC cultivars, labeled in green, red, and purple on the dendrogram, were found mostly in clusters

TABLE 7. Mean squares and variance components of Bacterial Wilt severity scores in F_1 and F_2 generations

Sources of variation	D.f	M.S F_1	Vc F_1	M.S F_2	Vc F_2
Rep stratum	2	0.047		0.024	
Female (GCA_f)	2	0.072 ns	0.026	0.291**	0.045
Male (GCA_m)	1	0.320 **	0.033	0.036 ns	0.001
Female x Male (SCA_{fxm})	2	0.072 ns	0.017	0.191**	0.056
Residual	10	0.020		0.024	
[a]BS CGD		0.792		0.810	
[b]NS CGD		0.616		0.367	
[c]BR		0.778		0.453	
C.V%		6.800		4.400	

*, **, *** significant at 0.05, 0.01, and 0.001 probability levels, respectively; [ns] not significant. [a] Broad sense coefficient of genetic determination for a fixed model (analogous to H); [b] Narrow sense coefficient of genetic determination for a fixed model. [C] Relative importance of GCA and SCA; All MS and CGD values are based on the mean of three replications

TABLE 8. Estimated genetic diversity of parameters obtained at each locus across 19 tomato genotypes

Marker	Genotype no.	Allele no.	Major allele frequency	Gene diversity	Heterozygosity	PIC
SLM12-2	4	5	0.500	0.676	1	0.634
SCU176-534	2	3	0.500	0.525	1	0.412
SCU176-1190F2R2	4	4	0.500	0.661	1	0.613
SCU176-1190F1R1	2	3	0.500	0.525	1	0.412
SLM12-10	3	4	0.500	0.644	1	0.586
TSCARAAG/CAT	2	3	0.500	0.525	1	0.412
TSCARAAT/CGA	3	4	0.447	0.630	1	0.558
TG564	4	4	0.447	0.614	0.947	0.536
TG230	2	3	0.500	0.525	1	0.412
LEaat002	1	2	0.500	0.500	1	0.375
LEat006	1	2	0.500	0.500	1	0.375
SSR 20	3	4	0.447	0.669	1	0.608
SSR128	2	3	0.500	0.525	1	0.412
SSR306	2	3	0.500	0.583	1	0.496
Total	35	47	6.842	8.101	13.947	6.843
Mean	2.500	3.429	0.489	0.579	0.996	0.489
S.E	32.500	44.571	6.354	7.522	12.951	6.355

