Genetic Diversity and resistance of African nightshade *Solanum nigrum L* Complex to Bacterial wilt *Ralstonia solanacearum* in Western Kenya

Nangila Janepher Mafuta

A Thesis submitted in partial fulfillment of the requirements for the award of the Doctor of Philosophy Degree in Horticulture of Masinde Muliro University of Science and Technology

December,2022

DECLARATION

This thesis is my original work prepared with no other than the indicated sources and support and has not been presented elsewhere for an award of degree in any other University.

Signature…… Date……

Nangila Janepher Mafuta

HTC/H/01-56515/2017

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a thesis entitled **"Genetic Diversity and resistance of African nightshade (*Solanum nigrum L complex)* to Bacterial wilt (*Ralstonia solanacearum*) in Western Kenya".**

Signature…… Date……

Dr. Rose Onamu, PhD.

Department of Agriculture and Land use management

School of Agriculture, Veterinary Sciences and Technology

Masinde Muliro University of Science and Technology

Signature…… Date……

Prof. Solomon I Shibairo

Department of Agriculture and Land use management

School of Agriculture, Veterinary Sciences and Technology

Masinde Muliro University of Science and Technology

Signature…… Date……

Prof. Leonard S Wamocho, PhD

Department of Agriculture and Land use management

School of Agriculture, Veterinary Sciences and Technology

Masinde Muliro University of Science and Technology

COPYRIGHT

DEDICATION

I dedicate this work to my daughter Destiney Joy, my parents Jacob S. Wafula and Joyce Enny Wafula and my siblings Stellar Chemwile Juma, Job Wafula, Juliet Wafula, Jimmy Wafula and John Wafula for the help they gave me throughout my study.

ACKNOWLEDGEMENTS

I give thanks to God almighty for his mercy and grace throughout the study period.

I thank my supervisors Dr. Rose Onamu, Prof. Shibairo I Solomon and Prof. Leonard Wamocho with much gratitude for their invaluable mentoring, suggestions, comments, guidance, inspiration and advice throughout the study period which enabled the writing of this thesis.

I'm grateful to Masinde Muliro University of Science and Technology and Kibabii University for providing facilities for screening of African nightshade against bacterial wilt. I wish to acknowledge Dr. Dennis Omayio (Masinde Muliro University of Science and Technology) and Dr.Emy Chepkoech (University of Eldoret), for guidance on statistical analysis I am grateful to Mr. Agrey Osogo (Kibabii University) and Nadhan Muasia (University of Eldoret), for assistance in the screen house and labaratory during screening and amplification of African nightshade respectively.

My humble thanks go to Prof. Hassan Were (MMUST), Prof. Francis Muyekho (MMUST), Prof Miriam Kinyua (University of Eldoret) and cannot forget to thank my friends and colleagues at Masinde Muliro University of Science and Technology, Bernard Khalinda (Sustainable Agriculture) and University of Eldoret Josphine Baraza (Seed and Horticultural Science Labaratory), I am humbled by the moral and material support they offered. My sincere appreciation to the Department of Agriculture and Land Use Management at Masinde Muliro University of Science and Technology for the humble study environment at the University library.

My unending thanks to my family for the encouragement and moral support that they gave me throughout my study, God bless you.

ABSTRACT

African nightshade, *Solanum nigrum L* .is one of the most significant leafy vegetable rich in nutritional and medicinal value, and can be used to feed people with human immune deficiency virus, HIV/AIDS in Kenya. There is limited information available of this species that hinders its sustainable conservation and development. Limited information on the crop pests and diseases also present major challenges that limit production of the African nightshade species since farmers are still using farm saved seed which is a danger of inadvertently spreading quarantine pest and diseases like *Ralstonia solanacearum*. The utilization of genetic diversity in breeding programs can be employed to enhance the development of African nightshade accessions that exhibit better yield potential for both leaf and fruit production, while also displaying resistance to various biotic and abiotic challenges. The objective of this study was to assess the presence of genetic diversity among African nightshade accessions by conducting morphological and genotypic characterisation. Additionally, the study aimed to determine the inherent resistance to bacterial wilt caused by Ralstonia solanacearum by field and greenhouse screening experiments. A total of 30 samples from three counties Bungoma, Kakamega and Trans Nzoia were evaluated. For morphological characterization the African nightshade accessions were planted at Kibabii University farm and scored for several agro morphological characters based on National Bureau of Plant Genetic Resource NBPGR descriptors on following qualitative traits; Leaf surface as Glaborous or pubscent, Colour of ripe fruit as Orange or Dark purple or Black, Stem ridge as Smooth ridges or Dented, Leaf shape as Lanceolate or Ovate or rhomboid, Leaf margin as Sinuate dented or Entire and Inflorescence orientation as Simple or Forked the plant type was scored as Semi erect or erect. Cluster analysis of morphological data was done using PASW Version 20 Statistical software. Results showed that there was phenotypic variation amongst accessions of African nightshade collected from the three counties since they were grouped into two major clusters A and B meaning that there is rich diversity both within and among African nightshade accessions which can be used for the crop breeding work. Molecular characterization was done using SSR markers on 30 African nightshade accessions. Each of the 6 SSR primers utilized produced a single polymorphic band. The mean polymorphic information content was 0.5881, with values ranging from 0.4215 to 0.8212. He=0.9111 is the mean heterozygosity for SSR markers utilized. The dendrogram indicated that the accessions were categorized into three primary clusters, each characterized by a high degree of diversity. Furthermore, it disclosed that the coefficient distance separating the majority of the accessions was below 79.56. These results indicate that cross-breeding was a possibility among the accessions; genetic variation between regions was not apparent. Screening of the 30 African nightshade accessions, to *Ralstonia solanacearum* was done in the screen house at Masinde Muliro University of science and Technology. Seedlings were inoculated at four to six leaf stages with 30 ml of 10^8cfu/ml per seedling in the pot and disease incidence was recorded. The different accessions of *Solanum nigrumL, Solanum villosum L* from Trans Nzoia, Bungoma and Kakamega counties were identified as susceptible. However, improved accessions of *Solanum scabrum L* sampled from the African nightshade growing areas in Western Kenya were resistant to bacterial wilt, the accessions that were found to be susceptible, symptoms appeared 4 days after inoculation, while the accessions that were found to be resistant/ tolerant no symptoms were observed even after 14 days after inoculation. The resistant accession of improved variety of *Solanum scabrum L* can be used in production and

also breeding programmes for developing new varieties of the African nightshade crops.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ACRONYMS AND ABBREVIATIONS

AFLP Amplified fragment length polymorphism

AIV African indigenous vegetables

AMF Arbuscular mycorrhizal fungi

AMOVA Analysis of molecular variance

ANOVA Analysis of Variance

ANS African nightshades

AVRDC Asian vegetable Research and Development centre

CCD Charge couple device

CPG Casamino acid peptone glucose

CRD CompletelyRandomizedBlockDesign

CTAB Cetyl-Trimethyl Ammonium Bromide

DNA Deoxyribonucleic acid

GOK Government of Kenya

GPS Global positioning system

KALRO Kenya Agricultural and Livestock Research organisation

LM Lower midland

MoA Ministry of Agriculture

PASW Power of Advanced Statistical Analysis

PCR Polymorphic Chain Reaction

PGPR Plant growth promoting rhizobacteria

PRA Participatory rural appraisal

RAPD Random amplified polymorphic

RDI Reference daily intake

SPSS Statistical package for social sciences

SSR	Simple sequence repeats
TTC/TZC	Triphenyl tetrazolium chloride agar
UH	Upper highland
UPGMA	Un weighted Pair-Group method
WHO	World health Organisation
YPGA	Yeast extract-peptone-glucose agar

CHAPTER ONE

INTRODUCTION

1.1 Background

African nightshade (*Solanum nigrum L*) is an important leafy vegetable in Kenya and its origin is Eurasia (Jagatheeswari *et al.,* 2013). The crop occupies a significant place in the economy contributing much to food and nutritional security (Nandhini *et al.,* 2014) and generating income for small holder farmers in Western Kenya.

Solanum nigrum L is the most diverse plant species within the genus *Solanum (*Matasyoh *et al.,* 2015). African nightshade (ANS) is a dicotyledonous crop in the *Solanaceae* family. *Solanum nigrum* complex (African nightshade) comprises of several species which include *S. douglasii*, *S. Schenopodioides*, *S. nigrum* L. subsp, *S. sarrachoides, S. furcatum*, *S. nigrum* L. subsp. *Nigrum, S. retroflexum*, *S. scabrum*, *S. villosum, S. americanum,* and *S. physalifolium,* (Edmond and Chweya, 1997a). The mainly grown African nightshade species in Kenya include *S. villosum, S. scabrum, S. nigrum, S.americanum, S. sarrachoides* and *S. physalifolium* (Ojiewo *et al*., 2013b; Matasyoh and Bosire Na, 2016).

Despite extensive research on African nightshade species, their accurate taxonomic identification remains unresolved. The observed phenomenon can be attributed to ongoing inter and intraspecific hybridization, which is a natural occurrence among African nightshade species, as well as to the presence of inconsistent genetic variation (Zebish et al., 2016). The taxonomic identification of morphological characteristics has been complicated by their vulnerability to phenotypic plasticity and the presence of several ploidy series (Poczai & Hyvonen, 2011). The utilization of distinct local

names by various populations to designate African nightshade species contributes to further complexity in distinguishing one species from another (Poczai and Hyvonen, 2011; Ojiewo et al., 2013a). Furthermore, it has been observed that certain species are assigned disparate names, while different species are labeled with identical names, leading to a perplexing situation in the classification of species (Ojiewo et al., 2013a). African nightshade has been classified as a weed, resulting in limited research conducted on the crop and a shortage of experts dedicated to the assessment and conservation of African nightshade germplasm (Zebish et al., 2016).Consequently, the scarcity of data has hindered the scientists' ability to cultivate enhanced cultivars.

Understanding the genetic closeness or distance between crops is essential for any breeding effort, and this can be gleaned from studies of diversity.

On average, one hundred grams of fresh African nightshade leaves contains 1.4 grams of fiber, 87.2% water, 3,660 micrograms of beta carotene, 20 milligrams of ascorbic acid, seventy-five milligrams of phosphorus, four hundred and forty-two milligrams of calcium, and 0.59 milligrams of riboflavin (Ojiewo et al., 2013a; Klocke et al.If 100 grams of the fresh vegetable are ingested, the consumer will receive 100% of the recommended daily amount for B-carotene, iron, calcium, and ascorbic acid, and 40% of the recommended daily allowance for protein (Abukutsa et al., 2005). Vitamins A and C, fat, fiber, and minerals including iron, calcium, and phosphorus can all be found in high concentrations in the green African nightshade (Zebish et al., 2016). However, the nutrient composition of African nightshade differs depending on the age of the plant, the plant type, and the soil fertility of the place where it is produced (Jagatheeswari et al., 2013).

Earaches, convulsion therapy, pain relief, anti-helminthes, antiseptic, ringworm, ulcers, blood pressure, and heart disease are only few of the conditions for which

African nightshade has been used medicinally over the world (Jagatheeswari et al., 2013; Matasyoh and Mwaura, 2014).