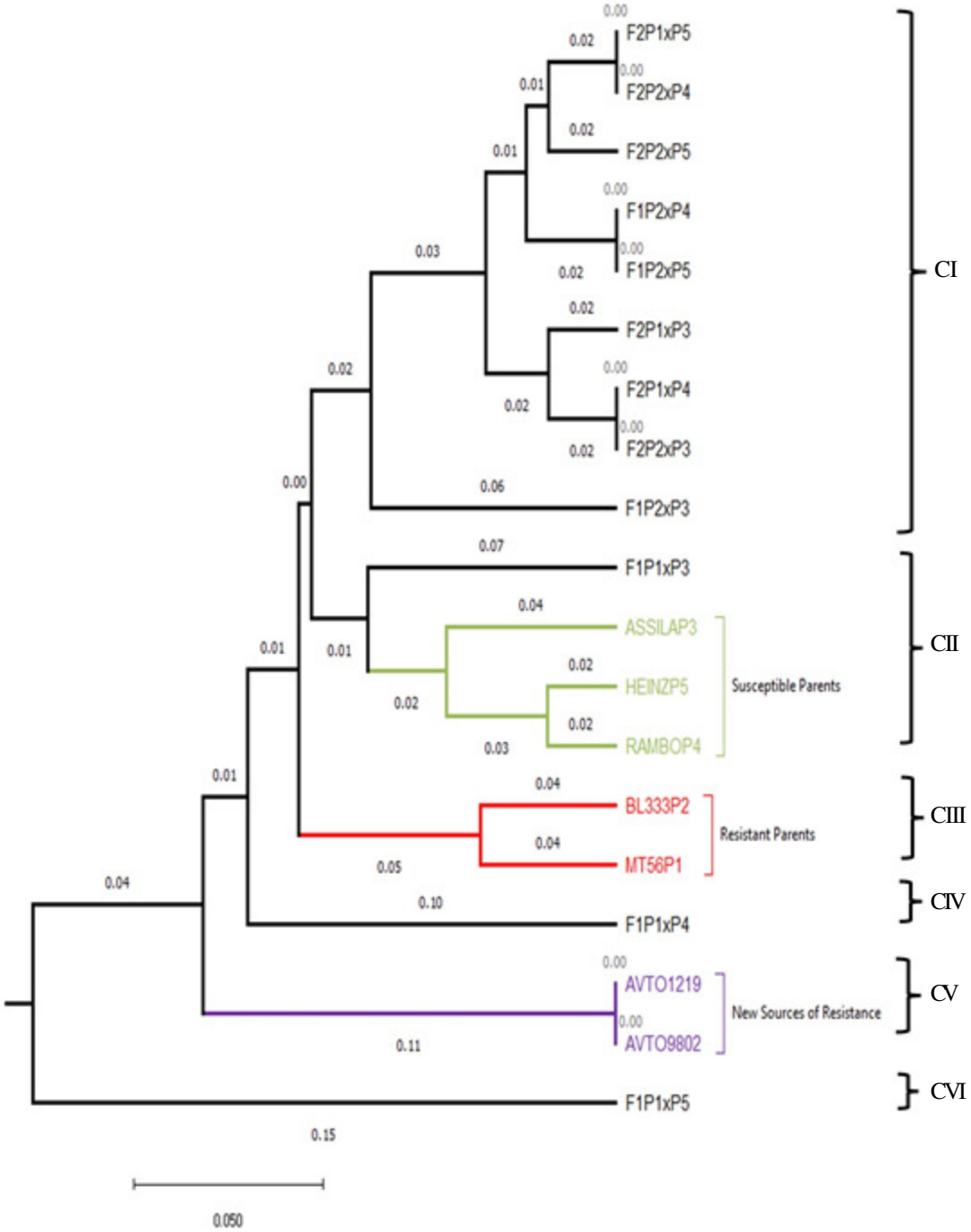

Figure 4. Dendogram showing genetic diversity among 19 tomato genotypes.

II, III and V, respectively. Cluster III comprised of closely related resistant lines (MT56 and BL333); while cluster V consisted of separate newly resistant lines screened in the study from AVRDC (AVTO1219 and AVTO9802).

Factor Component Analysis (FCA). Figure 5 shows the findings of the factor component analysis, used to examine genetic variation among released varieties and elite tomato genotypes. The results revealed that five genotype groups (A, B, C, D and E) were found to be distinct and in different quadrants (Fig. 5). The factor component accounted for 80.79% of the overall variance, with FC1 and FC2 accounting for 61.97 and 18.82%, respectively. Red labels denoted resistant tomato types (MT56 and BL333); whereas green labels represent the most susceptible advanced-level tomato cultivars (Assila, Rambo, and Heinz).

DISCUSSION

Bacterial wilt (BW) severity among tomato population. The observed variations in BW disease severity among 21 genotypes in six cross-combinations (Table 6), provided valuable insights into the resistance and susceptibility groups. These findings not only validated substantial differences in disease severity at both time points, but also indicated a progressive nature of resistance against *R. pseudosolanacearum*.

The three-generation study across six crosses not only confirmed this progressive disease trend, but also uncovered a consistent and high inoculum distribution in experimental pots. Notably, the cross means at F_1 displayed elevated severity compared to F_2, underscoring the developmental nature of resistance. Specifically, the lowest severity score at F_1 was evident in the cross between the resistant parent BL333 and the susceptible parents