Market demand has risen alongside rising consumer awareness of African nightshade's nutritional and medicinal value (Ojiewo et al., 2013), but supply has lagged behind due to a lack of new, improved varieties developed by scientists. African nightshade output must be raised for commercial purposes if it is to increase to a level that can hope to keep up with market demand.

Ojiewo et al. (2013) list consumer knowledge, human activity, climate change, disease, and pest as major challenges to African nightshade cultivation. High production costs and a dearth of disease- and pest-resistant varieties have also hampered trade (Ojiewo et al., 2013; Schafer et al., 2006).

The sustainable and cost-effective production of nightshade is in jeopardy due to bacterial wilt (Schafer et al., 2006). This disease is caused by the soil-borne pathogen Ralstonia solanacearum (Sikoru et al., 2004), which infects the crop through wounds, cracks, or roots (Pradhanang et al., 2005). The bacterial wilt induces wilting by impeding the upward movement of water up the plant (Genin, 2010; Kelman, 1954). As a result, the disease significantly restricts crop yield.

Furthermore, Ralstonia solanacearum exhibits some remarkable characteristics, including the capacity to persist in soils for extended periods of time and develop latent infections (Hayward, 1991; Wenneker et al., 1999).Despite being primarily found in tropical and subtropical regions (Hayward, 1991; Hayward et al., 2015), the pathogen has also been identified in North America and Europe, where strains tolerant to frigid temperatures were introduced during the 1990s (Janse et al., 2004; Swanson et al., 2005). The dissemination of Ralstonia solanacearum poses a threat to agricultural produce, and the pathogen is classified as a quarantine bacterium.

The crops most significantly impacted from this phenomenon are tobacco, tomato, and potato, where it can result in crop loss of up to 90% (Mallikarjun et al., 2008), and eggplant, where it can cause crop loss of up to 70% (Zebish et al., 2016). On nightshade, however, none have been reported.

The pathogen exhibits long-lasting persistence in soil, plant debris, and water (Muthoni et al., 2010). Frequently, smallholder farmers lack awareness regarding the severe nature of the pathogen (Coyne et al. 2006a, 2006b).Additionally, the home seed selection method does not guarantee clean planting materials. Smallholder farmers choose seeds that appear healthy to the naked eye but lack confirmation that they are free from bacterial wilt infection for production. They are unaware of the severity and prevalence of quarantine bacteria and contagious soil-borne pathogens (Coyne et al., 2006a, 2006b). As a result, it is challenging for them to comprehend the true nature of the problem and to devise intervention strategies for the crop. Therefore, it is necessary to assess the resistance of African nightshade *Solanum nigrum* L to Bacterial wilt Ralstonia solanacearum and its genetic diversity in Western Kenya.

1.2. Statement of the problem

Despite the significance of African nightshade as a nutritous vegetable and having inherent medicinal value (Nandhini *et al.,* 2014), African nightshade is still neglected, few farmers are growing it, there is potential reduction of food supply because very few improved varieties have been released. The reason for the inconsistent findings in studies on African nightshade is primarily due to the limited scope of genetic diversity investigations. These studies have been exploited, but the existing information lacks consistency across different studies. This inconsistency can be attributed to the fact that certain morphological traits of African nightshade are influenced by environmental conditions. Consequently, the expression of these traits may vary from one environment to another (Dhasmana *et al.,* 2007). The African nightshade complex comprises various species. However, due to the ploidy levels that differ among these species and the similarity of morphological traits among them resulting from their nearly identical genomes, numerous researchers have a tendency to classify these species as S. nigrum L. (Nandhini and Paramaguru, 2013).Although consumers are capable of distinguishing various *Solanum nigrum* varieties, this does not imply the presence of diversity. Despite farmers' diligent efforts to enhance production levels in response to the escalating demand for African nightshade, they encounter a primary obstacle in the form of limited availability of disease and pest-resistant varieties (Ojiewo et al., 2013) and substandard seed. Consequently, they are compelled to reuse saved seed, inadvertently facilitating the transmission of the quarantine bacterium, a soil-borne pathogen that inflicts severe damage on African nightshade plants (Coyne et al., 2006a, 2006b; Muthoni et al., 2010).Hence, in order to cater to the preferences of consumers, it is imperative to develop additional enhanced varieties that are resistant to diseases and pests, as well as resilient to the impacts of climate change.

1.3. General Research objective

The general objective of the study was to Reverage the importance of African nightshade *Solanum nigrum L* and the effect of bacterial wilt *Ralstonia solanacearum* which is rampart in Western Kenya for the purpose of future improvement.

1.3.1. Specific objectives

The specific objectives of the study were to:

I. Determine morphological characteristics of African nightshade accessions.
II. Determine molecular characteristics of African nightshade accessions using Simple Sequence Repeats markers.
III. Assess resistance of African nightshade accessions to bacterial wilt

1.3.2 Hypothesis

H_{01}: There are no phenotypic variations among African nightshade accessions grown in Western Kenya.

H_{02}: There are no genetic variations among African nightshade accessions grown in Western Kenya.

H_{03}: There is no resistance to bacterial wilt among African nightshade accessions grown in Western Kenya

1.4. Justification and significance

African nightshade types grown exhibit diverse characteristics with varied farmer preferences. Diversity studies provide information for the improvement of improved high yielding varieties to meet the increasing demand (Bhat and Kudesia, 2011), also for the management of effective conservation program and utilization of available germplasm (Nandhini *et al.,* 2014).

The production of African nightshade is threatened by bacterial wilt (Schafer *et al.,* 2006), caused by *Ralstonia solanacearum,* it is a soil borne disease (Sikoru *et al.,* 2004). Bacterial wilt (*Ralstonia solanacearum*) infects the susceptible crop (Pradhanang *et al.,* 2005) through roots, clogging the xylem vessels (Genin, 2010), and spreads rapidly to aerial parts of the plant through the vascular system where its high level of increase leads to wilting symptoms and, eventually, plant succumbs (Kelman, 1954).

The greatest economic loss due to *Ralstonia solanacearum* has been reported on tobacco, tomato and potatoes sharing family tree solanacae on which it causes up to 90% crop loss (Mallikarjun *et al.,* 2008), none has been reported on African nightshade. The lethality of *Ralstonia solanacearum* is enhanced by its ability to survive in the soil and water for several years (Mallikarjun *et al.,* 2018; Muthoni *et al.,* 2010) thus difficult to control once it establishes itself in the field.

Small holder farmers are often unaware of the severity of this economically significant disease of solanaceous vegetables (Coyne *et al.,* 2006a, 2006b) which is yield limiting disease thus difficult to understand the real problem and hard to plan strategies for intervention.

To sustain high yields, there is need to determine genetic diversity of African nightshade and develop resistant varieties to disease of quarantine importance such as *Ralstonia solanacearum.*

Findings from this study will add to the academic body. It will also elicit discussions on new areas of research to evaluation of genetic diversity of African nightshade and the development of consumer preferred disease resistant varieties of African nightshade to bacterial wilt for purpose of commercialisation of the crop and will help address food and nutrition security.

1.5 Scope of the study

The study covered the three regions (Bungoma, Kakamega, and Trans Nzoia) in Western Kenya. The study area was purposely selected due to high production and consumption of African nightshade. The study was limited to African nightshade growing farmers (farms). Genetic Diversity study and screening of *Ralstonia solanacearum* was limited to only African nightshade accessions collected from Bungoma, Kakamega and Trans NzoiaAfrican nightshade growing regions in Western Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter critically reviewed related literature organized according to the study objectives. The review was on the concept of different approaches and methods of assessing diversity studies of African nightshade and evaluation of resistance of African nightshade and to related crops against bacterial wilt.

2.2 Description of African nightshade species

The section Solanum contains a variety of taxa that comprise African nightshades (Shackleton et al., 2009). In sub-Saharan Africa, nightshades of African origin are predominantly utilized as leafy vegetables (Ojiewo et al., 2013; Binaryuy et al., 2002; Chweya and Eyaguirre, 1999; Edmonds and Chweya, 1997). Additionally, they possess medicinal properties (Schippers, 2000).Despite substantial variation, only the utilization of minor species is widely recognized.

Solanum scabrum, S. villosum, S. americanum, S. sarrachodes, and S. retroflexum are the most commonly cultivated species for vegetable production (Mwai et al., 2007; Ojiewo et al., 2013). Certain species have a restricted distribution, while the majority are found in diverse geographic regions (Maundu et al., 2009). African nightshade species were historically preserved and employed by farmers for cultivation purposes in gardens or for harvesting from the wild (Dinssa et al., 2014).

Due to the subsistence level of production, the area dedicated to cultivation remains modest (Tuwei et al., 2013). Based on empirical evidence, African nightshades are abundant in micronutrients (Kamga et al., 2013; Luoh et al., 2014), straightforward to cultivate, and thus possess the capacity to provide rural impoverished communities

with nutritional security and a means of subsistence. The recognition of its elevated nutrient content has resulted in a surge in consumer demand surpassing production capacity.

In Western Kenya, low yields may be the result of environmental factors, pests and diseases, and ineffective agronomic practices. Nonetheless, infection with the soil-borne pathogen Ralstonia solanacearum, a quarantine bacterium (Dinssa et al., 2013), and the lack of information regarding the taxonomy of the species, which has resulted in breeders releasing few consumer-preferred varieties, remain the primary obstacles to production. Certain scientists have employed common pinpointing characteristics to classify the species comprising section Solanum; however, these characteristics vary considerably, with some species within the section exhibiting variable morphology.

2.3 Taxonomy of species of African nightshade

Genetic diversity is the result of genetic variations occurring both within cultivars of a given species and between species. Certain species may exhibit morphological similarity, which could potentially lead to taxonomic confusion.

Despite the fact that African nightshade are morphologically similar, they differ genetically and that is why african nightshade has been disclosed to be rich in genetic diversity (Ojiewo et al., 2013 a). Diverse species exhibit morphological variation with regard to, among other things, pubescence, stem color, inflorescent orientation, plant growth behaviors, leaf shape, and stem ridging.

Varieties of African nightshade thrive in a wide range of environmental conditions, which explains their global distribution. They thrive at mild, high-moisture conditions at medium to high altitudes, with temperature ranges of 15–30ºC for germination and 20–30ºC for growth, and require an annual precipitation of 500–1200mm. (Ojiewo et

al., 2013) found that African nightshade genotypes with broad leaves are more susceptible to water stress than those with narrow foliage.

It is postulated that various species of African nightshade originate from disparate global regions. South America is considered to be the origin of diploid species such as S. americanum and S. sarrachoide, whereas Africa, Europe, and Asia are considered to be the birthplaces of tetraploid species S. villosum and S. retroflexum, and hexaploid species S. nigrum and S. scabrum, respectively. It is hypothesized that the hexaploid species of African nightshade resulted from the hybridization of the diploid Solanum americanum and the tetraploid Solanum villosum Mill (Edmond and Chweya, 1997).A genetic evaluation was conducted on thirty accessions of African nightshade that were collected in Western Kenya in order to ascertain their morphological and genetic distinctions as well as similarities.

2.4 Production, utilization and marketing of African nightshade in Kenya.

Kenya is confronted with significant food insecurity, as 56% of its population lives below the poverty line.A significant proportion (50%) of the Kenyan populace faces food insecurity due to factors such as protracted drought, extreme poverty, and rapid population growth (FAO, 17). Consequently, an excessive dependence on inadequately nourishing diets has ensued, resulting in infant mortality and malnutrition in rural and semi-urban regions. Enhancing food security and promoting appropriate nutrition in developing nations can be accomplished by increasing the production, awareness, and utilization of nutrient-dense indigenous leafy vegetables like African nightshade (Oniang'o et al., 2005; Ondieki et al., 2011).

Kenya has cultivated African nightshade for several centuries; it is one of numerous indigenous leafy vegetables that Kenyan producers from numerous communities continue to cultivate (Ondieki et al., 2011). The African nightshade is a native leafy vegetable that is widely consumed and predominantly available in the country. Kenyan producers produce yields ranging from 1.5 to 3 tons per hectare (Ojiewo et al., 2013). Leaf expansion is impeded by early flowering and excessive fruiting, which ultimately leads to reduced harvests. In Eastern Africa, there is evidence of a greater preference for indigenous vegetables over exotic ones. This preference can be attributed to the fact that indigenous vegetables require less labor to cultivate and are economically viable for rural households with limited financial resources (Ojiewo et al., 2013).

Varieties of African nightshade vegetables differ with regard to leaf yield, nutritional value, and growth pattern. S. solanum, S. scabrum, and S. sarrachoides are the typical species cultivated in Kenya. Solanum villosum subsp. Villosum is a species of S. villosum characterized by dentate leaf margins and orange-colored mature berries; similarly, Solanum villosum Miller subsp. Miniatum has entire, sinuate, sinuate-dented, or dentate leaf margins; and orange-colored mature berries. Solanum scabrum Miller is distinguished by mature berries that are dark purplish black and have entire to sinuate leaf margins. In contrast, Solanum sarrachoides produces mature berries that are light green in color and has dentate leaf margins that are densely pubescent with distinctly lobed leaf margins (Ashilenje et al., 2012).

The broad leafed African nightshade cultivar (Solanum scabrum) is a prevalent and auspicious species of African nightshade in Kenya. It is distinguished from others by its substantial purple berries and its wide leaves. Although its leaf size and plant

height vary, its leaf production remains superior to that of narrow leafed species such as Solanum villosum and Solanum eldoretianum. Solanum scabrum, as documented by Abukutsa et al. (2005), is a widely utilized and distributed species of African nightshade within the nation.

African nightshade is frequently cultivated in household gardens in Kenya, frequently accompanied by cereals, vegetables, or grains such as maize, sorghum, or millet. Particularly in urban areas, African nightshades are in high demand, and the supply is insufficient to meet that demand. According to a study conducted at the Kakamega municipal market, African nightshade ranked third in terms of quantities sold and was one of the ten most frequently consumed vegetables (Abukutsa et al., 2005).

Funding for research and heightened awareness regarding the significance of African indigenous vegetables, such as African nightshade, contributed to a tenfold increase in their production in the peri-urban regions of Nairobi between 1997 and 2007. As a result, farmers in these areas experienced a $200 annual net income surge (Biodiversity 2013). The annual leaf utilization of African indigenous vegetables in Nairobi was assessed at 31 tons in 2003, with a corresponding value of USD 6000. This figure has exhibited a consistent upward trend, reaching 600 tons in 2006 at a cost of USD 142,000 (Opiyo et al., 2015).

African nightshade production encounters significant obstacles, including neglect and stigmatization. This is primarily due to the perception that indigenous vegetables, which occasionally occur naturally, were antiquated and deemed the diet of the poor. In addition to being considered weeds, which impoverished individuals in rural regions gather for nutritional purposes (Mwangi and Kimathi, 2006), they are also

susceptible to pests and diseases, most notably the quarantine-destroying pest Ralstonia solanacearum. The aforementioned limitations have led to suboptimal vegetable yields ranging from one to three tons per hectare, which is significantly lower than the optimal yield range of twenty to forty tons per hectare (Abukutsa et al., 2005; Mwangi and Kimathi, 2006).Farmers continue to utilize conserved seed without being informed of the dissemination of the quarantine bacterium, a highly destructive soil-borne pathogen that significantly reduces crop yield.

2.5 Characterization of the African nightshade

Genetic diversity denotes the equilibrium between the loss and gain of genetic variation and is a quantitative measure of the variation within a population (Carvalho et al., 2019). Indirectly or directly, biodiversity erosion results in the extinction of plant species. The absence of diversity hinders breeders from creating novel cultivars that satisfy the preferences of consumers.

Biochemical characterization, molecular marker analysis, and morphological examination are methods utilized to ascertain genetic diversity both within and between plant populations (Govindaraj et al., 2015).

Diversity assessment through the utilization of morphological markers is predicated upon visual characteristics such as fruit color, leaf shape, development pattern, and flower hue, among others. While this method does not necessitate advanced technology, it does demand expansive land areas for experimental purposes.

A molecular marker is a quantifiable and trackable genetic locus that is typically linked to a specific trait of interest within a population (Hayward et al., 2015). These markers are capable of identifying variations in chromosomes caused by deletions, duplications, insertions, or inversions. Typically, they are situated in close proximity

to or interconnected with the genes that regulate the trait in question; the markers themselves do not exert any influence on the trait's phenotype.

Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) (Jonah et al., 2011), and Start codon targeted (SCoT) markers are a few of the molecular markers used to investigate genetic diversity in plants (Satya et al., 2016). The current investigation employed six SSR primers to assess the quality of African nightshade accessions obtained from three distinct localities in Western Kenya.

2.6. Genetic diversity of African nightshade in Kenya

The presence of variation among African nightshade species discovered in Kenya has been documented (Matasyoh et al., 2015). Previous research has demonstrated the presence of genetic diversity among African nightshade cultivars, both within and between species. For instance, Manoko et al. (2007) identified genetic variation among cultivars of S. scabrum and S. nigrum. However, there is a lack of studies that have specifically examined the genetic makeup of African nightshade species cultivated in Western Kenya.

2.7. Measuring diversity in African nightshade.

The examination of diversity is crucial in plant breeding because it provides the data necessary for the identification of desirable parental lines to cross in order to produce hybrids that produce higher yields than the parents. This process involves morphological and genetic characterization of existing cultivars (Matta et al., 2015). An increase in hybrid vigor observed in the offspring is proportional to the genetic distance between parental lines and genetic diversity (Khodadadi et al., 2011).

Hierarchical cluster analysis and multidimensional clustering based on principal component analysis, principal coordinate analysis (PCoA), and principal coordinate analysis (PCoA) are techniques used to quantify genetic diversity. Prior to calculating genetic distance, variable standardization is necessary; nevertheless, it diminishes the disparities between groups; consequently, the outcomes of principal component analysis and hierarchical cluster analysis may differ; thus, principle component analysis may be omitted when employing hierarchical cluster analysis (Khodadadi et al., 2011).

In the context of assessing hierarchical relationships and measuring genetic diversity, cluster analysis is generally favored over principal component analysis (Ravishanker et al., 2013). Utilizing descriptors, hierarchical analysis illustrates the relationship between or within genotypes; the outcomes are typically depicted in the form of a dendrogram, which further illustrates the genetic interaction within the clusters. Molecular, biochemical, pedigree, and morphological markers can all be employed to evaluate genetic diversity (Osawaru et al., 2015).

Morphological characterization is more labor-intensive than molecular characterization due to the need for a substantial area of land to conduct field experiments, despite the fact that it is more cost-effective. Morphological characteristics are typically susceptible to environmental influences, which consequently impact the genetic diversity under assessment. Biochemical analysis is a technique that separates proteins into distinct banding patterns using minute quantities of biological reagents. Nevertheless, a significant drawback of this method is the

restricted availability of enzymes, which significantly limits the observed diversity (Singh et al., 2011).

Morphological characterization permits comprehensive physical sampling and the use of large samples; however, due to their susceptibility to interference, these traits do not provide genetic information regarding a specific genotype nor do they quantify the precise genetic diversity present. In contrast to morphological characteristics, molecular markers offer a precise assessment of genetic diversity due to their immunity to environmental perturbations.

Molecular analysis involves the utilization of diverse DNA markers to evaluate genetic variation, thereby rectifying the errors that may occur during phenotypic characterization (Mondini et al., 2009). The correlation between molecular markers and the phenotypic manifestation of a genomic trait can vary. Molecular markers provide more accurate results than morphological characterization due to their stability and presence across all tissues, irrespective of cellular growth, differentiation, development, or defense status. Furthermore, these markers remain unaffected by environmental factors, pleiotropic influences, and epistatic effects (Mondini et al., 2009).

Historically, genetic diversity has been determined through phenotypic characterization; it remains an essential component in the evaluation and analysis of germplasm.

Additionally, molecular markers provide an enormous number of characters for analysis, enabling the differentiation of cultivars that were previously considered morphologically similar phenotypically. However, the high cost of molecular markers restricts the sample size that can be utilized for analysis (Ojiewo et al., 2013). By integrating molecular and morphological techniques for characterization, high-

resolution genetic diversity studies are possible (Tumbilen et al., 2011; Omondi et al., 2016). The current investigation employed cluster and hierarchical analysis techniques to assess the molecular and morphological diversity of accessions of African nightshade procured from Western Kenya.

2.8. Bacterial Wilt disease

Bacterial wilt, which is predominantly found in solanaceous cereals, is a soil-borne disease caused by Ralstonia solanacearum (Sikoru et al., 2004).As a quarantine bacterium, Ralstonia solanacearum is recognized for its ability to infect more than two hundred plant species and induce substantial agricultural setbacks, particularly in economically valuable solanaceous commodities. Except for Antarctica, it has been discovered on every continent (Swanson et al., 2005, 2007). Genetic diversity characterizes R. solanacearum, which comprises five distinct races, four phylotypes, and five biovars. Virulence, symptom manifestation, and host range vary among races, whereas biovars are distinguished by their distinct biochemical capabilities to oxidize a variety of disaccharides and hexose alcohols. In addition to racial and biovar variations, R. solanacearum can be subdivided into phylotypes. Race 1 R. solanacearum biovar 1 is a prevalent and widespread parasite in the southern region of the United States.

Ralstonia solanacearum poses a significant obstacle to the successful cultivation of these particular commodities in both tropical and temperate climates. The most significant financial setbacks have been attributed to tobacco, tomatoes, and potatoes, which Mallikarjun et al. (2008) estimate can result in a 90% reduction in crop yield. In most instances, the presence of root nematodes in conjunction with the pathogen R.

solanacearum exacerbates the severity of the disease (Deberdt et al., 1999). According to Chen (1984), nematode infestation in tobacco can lead to physiological changes that render the plant vulnerable to bacterial wilt. This phenomenon has also been documented in the case of *Solanum nigrum*.