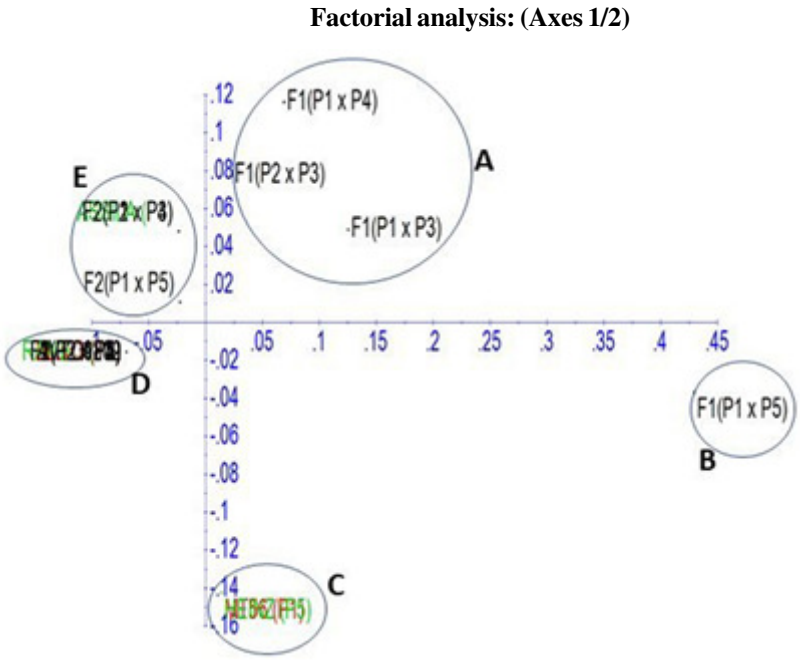

Figure 5. Factor loadings and scores for 19 tomato genotypes. Red labels resistant varieties and green labels susceptible.

Assila, Heinz and Rambo; registering a score of 1.167. In F_2, the cross between BL333 and Assila maintained the lowest observed severity value at 1.

Scientifically, these trends imply a dynamic interplay between genetic factors and disease resistance (Sun *et al*.,2023). The progressive nature of resistance observed, suggests ongoing genetic adaptations within the population, contributing to enhanced resistance over successive generations. The contrast in severity scores between F_1 and F_2, emphasizes the importance of evaluating resistance development over time, shedding light on the heritability dynamics within the population (Heidari *et al*., 2020).

In terms of specific tomato varieties, variety AVTO1219 emerged as the most resistant, exhibiting a flat disease progression curve (Fig. 3A); while Heinz, with a steep slope, demonstrated greater vulnerability. The hybridisation outcomes further contributed to our understanding of heritability of resistance to BW in tomato, with the cross between BL333 and sensitive varieties producing the most resistant hybrids. The F_1 generation from this cross, displayed the lowest mean disease severity (Table 6), a finding corroborated by the disease progression curve in Figure 3B, the former which illustrated a moderate level of resistance in the F_1 generation.

These collective results align with the study's objective of exploring heritability for tomato resistance against bacterial wilt and genetic variation within the population for breeding purposes. They not only offer valuable insights into the dynamic nature of resistance, but also provide actionable information for targeted breeding strategies to develop more resilient tomato varieties.

The observed differences in disease progression and severity among genotypes; and hybrid generations, indicate the presence of diverse genetic factors influencing resistance. Understanding these variations contributes considerably to unraveling the genetic diversity and inheritance patterns related to bacterial wilt in the desired tomato genotypes.

This knowledge serves as a foundation for future research endeavors aimed at uncovering specific genetic markers, mechanisms, and inheritance patterns that can be harnessed for more effective breeding strategies, ultimately advancing our ability to cultivate tomato varieties with enhanced resistance to bacterial wilt.

These findings are consistent with a previous study by Fegan and Prior (2005), which found that the BW initially appeared phenotypically around 14 DAI. Furthermore, the study revealed that the severity of the disease is correlated with the sensitivity of the genotype.

Combining ability. The substantial positive General Combining Ability (GCA) effects (0.033) observed in F_1s, underscore the significant contribution of male-resistant parents, MT56 and BL333, to resistance in the F_1 progeny (Table 7). Conversely, female-susceptible parents exhibited modest GCA effects (0.026), leading to susceptibility across both F_1 and F_2 generations in their crosses. The notable negative GCA effects observed in male parents contribute to imparting resistance in their involved crosses(Sun *et al*., 2023).