2.8.1 Biology and Ecology

Gram-negative soil and waterborne pathogen R. solanacearum R3 Bvr2 (Wenneker et al., 1999; Swanson et al., 2007; Marco-Noales et al., 2008). Resistance to R. solanacearum has been observed in non-hosts, the rhizosphere, infected plant detritus, and the roots of hosts (Wenneker et al., 1999; Janse et al., 2004). Yeast extract-peptone-glucose agar (YPGA), non-selective media including triphenyl tetrazolium chloride (TTC/TZC) agar and casamino acid peptone glucose (CPG), or semi-selective media (SMSA) are common media used to cultivate the bacterium. SMSA is maintained at temperatures ranging from 28°C to 29°C (82.4°F to 84.2°F) (Cook and Sequeria, 1991; Caruso et al., 2003; Ozakman and Schaad, 2003; Marco-Noales et al., 2008).

Due to its ability to persist for extended periods of time in soil and water, R. solanacearum R3 Bvr2 is capable of infecting its host through a variety of mechanisms. By penetrating the plant's xylem tissue via incisions, the pathogen can cause colonization, wilting, and eventual demise (Milling et al., 2009). Young root filaments are another means by which R. solanacearum can infiltrate the plant (Swanson et al., 2007). Bacteria are discharged into the environment during the decomposition of infected plants; this can also transpire via plant lesions, where bacteria generate a "matrix of protective polysaccharides" that facilitates their survival

(Van Elsas et al., 2000). Upon being discharged, irrigation water can readily disseminate millions of bacterial cells (Swanson et al., 2007).

Additionally, Ralstonia solanacearum R3 Bvr2 is colder tolerant than the native/endemic species of the United States, race 1 biovar 1 (R1 Bvr1). R3 Bvr2 is a highly dangerous pathogen in regions that cultivate solanaceous commodities (tomato, potato, etc.) due to its resistance to cold. R. solanacearum R1 Bvr1 is predominantly observed in temperate regions of the Southern United States; however, R3 Bvr2 might have the potential to persist both above and below the mid-Atlantic line. R. solanacearum R3 Bvr2 has the ability to endure winter temperatures as low as 4°C (39.2°F) in dormant soils in Australia (Milling et al., 2009).

According to Swanson et al. (2005), this pathogen has the ability to remain undetected in latent hosts or symptomless hosts that do not exhibit symptoms when exposed to unfavorable environmental conditions. This attribute may pose challenges in conducting comprehensive surveys for this parasite. Subsequent to the removal of contaminated crops, this pathogen has been observed to persist for a duration of up to two years in deep soil in Australia, Kenya, Sweden, and the United Kingdom, all of which have moderate environmental conditions (Wenneker et al., 1999; Van Elsas et al., 2000). Regarding host resistance, little is known. Gorissen et al. (2004) discovered that the survival and population size of R. solanacearum R3 Bvr2 in soil can be diminished through the use of solarization and swine waste.

2.8.2 Symptoms and Signs

Wilted leaves are usually the first symptom observed followed by chlorosis and plant death (Champoiseau *et al.,* 2009).

2.9 Bacterial wilt (*Ralstoniasolanacearum*) disease management

Once R. solanacearum has infiltrated the soil, it is difficult to control it (Jones, 2008). This is true for a wide variety of solanaceous commodities, including potatoes, eggplants, tomatoes, and weeds like Jimson weed (Datura spp.). (Smith et al., 1995) State that the incidence of this disease can be substantially diminished through the use of resistant cultivars and crop rotation every 5 to 7 years.

It has been demonstrated that crop rotation can reduce the incidence of diseases; however, this management technique remains inconsequential due to the pathogen's ability to infect volunteer crops in the solanaceae family and Jimson weed (Datura spp.), which renders it problematical (Fajinmi et al., 2010). Weed management, when combined with crop rotation, has the potential to significantly decrease the occurrence of diseases (Allen et al., 2005).

The spread of the disease can be prevented by planting certified disease-free seedlings from registered propagators, disinfecting equipment after working in a field, employing controlled flood irrigation, and avoiding overhead irrigation on solanaceous crops (McCarter, 1991).

Chemical control of bacteria presents a formidable obstacle due to the pathogen's ability to persist in the soil and its location within the xylem.The bacterial wilt disease is not amenable to any known chemical control (Hartman et al., 1994), and bacterial control with chemicals is also challenging (Grimault et al., 1994).

2.10 Host resistance

Due to the character of the pathogen and the lack of resistance in solanaceous crops, it has been difficult to implement host plant resistance as a field control measure for Ralstonia solanacearum. The emergence of tobacco genotypes with hypersensitive responses, which are indicative of gene-for-gene interaction genes, has been facilitated by the existence of a significant resistance gene (Robertson et al., 2004). Nevertheless, the existence of a comparable significant resistance gene in *Solanum nigrum* C remains unknown, and plant scientists remain perplexed by the wide range of variants exhibited by this pathogen.

The wide diversity of pathogenic Ralstonia strains has resulted in the emergence of resistant strains that exhibit efficacy in specific growing regions while remaining ineffective in others (Scott et al., 2005).

Initial investigations into the physiological processes underlying bacterial wilt resistance in the Solanaceae family postulated that genotypes resistant to the host restrict the migration of bacteria from the soil environment to the collar and mid-stem. While host resistance is a cost-effective control method, developing cultivars that exhibit resistance in different geographical areas can be difficult (Abedayo et al., 2009). Resistance of the crop is frequently surmounted by the pathogen's genetic diversity and environmental interactions influenced by genotype (Nguyen and Ranamukhaarachchi, 2010).

Integrated management of bacterial wilt disease includes host resistance as an efficient and effective component; certain tomato cultivars offer moderate resistance to bacterial disease (Peregrine, 1982). It has been reported that resistant varieties are

the most practical and effective method for preventing bacterial wilt (Black et al., 2003; Grimault et al., 1994). The extensive host range of the diverse species group Ralstonia solanacearum (Kelman et al., 1961) presents a challenge in the development of resistant breeding techniques. Resistance to Ralstonia solanacearum in certain solanaceous cultivars, including tomato genotypes, has been documented (Gomes et al., 1998). However, there is a lack of research examining resistance in African nightshade.

The selection of resistant material may be influenced by factors such as the strain type of Ralstonia solanacearum, the genetic variability of the plant, and the reproducibility of the inoculation technique (Prior et al., 1990a). Some cultivars of Solanum spp. that are resistant to Ralstonia solanacearum were developed at the Asian Vegetable Research and Development Center. Nonetheless, their resistance is limited to specific factors such as soil characteristics, climate, locations, and strains of the pathogen (AVRDC, 2003). Certain tomato varieties have been bred to exhibit notable resistance to particular environments (Gomes et al., 1998). However, stability in lowland tropics and regions characterized by high temperatures and humidity is often unattainable due to the deterioration of resistance that occurs during variety transfer (Hayward et al., 1991; Hanson et al., 1996). There have been no investigations conducted on African nightshade species pertaining to the development of substantial levels of resistance in specific environments.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter outlines the materials and methods adopted for this study. It outlines the study area, research design and describes the data collection methods and statistical analyses methods.

3.2 The Study area

The study was carried out in Bungoma, Kakamega and Trans Nzoia counties of Western Kenya.

3.2.1 Bungoma county

The geographical coordinates of the county are longitude 33058' east and 34033' west, latitude 0o26' to $0^0$18' north, and elevations 1384–2100 meters above sea level (GOK, 2013). The region experiences a bimodal precipitation pattern, consisting of heavy downpours from March to June/July and light precipitation from September to November; the annual total precipitation ranges from 1500mm to 2400mm (GOK, 2014). The ambient temperature is 20–32°C. The region's subsurface soils vary in texture from friable loamy clays to sandy clays. The county cultivates a variety of common food commodities, including cassava, cassava, maize, common beans, bananas, sweet potatoes, and even vegetables like African nightshade (GOK, 2014).

3.2.2 Kakamega County

Kakamega County is situated within the geographical coordinates of 34° and 35° east longitude and 0° and 1° north latitude, as well as an elevation ranging from 1,250 to 2,000 meters above sea level (Barasa et al., 2015a and b). The region experiences a

generally hot and humid environment during the majority of the year, characterized by an average annual precipitation ranging between 1,800 and 2,000 mm. The region experiences a bimodal precipitation pattern, with the occurrence of two distinct rainy seasons. The first rainy season, commonly referred to as the "long rains," takes place from March to May. The second rainy season, known as the "short rains," occurs between October and December (Kabubo-Mariara & Karanja, 2007). The mean temperature within the county is recorded as 22.5°C. According to Barasa et al. (2015a), January and February are typically characterized as months with low precipitation. The region experiences consistently elevated temperatures throughout the year, exhibiting marginal fluctuations in average maximum and lowest temperatures, ranging from 28°C to 32°C and 11°C to 13°C, respectively. The average annual evaporation exhibits a significant magnitude and varies between 1,600 mm and 2,100 mm, accompanied by elevated levels of humidity. (Ngetich, 2013). The soils in this region range from loamy to sandy.The common crops grown in the county are; maize, common bean, cassava, sweet potato, groundnut and vegetables such as African nightshade.

3.2.3Trans Nzoia county

With mean maximum (daytime) temperatures varying from 23.4^{o}C to 28.40^{o}C and mean minimum (nighttime) temperatures ranging from 11.00^{o}C to 13.50^{o}C, Trans Nzoia County has a temperate and mild climate. The months of February and January exhibit the highest and lowest recorded extreme temperatures, approximately 34.20C and 6.50C, respectively.

The annual precipitation in the county varies between 1000mm and 1700mm. Long precipitation season (March, April, and May) comprises the annual precipitation; intermediate precipitation season (June-July-August); and short precipitation season

(October-November-December). The region is characterized by deep soils that vary in hue from dark red to reddish brown and are composed of friable granular clay to clay.Common agricultural products in the county include maize, common bean, sweet potato, potato, arrowroot, and African nightshade, among others.

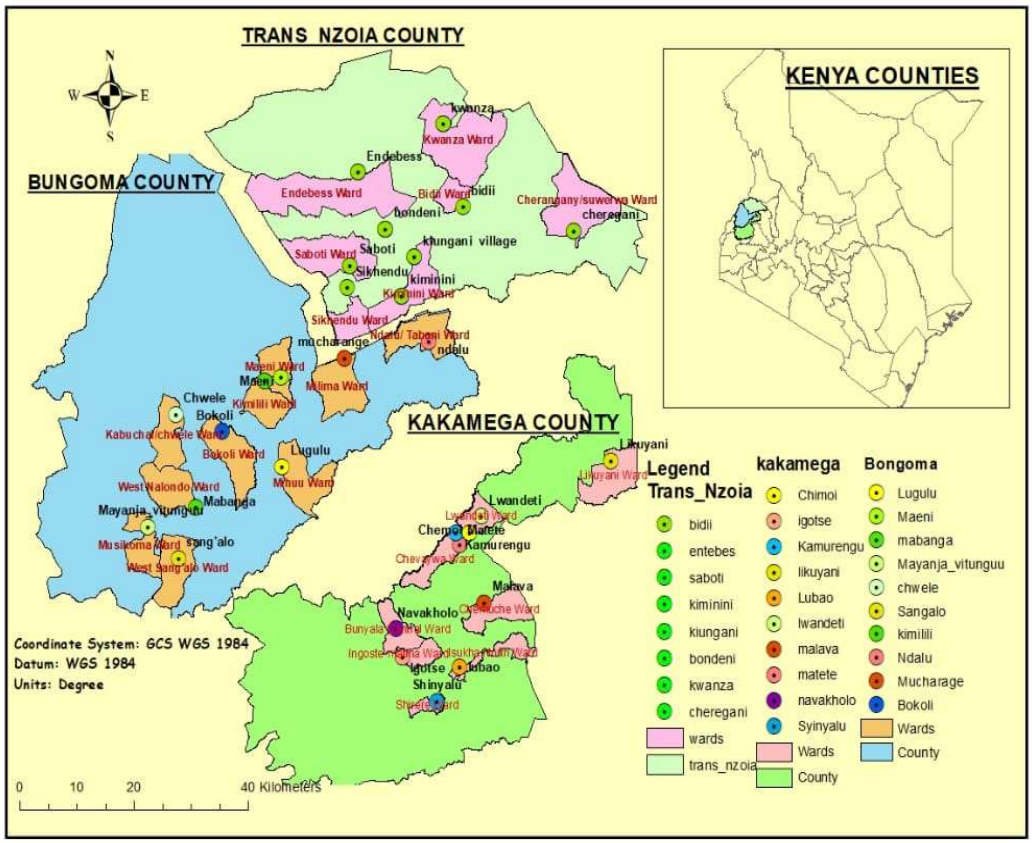


Figure 3. 1: Map showing three counties in Western Kenya where the African nightshade samples were collected. (Source Author, 2021)

Table 3. 1Data description

Data	Data description	Source
Points data	Gps coordinates Longitudes and latitudes	Researcher field data collection
Boundary line data	Kenya administrative boundary: county, wards shapefiles	Kenya Data IEBC

3.3. Sampling criteria

Purposive sampling was employed to select the counties of Bungoma, Kakamega, and Trans Nzoia on the basis of their notable African nightshade production and consumption. A random sampling procedure was implemented, utilizing pre-existing data on the species cultivated and in collaboration with producers. Pre-material collection consultations were conducted with agricultural personnel in the region. Samples were collected within a 0.5-kilometer radius of each location. The farms that cultivated African nightshade were chosen at random. The chosen farms varied in dimensions from 0.3 to 2 hectares, and their African nightshade production was conducted on a modest scale. A random selection was made of five mature plants bearing ripe fruits from each farm, and seeds of the identical accession were obtained from the producers as well. The majority of farms and producers engaged in African nightshade cultivation across the three regions were incorporated into this research endeavor. Furthermore, a grand total of thirty seed samples representing the accessions were gathered from the three designated counties, with each county contributing ten samples. The aforementioned materials were designated with the farm (farmer) number and collection area as follows: Bungoma (B), Kakamega (K), and Trans Nzoia (T). (B Appendix)

3.4 Morphological characteristics of African nightshade in Western Kenya

3.4.1 Experimental design

The experiment was laid out in a randomized complete block design with three replications each.

3.4.2 Experimental layout

The land underwent ploughing and harrowing processes until it reached a state of fine tilth. A total of thirty accessions of African nightshade were planted at the Kibabii University Agricultural farm, employing a randomised full block design. The experiment consisted of three replications, with each accession being represented once inside each block. The seeds were sown at a depth of 1cm in single row plots measuring 3 meters by 4 meters. The spacing between plants was 30cm, while the spacing between rows was 40cm. Additionally, there was a distance of 1 meter between blocks. This sowing practice was followed for both the short and long rainy seasons. Diammonium phosphate (DAP) was administered at a rate of 0.012 kilograms per hectare (kg/ha) and was effectively incorporated into the upper layer of soil during the planting process. The assessment of plant stand count, specifically the germination percentage, was conducted at the two-week mark following the planting process. Thinning procedures were implemented to guarantee the attainment of a desired plant stand consisting of 10 plants per plot..

3.4.3 Data Collection

The data was gathered from four plants that were selected at random from the midpoint of each allotment, in accordance with the criteria outlined by Nandhini et al. (2014).A phenotypic descriptor list was compiled and constructed based on the phenotypic characteristics outlined by Singh et al. (2003) of the National Bureau of Plant Genetic Resources (NBPGR) for the four seedlings.

i. Leaf margin: The margins of the leaves were observed when the plant started flowering and scored as Entire or Sinuate or sinuate dentate.

ii. Leaf surface was determined by feeling the texture of leaf surface using the hand and scored for presence or absence of hairs.

iii. Stem ridge: Was evaluated at maturity when plants started fruiting and was determined by feeling the texture of the entire stem of the plant using hand and scoring for presence or absence of ridges.

iv. Colour of the berry/ fruit: Was evaluated when berry change colour from green. The colour was determined by visually observing and recording the colour of mature and ripe fruits against fruit colour chart. The colours of the mature fruits were scored as green or purple or orange.

v. Inflorescent type: Was determined when the flower was mature and scored as Forked or simple

vi. Leaf shapes: Was determined at flowering and scored as Ovate or Lanceolate

vii. Plant type: Was evaluated at flowering stage and scored as Semi erect or erect

Table 3. 2: Characters used in morphological analysis of Western Kenya accessions of African nightshade

Character	Scoring method
Leaf margin	Entire or Sinuate or Sinuate dentate
Leaf hair surface	Presence or absence
Stem ridge	Presence or absence
Berry colour	Green or purple or orange
Inflorescent type	Forked or simple
Leaf shape	Ovate or lanceolate

Plant type	Erect or Semi erect

Data collected (Appendix C) was analysed using PASW Version 20 Statistical analysis package and a dendrogram drawn using hierarchical cluster analysis procedure and Euclidian average distance.

3.5 Molecular characteristics of African nightshade in Western Kenya

The study utilized thirty African nightshade accessions' seeds procured from farmers residing in African nightshade-growing regions of Kakamega, Bungoma, and Trans Nzoia (see Appendix B).In a glasshouse, the African nightshade accessions were sown in containers containing extremely fine soil that had been sieved through a 60-gauge mesh.

3.5.1 Leaf harvesting and DNA extraction

The leaves of African nightshade plants, which were four weeks old and in a young, tender, and healthy state, were collected from each accession. These leaves were carefully wrapped in foil paper and promptly transported to the laboratory. Upon arrival, they were stored in a refrigerator set at a temperature of -20°C to preserve their quality. The leaves were subsequently washed with distilled water in order to eliminate any soil particles present on their surfaces, following the methodology employed by Agbagwa et al. (2012). The leaves of African nightshade accessions were measured and 200mg of leaves from each accession were carefully pulverized into a smooth paste using a mortar and pestle in 500µl of CTAB buffer. The sample was thereafter placed into a microcentrifuge tube and subjected to incubation for a duration of 15 minutes at a temperature of 55°C within a recirculating water bath. The CTAB/plant extract mixture was subsequently subjected to centrifugation at a speed

of 12000 revolutions per minute (rpm) for a duration of five minutes in order to separate and sediment the cellular debris. Subsequently, the liquid portion (supernatant) was carefully transferred into sterile microfuge tubes, with an added volume of 250 µl of chloroform. Isoamyl alcohol (24:1) was introduced into each tube, and the solution was thoroughly mixed by gently and repeatedly inverting the tubes. Subsequently, the combination underwent centrifugation at a speed of 13000 revolutions per minute for a duration of one minute. The resultant upper aqueous phase, which harbors the DNA, was meticulously transferred into a sterile microfuge tube. Each tube was supplemented with 50µl of 7.5M ammonium acetate, and then, 500µl of ice-cold 100% ethanol was introduced. The tubes were subsequently inverted in a deliberate and cautious manner on multiple occasions in order to induce the precipitation of DNA. The DNA that had precipitated was observed to gather at the base of the tubes, and the liquid portion above, known as the supernatant, was cautiously decanted by pouring it out gently from the tubes, ensuring that the DNA pellets remained undisturbed. Subsequently, the DNA pellet underwent two rounds of washing with ice-cold 70% ethanol. After the washing process, the DNA sample was subjected to centrifugation at a speed of 13000 revolutions per minute (rpm) for a duration of 1 minute. Subsequently, the liquid portion above the sedimented material, known as the supernatant, was carefully eliminated. The DNA was subsequently desiccated by inverting the tube housing the DNA onto a sterile paper towel for a duration of 14 minutes, with precautionary measures implemented to prevent the DNA pellet from dislodging from the tube. The tubes containing the DNA pellets were subsequently positioned in an upright orientation and, while being shielded by paper towels, were allowed to remain undisturbed for a duration of 30 minutes to ensure the thorough desiccation of the pellets. Subsequently, the DNA that was

obtained was reconstituted in 400µl of sterile water that was free from DNase. A solution containing 10µl/ml of RNase (10µl of RNase in 10ml of H2O) was subsequently used to eliminate any potential presence of RNA in the sample. Following the process of resuspension, the DNA was subjected to incubation at a temperature of 65°C for a duration of 20 minutes, with the purpose of eliminating any potential DNases that may have been present. The DNA was subsequently kept at a temperature of 4°C to facilitate its utilization in the Polymerase Chain Reaction (PCR).

Section 3.5.2 focuses on the topic of DNA. The process of quantification

The DNA amount and quality of each entry were assessed by subjecting the samples to electrophoresis on 1% (w/v) agarose gels for a duration of 1 hour at 80 volts. The agarose gel was prepared by diluting it in a 100 mL solution of 1 x TAE buffer, which consisted of 0.89 M Tris base, 0.89 M boric acid, and 20 mM EDTA at pH 8.0. The remaining volume of the solution was made up to 900 mL using distilled water. In order to determine the DNA concentration of the African nightshade accessions, a comparison was made using a standard undigested lambda DNA with varying concentrations of 10, 20, 50, 80, and 100 ng. This comparison involved analyzing the band widths and intensities. The gel underwent staining with ethidium bromide (10mg/ml) for a duration of 30 minutes, followed by destaining in distilled water for a period of 20 minutes, prior to being seen using a UV transilluminator. A quantity of high quality DNA ranging from 0.5µg to 1µg was acquired and subsequently diluted to a concentration of 0.01µg/µl using deionized distilled water in preparation for PCR amplification.

The confirmation of DNA extraction quality was conducted using agarose gel electrophoresis. A 3% agarose gel was prepared by weighing 3g of agarose powder

and dissolving it in a conical flask containing 100ml of 1x TBE buffer. The mixture was then heated in a microwave for 3 minutes until the agarose was completely dissolved and a rolling boil was achieved. Subsequently, the conical flask containing the mixture was extracted and subjected to a cooling period of five minutes. The concentration of the substance is 0.5 micrograms per milliliter. Subsequently, ethidium bromide was meticulously introduced into the gel for the purpose of visualization and thoroughly agitated to ensure uniform distribution. The gel combs were subsequently organized within a gel tray to facilitate the formation of wells, which would serve as the designated locations for loading the DNA samples. Subsequently, the gel was meticulously poured onto the tray and left undisturbed for a duration of 20 minutes at ambient temperature on a level platform, until it achieved full solidification. During the gel casting process, precautions were taken to prevent the formation of bubbles inside the gel matrix. This was done because to the potential interference that bubbles could cause to the movement of DNA molecules during electrophoresis. The combs utilized were selected in order to generate a minimum of 31 wells, with one well designated for loading the ladder and the remaining wells allocated for loading the 30 samples. Subsequently, the combs were cautiously extracted subsequent to the solidification of the gel. The gel was subsequently transferred into a gel electrophoretic tank that was filled with a 0.5x TBE buffer, facilitating the loading of the DNA samples.