Additionally, Specific Combining Ability (SCA) effects at F_2 are substantial, indicating that specific parental combinations influence resistance levels more or less than predicted by their GCA values. According to Dabholkar (1992), all cross combinations exhibit the most negative SCA impact for *R. pseudosolanacearum* resistance, suggesting their favourability in hybridisation and the potential to produce a high frequency of resistant offspring. These findings offer critical insights into the genetic variation within the population, emphasizing the importance of understanding how different parental combinations influence resistance levels.

The evaluations further reveal high Broad-sense Coefficient of Genetic Determination

(CGD) values, consistently at 79.2% for F_1 and 81% for F_2; indicating that efficient selection was likely greater in subsequent generations (da Silva Costa *et al*., 2018) These values approximate heritability, reflecting all genetic contributions to phenotypic variance, including additive and non-additive effects (Falconer and Mackay, 1996).

High broad-sense CGD values suggest that the environment played a limited role in expressing resistance to *R. pseudosolanacearum* (da Silva Costa *et al*., 2018). Notably, the utilisation of narrow-sense heritability for prediction purposes highlights a nuanced picture; namely a high narrow-sense heritability of 61.6% for F_1s, suggests that resistance was mainly controlled by additive genetic variation; while the lower value of 36.7% for F_2s implies that non-additive genetic and environmental factors dominated the control of resistance, thus making phenotypic value a poor predictor of breeding value (Haq *et al*., 2008). This distinction emphasizes the multifaceted nature of resistance inheritance, making it imperative for breeders to carefully consider genetic variation and environmental influences in their breeding strategies.

These observations collectively contribute significantly to our understanding of genetic diversity and inheritance in tomato genotypes, regarding BW resistance. Leveraging this knowledge can guide targeted breeding strategies, leading to the development of more resilient tomato varieties. Ultimately, these findings provide a pathway for advancing bacterial wilt management practices in tomato cultivation.

Molecular diversity among tomato population. The tested tomato varieties showcased a notable genetic diversity (Table 8), evident in the average polymorphic information content (PIC) of 0.48. This surpassed the typically low diversity found in cultivated tomatoes, as reported in previous studies (Tam *et al*., 2005; Benor *et al*., 2008).

The selection process, prioritising high polymorphism, revealed 47 alleles among the evaluated genotypes, averaging 3.43 alleles per locus. However, allelic diversity varied between 2 and 5, indicating a relatively low diversity within the current set of tomato genotypes (Hipparagi *et al*., 2017). To enhance allelic richness, the introduction of more landraces into the breeding programme is recommended. Notably, among the 12 markers with a PIC value exceeding 0.4, the SSR primer SLM12-2 proved to be the most informative with a PIC value of 0.6357, underscoring its effectiveness as a valuable tool for discerning genetic differences and studying phylogenetic relationships among tomato germplasms.

Hierarchical clustering analysis categorised the tomato genotypes into six distinct clusters (Fig. 4). The grouping of susceptible and resistant varieties in clusters II and III, suggested common parentage among commercially available varieties. This observation raises concerns about potential susceptibility loss in released varieties in the event of pathogen changes, particularly for those in cluster III.

The cluster analysis deviated from traditional classification methods (Blanca *et al*., 2012), revealing a genetic diversity pattern, consistent with the broad diversity in cultivated tomato varieties (Dossoumou *et al*., 2021). Notably, some exceptional materials such as F_1 (P1×P5), stood relatively distant from other populations, indicating unique tomato lines with distinct breeding values (Sauvage *et al*., 2020).

Further research is essential to comprehend the underlying reasons for this behavior. Comparing the UPGMA dendrogram (Fig. 4) with the 3-dimensional factorial component plot emphasised that systematic clustering and FCA could offer a comprehensive understanding of how different genotypes; and varieties interact within germplasm pools.