In the first well, 10µl of a 1kb ladder was introduced, while the remaining wells were filled with a combination of 5µl of each ANS genotype's extracted DNA sample, 5µl of water, and 2µl of 6x loading buffer. The loading process facilitated the sedimentation of DNA samples within the gel wells, preventing their diffusion into the buffer solution. The gel electrophoresis procedure was thereafter performed for a

duration of 30 minutes at a voltage of 100. Following this, the gel was cautiously extracted from the gel tanks and subjected to ultraviolet light exposure, after which it was documented through photography. The confirmation of good DNA extraction quality was established through the observation of high resolution molecular weight bands.

3.5.3 PCR Amplification

The PCR amplification was conducted using a reaction mixture with a volume of 25 µl. This mixture included 2 µl of DNA sample template, 5 µl of 5x PCR buffer, 0.1 µl of Taq polymerase, 0.5 µl of reverse primer, 0.5 µl of forward primer, and 17 µl of double distilled water. In this study, a total of six distinct primers were employed for the polymerase chain reaction (PCR) process. Each reaction involved the utilization of a primer pair, consisting of both a reverse and a forward primer. Following an initial denaturation step of 4 minutes at a temperature of 94°C, a series of 30 cycles were conducted. Each cycle consisted of a 1-minute denaturation step at 94°C, followed by a 1-minute annealing step at a temperature range of 49-55°C, and finally a 1-minute extension step at 72°C. Subsequently, a final extension step of 10 minutes at 72°C was carried out. The amplifications were conducted with the Applied Biosystems 2720 Thermo cycler.

3.5.4 DNA Data analysis

The manual scoring of allele sizes was conducted for each repeatable band observed, utilizing a molecular size ladder. A score of 1 was assigned to indicate the presence of a band, while a score of 0 was assigned to indicate the absence of a band for each primer. The band's size was estimated using a comparative analysis with a 100 base pair molecular size ladder. The matrix data that was generated was utilized for the

purpose of conducting statistical analysis. The researchers conducted cluster analysis in order to determine the genetic relationships between accessions. The resulting genetic distance dendrogram was generated using the Numerical Taxonomy Multivariate Analysis System package (NTSYS-pc) software, specifically version 2.1. The genetic relationships were assessed by computing the genetic similarity matrix using the Euclidean distance metric. This matrix was then utilized in the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) clustering algorithm, which was implemented using the Sequential Agglomerative Hierarchical Nested (SAHN) software. The resulting clustering analysis was visualized using a tree plot.The matrix data was analyzed using Power Marker (version 3.0) software to calculate the major allele frequency, genetic diversity, and polymorphic information content (PIC) indices.

3.6 Screening African nightshade accessions for resistance to Bacterial wilt

3.6.1 Bacterium *Ralstonia solanacearum* sample Isolation and preservation

Diseased African nightshade plants and soil samples were collected through field observations of bacterial wilt symptoms. These symptoms were differentiated from vascular wilts caused by fungal pathogens through visual examination of primary indicators such as wilting and vascular discoloration. Additionally, a bacterial streaming test was conducted by observing the presence of bacterial movement in a glass of water.

Plants that exhibited the exudation of milky white filaments from the severed ends of their xylem, while immersed in a container of water, were selected. A minimum of ten samples of the afflicted plants were gathered from each of the studied agricultural establishments situated in the African nightshade cultivation regions of Western Kenya, specifically Bungoma, Kakamega, and Trans Nzoia county.

The afflicted plants were transported to the Microbiology laboratories at Masinde Muliro University of Science and Technology (MMUST) and Kibabii University, respectively.

In the laboratory, the samples obtained from the fields underwent a washing process using running tap water to eliminate any sand and dust particles. The stems were then subjected to surface sterilization using a 70% alcohol solution. Following this, the stems were crushed and the bacteria were cultivated on Casamino acid Peptone Glucose media.The colonies were grown individually and subsequently transferred to fresh media in order to obtain pure cultures on triphenyl tetrazolium chloride (TTC) agar medium, which is a semi-selective medium.

3.6.2 Preparation of bacterial Inoculum

Bacterial cells were grown in casamino acid, peptone, glucose (nutrient broth) and multiplied by shaking at 28^{o} C for 48 hours, the cells were suspended in distilled water and adjusted to 108 cfu/ml (OD600 = 0.8).

3.6.3 Screening of African nightshade accessions for Resistance to bacterial wilt.

The 30 accessions were cultivated individually in sterile potting mixture within germination trays in a greenhouse. The potting mixture consisted of a ratio of 3 parts sand, 1 part silt, and 1 part compost. The trays received daily irrigation.At the age of four weeks, three seedlings from each accession, each with four to five leaves, were transplanted from the tray to individual pots. The experiment was conducted with three replications for each treatment. The pots were distributed in a completely randomized manner within a greenhouse and were adequately watered on alternating days. One week post-transplantation, inoculations were conducted by applying a 30 ml bacterial suspension with a concentration of 107 CFUml–1 to each pot through soil drenching. Following the inoculation process, the plants were then watered on

alternating days. The plants that were affected by infection were evaluated for indications of wilting, and the presence of pathogens was additionally verified on vulnerable varieties of African nightshade plants, following the methodology outlined by Elphinstone et al. in 1996.

3.6.5 Data collection

The assessment of bacterial wilt status was conducted using a severity scale previously described by Horita and Tsuchiya (2001). Additionally, a disease index was employed, with the following categories: 1 = No symptom (indicating high resistance), 2 = Wilted top young leaves (indicating 25% resistance), 3 = Wilted two leaves (indicating 50% susceptibility), 4 = Wilted four or more leaves (indicating 75% susceptibility), and 5 = Complete plant death with wilted canopy (indicating high susceptibility, 100%).

3.6.6 Data analysis

The disease severity score was subjected to excel and frequency distribution graphs drawn to establish bacterial wilt disease progression of African nightshade accessions.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1. Introduction

This chapter presents the results for the study.

4.2 Morphological characteristics of African nightshade accessions grown in Western Kenya

The dendrogram (Fig. 4.1.1 has two major clusters **A** and **B** linked at a square Euclidean distance of 25 showing that there is a wider variation among the species collected from the three regions of Western Kenya. The dendrogram exhibited a complex clustering pattern, indicating a significant level of diversity among the African nightshade cultivars under investigation. This observation aligns with the principle that a shorter Euclidean distance between dendrogram branches corresponds to a higher degree of similarity among cultivars, while longer branches indicate greater genetic diversity among cultivars (Kalinowski, 2009).These findings suggest that the cultivars within a specific cluster exhibit a higher degree of genetic similarity compared to cultivars belonging to different cluster groupings. The findings presented here are consistent with the research conducted by Nyadanu et al. (2014) on the agro-morphological characterization of eggplant. Cluster **A** links two major clusters at a square Euclidean distance of 16, **A1** links three clusters at a square Euclidean distance of 8. **A2** links two clusters at a square Euclidean distance of 8 while the selection from the counties of Trans Nzoia, Kakamega and Bungoma shows geographic spread but genetic similarities; $\mathbf{A1_1}$ comprises of *Solanum nigrum* (Trans Nzoia 9), *Solanum nigrum* (Trans Nzoia 10), *Solanum nigrum* (Trans Nzoia 1), *Solanum nigrum* (Trans Nzoia 6), *Solanum nigrum* (Kakamega 10), *Solanum nigrum* (Trans Nzoia 3),

Solanum nigrum (Kakamega 8).**A1$_2$** comprises of *Solanum nigrum* (Kakamega 7),*Solanum nigrum* (Trans Nzoia 5), *Solanum nigrum* (Bungoma 2), *Solanum nigrum* (Bungoma 6), *Solanum nigrum* (Bungoma 8), *Solanum nigrum* (Bungoma 5), *Solanum nigrum* (Kakamega 6), *Solanum nigrum* (Bungoma 4) whose accessions had rhomboid shaped leaves with sinuate to dentate and entire margins, and had small dark purple fruit when ripe and a semi erect plant type (Plate 4.1. 1,b and Plate 4.1.2b,f).**A1$_3$**comprises of *Solanum villosum* (Trans Nzoia 8).**A2$_1$** comprises of (*Solanum villosum* (Trans Nzoia 2), *Solanum villosum* (Bungoma 8), *Solanum vilosum* (Kakamega 1), *Solanum villosum* (Kakamega 4),**A2$_2$** comprises of (*Solanum villosum* (Bungoma 10), *Solanum villosum* (Trans Nzoia 7), *Solanum villosum* (Bungoma 1), *Solanum villosum* (Bungoma 3), *Solanum villosum* (Kakamega 5).These had lanceolate leaves with lobed sinuate dentate leaf margins that produced green berries which turned Orange when mature and had an erect plant type (Plate 4.1.1 b and Plate 4.1.1 2 c,f,d). Cluster **B** comprises of *Solanum scabrum* improved (Trans Nzoia 4), *Solanum scabrum* improved (Kakamega 9), *Solanum scabrum* improved (Kakamega 2), *Solanum scabrum* improved (Kakamega 3), *Solanum scabrum* improved (Kakamega 7) these were those species collected from Trans Nzoia and Kakamega County. These cultivars had ovate leaves with entire margins, produced large dark purple berries when mature and had an erect plant type. (Plate 4.1.1, a and Plate 4.1.2, a, b)