Contrary to genetic diversity reported in other tomato populations (Gonias *et al*., 2019; Schouten *et al*., 2019; Marefatzadeh-

Khameneh *et al*., 2021) the 19 tomato varieties in this study exhibited lower genetic diversity, ranging from 0.5 to 0.6759. This variance perhaps stemed from the cultivation of these varieties in distinct geographic regions in other countries (Schouten *et al*., 2019).

Factorial analysis grouped the genotypes into five clusters based on factor bases, with the first two axes explaining 80.79% of the total variation; suggesting moderate genetic variation (Wang *et al*., 2019). Notably, Cluster A, situated in the first positive by positive quadrat, included 3 F_1 genotypes, indicating a close relationship between these specific genotypes (Korir *et al*., 2013).

The diverse genotypes identified in this study represent a potential source of new alleles for Uganda's tomato breeding programmes, offering opportunities for enhancing genetic diversity and resilience against the BW. Leveraging these diverse alleles will be instrumental in developing tomato varieties with improved resistance traits, thus contributing to the overall management and sustainability of tomato cultivation in the face of BW challenges.

In a nutshell, these observations provide valuable insights into the genetic diversity and inheritance of bacterial wilt resistance in tomato genotypes. The identified markers, allelic patterns and clustering dynamics offer a foundation for exploring heritability, understanding genetic relationships, and guiding targeted breeding strategies. This knowledge can be leveraged for future breeding programmes to introduce diverse alleles to enhance resistance traits, ultimately contributing to the sustainable management of bacterial wilt in tomato cultivation.

CONCLUSION

We have uncovered resilient tomato champions (MT56, BL333, AVTO1219 and AVTO9802) standing strong against *R. pseudosolanacearum.* AVTO1219 emerged with the highest resistance. Their defense is an interplay between additive and non-additive gene actions, with additive genes taking the lead. The SSR maestro, SLM 12-2, played its part with a polymorphism of 0.6357, unveiling a rich genetic tapestry within our tomato comrades.

In the ever-evolving battle against bacterial wilt, our findings underscore the importance of a diverse genetic arsenal. In summary, the integration of landraces, could further enrich the allelic landscape and fortify our tomato defenses. Through this study, with its genetic revelations, we wish to pave way for a strategic approach to breeding programmes, offering a roadmap for a future where tomatoes can steadfastly withstand the challenges posed by *R. pseudosolanacearum*.

ACKNOWLEDGEMENT

This study was funded by the World Bank fund, through the Makerere University Regional Centre for Crop Improvement (MaRCCI), through project Number 001. Dr. Amponsah Emmanuel Adjei, significantly contributed to the first thoughts and reflections on this paper.

REFERENCES

Anderson, J.A., Churchill, G.A., Autrique, J.E., Tanksley, S.D. and Sorrells, M.E. 1993. Optimizing parental selection for genetic linkage maps. *Genome* 36(1):181-186.

Baker, R.J. 1978. Issues in Diallel Analysis. *Crop Science* 18(4):533-536.

Benor, S., Zhang, M., Wang, Z. and Zhang, H. 2008. Assessment of genetic variation in tomato (*Solanum lycopersicum* L.) inbred lines using SSR molecular markers. *Journal of Genetics and Genomics* 35(6):373-379.

Bisen, A., Khare, D., Nair, P. and Tripathi, N. 2015. SSR analysis of 38 genotypes of soybean (*Glycine max* (L.) Merr.) genetic

diversity in India. *Physiology and Molecular Biology of Plants* 21(1):109-115.

Blanca, J., Cañizares, J., Cordero, L., Pascual, L., Diez, M.J. and Nuez, F. 2012. Variation revealed by SNP genotyping and morphology provides insight into the origin of the tomato. *PLoS ONE* 7(10): e48198. doi: https://doi.org/10.1371/journal.pone.0048198

Bradbury, J.F.J.F. 1986. Guide to plant pathogenic bacteria. *CAB International Mycological Institute*. 332pp.

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

Choi, K., Choi, J., Lee, P.A., Roy, N., Khan, R., Lee, H.J., Weon, H.Y., Kong, H.G. and Lee, S.W. 2020. Alteration of Bacterial Wilt Resistance in Tomato Plant by Microbiota Transplant. *Frontiers in Plant Science* 11:1-12.