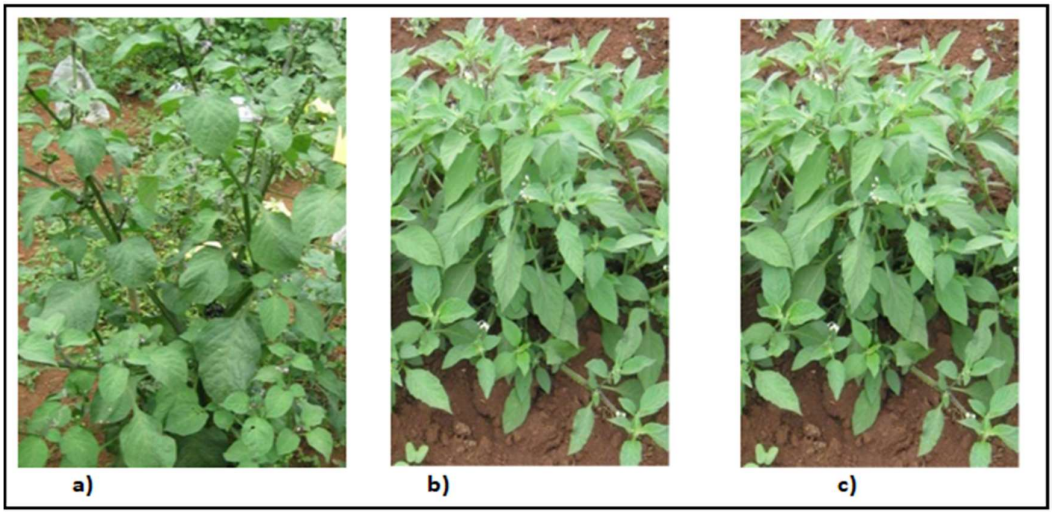


Plate 4.1. 1: African nightshade accessions exhibiting diversity in plant type;

a- Erect. b- Semi erect (c) Semi erect

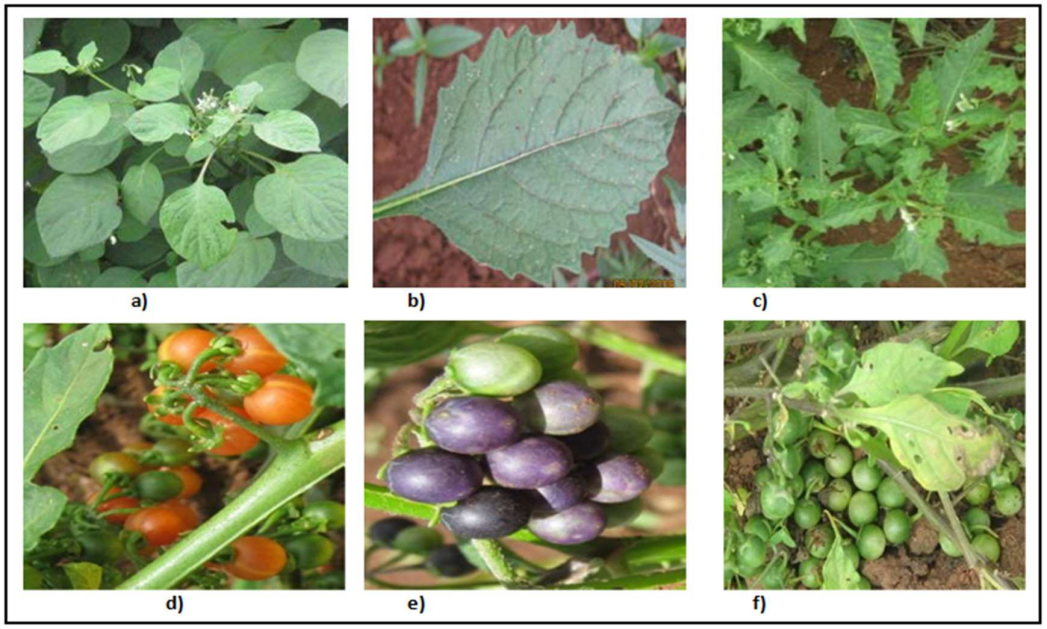


Plate 4.1. 2: African nightshade accessions exhibiting diversity in leaf.

Shape, leaf margin, leaf pubescent and fruit colour; a-Ovate leaves with entire margins, b- Rhomboid leaf with sinuate-dentate margins, c- Lanceolate leaves with sinuate to dentate margins, d- Orange berries, e- Purple berries, f- unripe Green berries

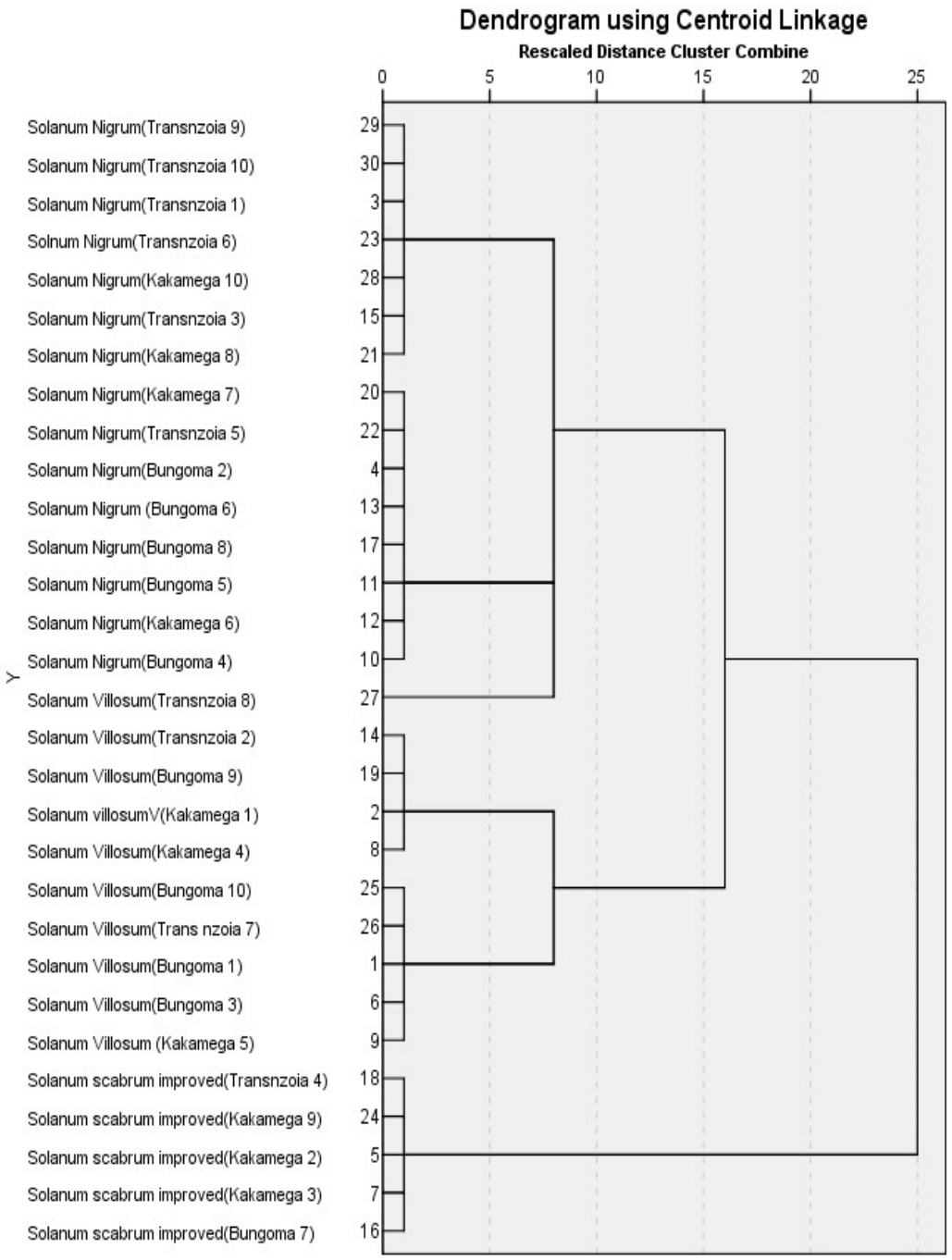


Figure 4.1.1 Hierarchical cluster analysis dendrogram displaying relationship among 30African nightshade accessions using qualitative traits.

Discussion

The morphological characterization of plant species plays a crucial role in crop breeding programs, as it enables scientists to discern and choose superior lines for

subsequent crop development and enhancement (Adebola and Morakinyo, 2006; Das and Kumar, 2012; Julia et al., 2016; Peratoner et al., 2016; Ngomuo et al., 2017). This process facilitates the examination of plant diversity through the observation of discernible characteristics. This study revealed notable diversity across the 30 African nightshade accessions in terms of qualitative features, suggesting that these traits can serve as an indicator of diversity among African nightshade cultivars. The findings shown here align with the research conducted by Olet (2004), which posited that qualitative features are more dependable in discerning genetic relationships among African nightshade compared to quantitative traits.

Considerable variability in qualitative features across African nightshade cultivars were also discovered by Nandhini et al. (2014). The observed variation may arise from either genetic factors or the influence of environmental conditions on the cultivars' genes. The variation in fruit colors observed among various African nightshade accessions may be attributed to variations in anthocyanin levels within the plants, potentially influenced by environmental factors (Manoko, 2008). These environmental influences have a significant impact on the phenotype, which explains the similarities in genetics observed across different locations.

The utilization of a cluster dendrogram serves as a reliable metric for assessing the level of diversity present both among and within species, as it effectively consolidates comparable entries into cohesive clusters (Malek et al., 2014). The results of the cluster analysis indicate that there is observable variation among the 30 African nightshade accessions in relation to the physical parameters that were examined (see Figure 4.1.1). Zhang et al. (2012) and Mekonnen et al. (2014) employed a comparable approach in their separate studies, focusing on the morphological characterization of Cucumis melo and lentil accessions.

The observed clustering pattern indicates that the Solanum scabrum accessions exhibit significant genetic divergence from the Solanum villosum and *Solanum nigrum* accessions. This finding suggests that these accessions have the potential to be utilized in reciprocal improvement efforts. The accessions originating from identical counties were aggregated, but further sub-clustering was observed within the primary clusters, indicating the presence of intra-cluster variability. The clustering analysis also identified a few individual instances of Solanum villosum, specifically Trans Nzoia 8. Singletons refer to accessions that are segregated from the remaining accessions within a cluster. According to Choudhary et al. (2013), these accessions exhibit greater diversity and hence need special consideration throughout the selection process due to their perceived superiority over other accessions.

Singletons were also identified in other genetic characterisation investigations, such as those conducted by Dube et al. (2018) on Corchurus, Chowdhury et al. (2015) on Chickpea, and Gerrano et al. (2015) on Amaranthus.The presence of intricate clustering patterns in the dendrogram indicated a significant level of diversity among the African nightshade cultivars that were evaluated. This is because, according to Kalinowski (2009), a shorter Euclidean distance between the branches of a dendrogram signifies a higher degree of similarity between the cultivars, whereas longer branches indicate greater genetic diversity among the cultivars.These findings suggest that the cultivars within a particular cluster exhibit a higher degree of genetic similarity compared to cultivars belonging to different cluster groupings. The findings presented here align with the conclusions reported by Nyadanu et al. (2014) in their study on the agro-morphological characterization of eggplant.

All specimens classified under the African nightshade species Solanum scabrum exhibited the presence of trichomes on their leaf surfaces. According to previous

research conducted by War et al. (2012), it has been demonstrated that cultivars possessing pubescence exhibit a higher level of tolerance towards pests and insects. This is due to the fact that the presence of hairs on the plant surface serves as a hindrance to insects and pests, preventing them from laying eggs and eating. Additionally, the pubescence also interferes with the larval feeding of these organisms, further contributing to the cultivars' ability to withstand infestations (Steinite and Ievinsh, 2003). The presence of hairs on the plant surface has been seen to impede the movement of insects and pests, resulting in reduced contact with the leaf epidermis and subsequently reducing harm to the leaves (War et al., 2012). The primary obstacle faced by farmers during the cultivation of African nightshade is the presence of pests and illnesses, as noted by Onyango et al. (2016).

The leaf shape and leaf pubescence features possess significant importance in the development of African nightshade cultivars that exhibit resistance to drought, pests, and diseases.