Clever, M. 2019. Genetic diversity and genotype x environment interaction of advanced elite soybean genotypes in Uganda. M.Sc. Thesis. Makerere University, Uganda. 72pp.

Dabholkar, A.R. 1992. Elements of Biometrical Genetics. Concept Publishing Company, New Delhi, India. pp. 57-116.

da Silva Costa, K.D., dos Santos, A.M.M., dos Santos, P.R., Nascimento, M.R., Silva, A.M.F., Albuquerque, G.M.R., Batista, R.O., de Lima Pereira, J.W. and de Carvalho Filho, J.L.S. 2018. Inheritance of resistance to *Ralstonia pseudo solanacearum* in tomato. *Euphytica* 214(8):0-11.

Denny, T.P. 2006. Plant pathogenic *Ralstonia* species. In: Gnanamanickam, S.S. (Ed.). Plant-associated bacteria. *Springer, Dordrecht*. pp. 573-644.

Dossoumou, M.E., Sikirou, R., Adandonon, A., Gonroudobou, J. and Baba-Moussa, L. 2021. Tomato hybrid and local varieties screened for resistance to bacterial wilt caused by <i> *Ralstonia solanacearum* </i> under screen house and field conditions. *American Journal of Plant Sciences* 12(08):1222-1235.

Falconer, D.S. and Mackay, T.F.C. 1996. Introduction to Quantitative Genetics. 4th Edition, Addison Wesley Longman, Harlow. 464pp.

Fasahat, P., Abazar, R., Javad, Mohseni, R. and John, D. 2016. Principles and utilization of combining ability in plant breeding. *Biometrics & Biostatistics International Journal* 4(1):1-22.

Fegan, M. and Prior, P. 2005. How complex is the "*Ralstonia solanacearum* species complex"? Bacterial wilt disease and the *Ralstonia solanacearum* species complex. pp. 449-461.

Garæia, R.O., Kerns, J.P. and Thiessen, L. 2019. *Ralstonia solanacearum* species complex: A quick diagnostic guide. *Plant Health Progress* 20(1):7-13.

Gomez, K.A.G.A.A. 1984. Statistical procedures for agricultural research. second edition. pp. 75 - 83.

Gonias, E.D., Ganopoulos, I., Mellidou, I., Bibi, A.C., Kalivas, A., Mylona, P. V., Osanthanunkul, M., Tsaftaris, A., Madesis, P. and Doulis, A.G. 2019. Exploring genetic diversity of tomato (*Solanum lycopersicum* L.) germplasm of genebank collection employing SSR and SCAR markers. *Genetic Resources and Crop Evolution* 66(6):1295-1309.

Haq, W.U., Malik, M.F., Rashid, M., Munir, M. and Akram, Z. 2008. Evaluation and estimation of heritability and genetic advancement for yield related attributes in wheat lines. *Pakistan Journal of Botany* 40(4):1699-1702.

Heidari, N., Sedaratian-Jahromi, A., Ghane-Jahromi, M. and Zalucki, M.P. 2020. How bottom-up effects of different tomato cultivars affect population responses of *Tuta absoluta* (Lep.: Gelechiidae): A case

study on host plant resistance. *Arthropod-Plant Interactions* 14(2):181-192.

Hipparagi, Y., Singh, R., Choudhury, D.R. and Gupta, V. 2017. Genetic diversity and population structure analysis of Kala bhat (*Glycine max* (L.) Merrill) genotypes using SSR markers. *Hereditas* 154(1):9. https://doi.org/10.1186/s41065-017-0030-8

Huang, W. and Mackay, T.F.C. 2016. The genetic srchitecture of quantitative traits cannot be inferred from variance component analysis. *PLoS Genetics* 12(11):1-15.

Huet, G. 2014. Breeding for resistances to *Ralstonia solanacearum*. *Frontiers in Plant Science* 5:1-5.

Hussain, M.Z., Rahman, M.A. and Bashar, M.A. 2005. Screening of brinjal accessions for bacterial wilt caused by *Ralstonia solanacearum*. *Bangladesh Journal of Botany* 34(1):55-58.

Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology* 44:693-695.

Korir, N.K., Han, J., Shangguan, L., Wang, C., Kayesh, E., Zhang, Y. and Fang, J. 2013. Plant variety and cultivar identification: advances and prospects. *Critical Reviews in Biotechnology* 33(2):111-125.

Kumar, R.E.R. and Yadav, K. 2019. Phylotype analysis of *Ralstonia solanacearum* causing bacterial wilt in eggplants in Orissa in India. *International Journal of Trend in Scientific Research and Development* 3(3):1-3.

Kumar, A., Kumar, V., Gull, A. and Nayik, G.A. 2020. Tomato (*Solanum lycopersicon*) In: *Antioxidants in Vegetables and Nuts - Properties and Health Benefits*. Singapore: Springer Singapore. pp. 191-207.

Marefatzadeh-Khameneh, M., Fabriki-Ourang, S., Sorkhilalehloo, B., Abbasi-Kohpalekani, J. and Ahmadi, J. 2021. Genetic diversity in tomato (*Solanum lycopersicum* L.) germplasm using fruit variation implemented by tomato analyzer software based on high throughput phenotyping. *Genetic Resources and Crop Evolution* 68(6):2611-2625.

Mathai, Faith, W., Pascal, P.O.O. and Robert, M.G. 2022. Combining ability and gene action for bacterial wilt disease resistance in wild tomato (*Solanum pimpinellifolium*) and cultivated tomato (*Solanum lycopersicum*) genotypes. *African Journal of Plant Science* 16(4):34-46.

Maughan, P.J., Maroof, M.A.S. and Buss, G.R. 1995. Microsatellite and amplified sequence length polymorphisms in cultivated and wild soybean. *Genome* 38(4):715-723.

Opina, N., Tavner, F., Hollway, G., Wang, J.F., Li, T.H., Maghirang, R., Fegan, M., Hayward, A., Krishnapillai, V., Hong, W., Holloway, B. and Timmis, J. 1997. A novel method for development of species and strain-specific DNA proves and PCR primers for identifying *Burkholderia solanacearum* (formerly *Pseudomonas solanacearum*). *Asia Pacific Journal of Molecular Biology and Biotechnology* 5(1):19-30.

Osawaru, M.E., Ogwu, M.C. and Aiwansoba, R.O. 2015. Hierarchical approaches to the analysis of genetic diversity in plants: A systematic overview. *University of Mauritius Research Journal* 21(0):1-36.

Peakall, R. and Smouse, P.E. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28(19):2537-2539.

Peralta, I., Knapp, S., Spooner, D., Peralta, I., Knapp, S. and Spooner, D. 2007. The taxonomy of tomatoes: A revision of wild tomatoes (*Solanum* L. section *lycopersicon* (Mill.) Wettst.) and their outgroup relatives (*Solanum* sections *Juglandifolium* (Rydb.) Child and Lycopersicoides (Child) Peralta). *Systematic Botany Monographs* 84:1-186.

Perrier, X. and Jacquemoud-Collet, J.P. 2006. DARwin software: Dissimilarity analysis and representation for windows. http://darwin.cirad.fr/darwin.

Sauvage, C., Arnoux, S. and Causse, M. 2020. Population genomics of tomato In: *Springer, Cham* [Online]. https://link.springer.com/10.1007/13836_2020_79.

Schouten, H.J., Tikunov, Y., Verkerke, W., Finkers, R., Bovy, A., Bai, Y. and Visser, R.G.F. 2019. Breeding has increased the diversity of cultivated tomato in the Netherlands. *Frontiers in Plant Science* 10:500236. https://doi.org/10.3389/fpls.2019.01606

Singh, N., Phukan, T., Shanna, P.L., Kabyashree, K., Barman, A., Kumar, R., Sonti, R. V., Genin, S. and Ray, S.K. 2018. An innovative root inoculation method to study ralstonia solanacearum pathogenicity in tomato seedlings. *Phytopathology* 108(4):436-442.

Singh, R.K. and Chaudhary, B.D. 1985. Biometrical methods in Quantitative Genetic Analysis. Kalyani Publishers Ludhiana, New Delhi, India. 318pp.

Sun, Y., Su, Y., Meng, Z., Zhang, J., Zheng, L., Miao, S., Qin, D., Ruan ,Y., Wu, Y., Xiong, L., Yan, X., Dong, Z., Cheng, P., Shao, M. and Yu, G. 2023. Biocontrol of bacterial wilt disease in tomato using *Bacillus subtilis* strain R31. *Frontiers in Microbiology* 14:1281381. doi: https://doi.org/10.3389%2Ffmicb.2023.1281381 . PMID: 37840725; PMCID: PMC10568012.

Suvi, W.T., Shimelis, H., Laing, M., Mathew, I. and Shayanowako, A.I.T. 2021. Determining the combining ability and gene action for rice yellow mottle virus disease resistance and agronomic traits in rice (*Oryza sativa* L.). *Agronomy* 11(1):12 https://doi.org/10.3390/agronomy11010012

Tam, S.M., Mhiri, C., Vogelaar, A., Kerkveld, M., Pearce, S.R. and Grandbastien, M.-A. 2005. Comparative analyses of genetic diversities within tomato and pepper collections detected by retrotransposon-based SSAP, AFLP and SSR. *Theoretical and Applied Genetics* 110(5):819-831.

Tantasawat, P., Trongchuen, J., Prajongjai, T., Jenweerawat, S. and Chaowiset, W. 2011. SSR analysis of soybean (*Glycine* max (L.) merr.) genetic relationship and variety identification in Thailand. *Australian Journal of Crop Science* 5(3):283-290.

Temesgen, B. 2021. Combining ability and heterosis in plant improvement. *Open Journal of Plant Science* 6:108-117.

Vanitha, S.C., Niranjana, S.R., Mortensen, C.N. and Umesha, S. 2009. Bacterial wilt of tomato in Karnataka and its management by Pseudomonas fluorescens. *Biocontrol* 54(5):685-695.

Wang, J.-F., Olivier, J., Thoquet, P., Mangin, B., Sauviac, L. and Grimsley, N.H. 2000. Resistance of tomato line Hawaii7996 to *Ralstonia solanacearum* Pss4 in Taiwan is controlled mainly by a major strain-specific locus. *Molecular Plant-Microbe Interactions* 13(1):6-13.

Wang, T., Li, H.T., Zhu, H., Qi, S.Y., Zhang, Y.M., Zhang, Z.J. and Zou, Q.D. 2019. Comparative analyses of genetic variation in a tomato (*Solanum lycopersicum* L.) germplasm collection with single nucleotide polymorphism and insertion-deletion markers. *Russian Journal of Genetics* 55(2):204-211.

Xian-Qui, Y., Jian-Hua, L., Guang-Hui, P., Yun, Z. and Qi-Feng, Y. 2006. Breeding of promising tomato genotypes and hybrids against bacterial wilt. *Southwest China Journal of Agricultural Science* 19:103-107.

Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H. and Nishiuchi, Y. 1995. Transfer of two burkholderia and an alcaligenes species to *Ralstonia* Gen. Nov. proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) Comb. Nov., *Ralstonia solanacearum* (Smith 1896) Comb. Nov. and Ralstonia eutropha (Davis 1969) Comb. Nov. *Microbiology and Immunology*. 39(11):897-904.

Yu, J.-K., Dake, T.M., Singh, S., Benscher, D., Li, W., Gill, B. and Sorrells, M.E. 2004.

Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. *Genome* 47(5): 805-818.

Zohoungbogbo, H., Quenum, A., Honfoga, J., Chen, J.R., Achigan-Dako, E., Kenyon, L. and Hanson, P. 2021. Evaluation of resistance sources of tomato (*Solanum lycopersicum* l.) to phylotype in strains of *Ralstonia solanacearum* species complex in Benin. Agronomy 11(8):1513. https://doi.org/10.3390/agronomy11081513