The primary factor contributing to crop improvement is in the inherent differences within and between cultivars, as highlighted by Nyadanu et al. (2014) and Ojiewo et al. (2013). This is mostly due to the potential for developing cultivars with enhanced yield characteristics through selective breeding methods, therefore leading to improved vegetable productivity. Various communities have distinct preferences for different species of African nightshade. For instance, the Abagusii community favors genotypes characterized by a spreading plant type, which yield small lanceolate leaves with a mild bitterness. On the other hand, the Abaluhya community prefers genotypes with an erect plant type, resulting in broad leaves with a bitter taste. This distinction in preference is exemplified by the S. sarrachoides species for the Abagusii and the S.

scabrum species for the Abaluhya (Onyango et al., 2016). This observation suggests that there exists variability in the favored African nightshade species among different communities. Therefore, while undertaking breeding efforts to enhance the production of African nightshade, it is crucial to take into account the individual interests of each community.

In the case of qualitative features, the majority of observed changes were found to occur across different species rather than within a single species. For example, all cultivars belonging to the species S. villosum exhibited a semi-erect plant type and produced mature orange berries, while cultivars of S. scabrums produced mature purple berries. This observation suggests that the observed variations are likely due to genetic factors rather than environmental factors, as they remain consistent across different locations. For instance, the leaf shape of cultivars within the S. scabrum species was found to be ovate in both the short and long rain periods, which contradicts the findings of Madic et al. (2016).

4.3 Molecular characteristics of African nightshade accessions in Western Kenya

The presence of clustering and sub-clustering patterns observed in the dendrogram suggests the potential for crossability among the African nightshade accessions under investigation. The dendrogram, as depicted in Figure 4.2.1, exhibited three primary clusters that were connected at a Euclidean distance of 198.91. The initial cluster consists of Trans Nzoia 5, which refers to the plant species *Solanum nigrum*. The second cluster consisted of Kakamega 10 (*Solanum nigrum*). The final cluster consisted of the remaining 28 accessions of African nightshade. The third cluster is subsequently partitioned into two more clusters, with a spatial separation of 115.49 units. The third cluster consisted of various Solanum species, namely Trans Nzoia 7

(Solanum villosum), Trans Nzoia 6 (*Solanum nigrum*), Kakamega 7 (*Solanum nigrum*), Bungoma 9 (Solanum villosum), Trans Nzoia 4 (Solanum scabrum improved), Trans Nzoia 10 (*Solanum nigrum*), Trans Nzoia 9 (*Solanum nigrum*), Trans Nzoia 8 (Solanum villosum), Bungoma 10 (Solanum villosum), Kakamega 9 (Solanum scabrum improved), Bungoma 8 (*Solanum nigrum*), Bungoma 7 (Solanum scabrum improved), Bungoma 5 (*Solanum nigrum*), Kakamega 8 (*Solanum nigrum*), Trans Nzoia 3 (*Solanum nigrum*), Trans Nzoia 2 (Solanum villosum), Bungoma 6 (*Solanum nigrum*), Kakamega 6 (*Solanum nigrum*), Bungoma 4 (*Solanum nigrum*), Kakamega 4 (Solanum villosum), Kakamega 2 (Solanum scabrum improved), Kakamega 3 (Solanum scabrum improved), Trans Nzoia 1 (*Solanum nigrum*), Kakamega 5 (Solanum villosum), Bungoma (*Solanum nigrum*), Kakamega 1 (Solanum villosum), and Bungoma 1 (Solanum villosum). The third cluster consists of two clusters connected at a Euclidean distance of 58.02, along with eleven subclusters connected at a Euclidean distance of 39.78.

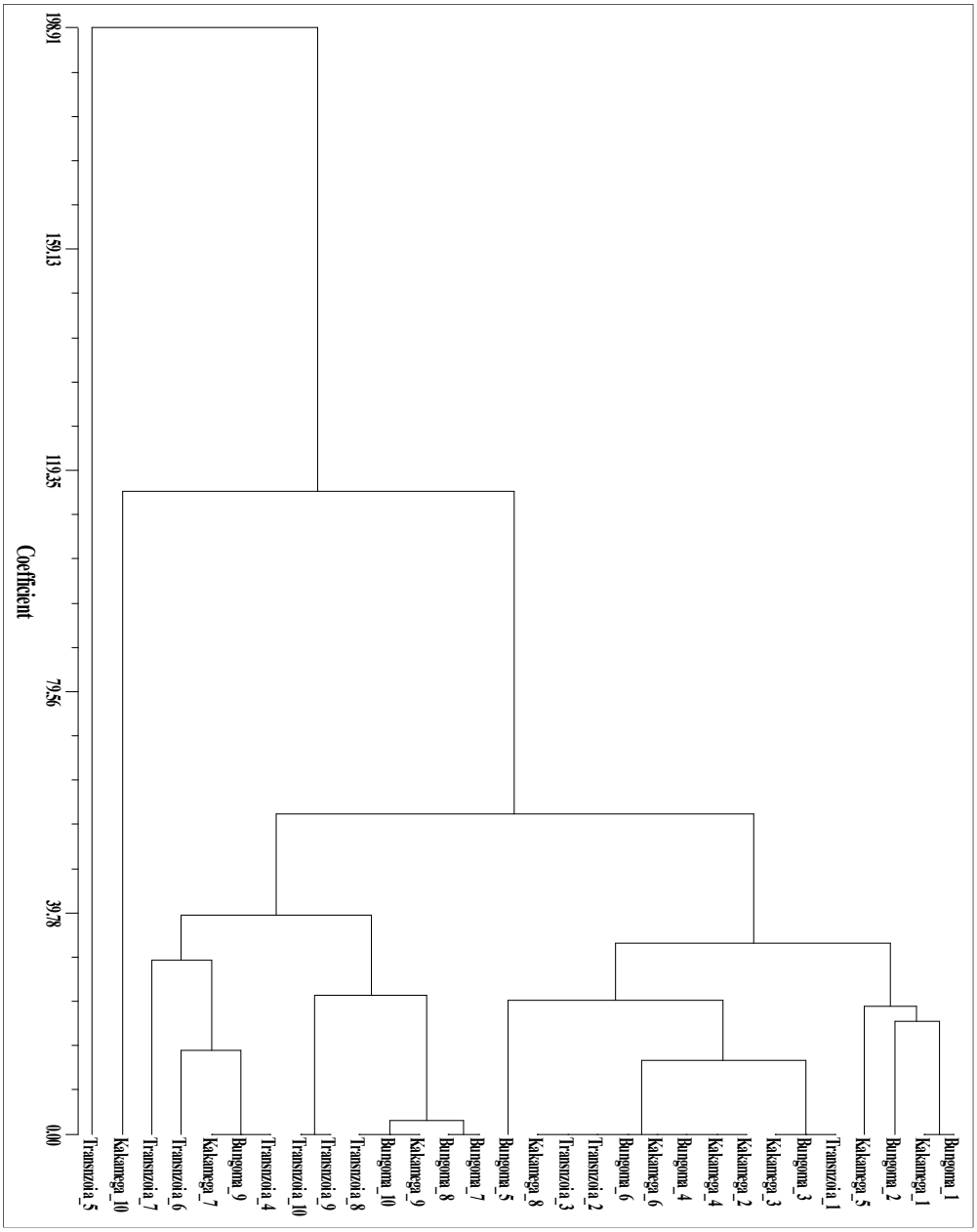


Figure 4.2. 1Genetic distance among accessions

The estimation was conducted using the Numerical Taxonomy Multivariate Analysis System package (NTSYS-pc) software, specifically version 2.1.

The genetic correlations were assessed by computing the genetic similarity matrix using the Euclidean algorithm calculator. This matrix was then utilized in the Unweighted Pair-Group method (UPGMA) clustering, which was performed using the sequential agglomerative hierarchical nested (SAHN) program. The resulting clustering analysis was visualized by a tree plot analysis.

Discussion

Molecular markers, specifically SSR markers, have been effectively utilized in various studies to elucidate the genetic diversity present within crops and their wild counterparts. These markers have also been employed to assess the genetic variation between different accessions of cultivated or semi-cultivated plants originating from diverse geographical or ecological regions. Furthermore, they have served as valuable tools for selection processes and the preservation of genetic diversity. This has been demonstrated in several studies conducted by Dehmer (2001), Hammer et al. (2003), Lanteri and Barcaccia (2005), Mace et al. (1999), Muluvi et al. (1999), McGregor et al. (2002), Perera et al. (1998), Potokina et al. (2002), Shan et al. (2005), Vergara and Bughrara (2003), and de Vicente et al. (2005).

The current investigation utilizes Simple Sequence Repeat (SSR) analysis to examine the genetic diversity within African nightshade accessions from Western Kenya. The clustering pattern seen in this analysis for the African nightshade accessions suggests a substantial level of genetic heterogeneity among the accessions. The absence of grouping based on regional origin suggests that there is no significant genetic differentiation among accessions from various locations, namely Bungoma, Kakamega, and Trans Nzoia counties. Olet (2004) identified a clustering pattern that was found to be similar among Ugandan, Indonesian, and European material.

The clustering pattern observed in this study did not align with the previously reported morphological differences. The absence of congruity between morphological and genetic disparities implies that the morphological disparities cannot be accounted for by selection for distinct plant types. Consequently, these disparities must be attributed to environmental factors and the interplay between the plant's genetics and its surrounding environment, as supported by the findings of Onyango et al. (2016).

The study conducted by Osei et al. (2013) found that African nightshade accessions within a cluster exhibited a higher degree of genetic similarity compared to accessions from different clusters.

4.4 Response of different African nightshade accessions to bacterial wilt (*Ralstonia solanacearum)* in Western Kenya

The individual in question is susceptible. By the seventh day after inoculation, it was observed that *Solanum nigrum* plants from various locations (Bungoma 5, Bungoma 8, Bungoma 2, Bungoma 4, Bungoma 6, Trans Nzoia 2, Trans Nzoia 10, Trans Nzoia 5, Trans Nzoia 3, Trans Nzoia 9, Trans Nzoia 8, Kakamega 6, Kakamega 10) exhibited wilting of all leaves, except for the top 2 to 3 leaves.The plants exhibited a persistent state of wilting, culminating in the complete wilting of all leaves over the entire plant by the 14th day. The plant exhibited a persistent state of wilting till its eventual demise after a period of 21 days in the presence of highly susceptible accessions. Conversely, *Solanum nigrum* (Trans Nzoia 10) plants experienced mortality on the 14th day following inoculation, as depicted in Figure 4.1.3. Solanum villosum specimens collected from various locations (Bungoma1, Bungoma 3, Bungoma 9, Bungoma 10, Trans Nzoia 1, Trans Nzoia 7, Kakamega 1, Kakamega 5, Kakamega 4, Kakamega 7, Kakamega 8) exhibited complete leaf wilting by the

seventh day of observation. The wilting persisted, resulting in plant mortality within 14 days, indicating a high susceptibility of these specimens. This information is visually represented in Figure 4.3.1, 4.3.2, 4.3.4, 4.3.5, 4.3.7, and 4.3.8, respectively.

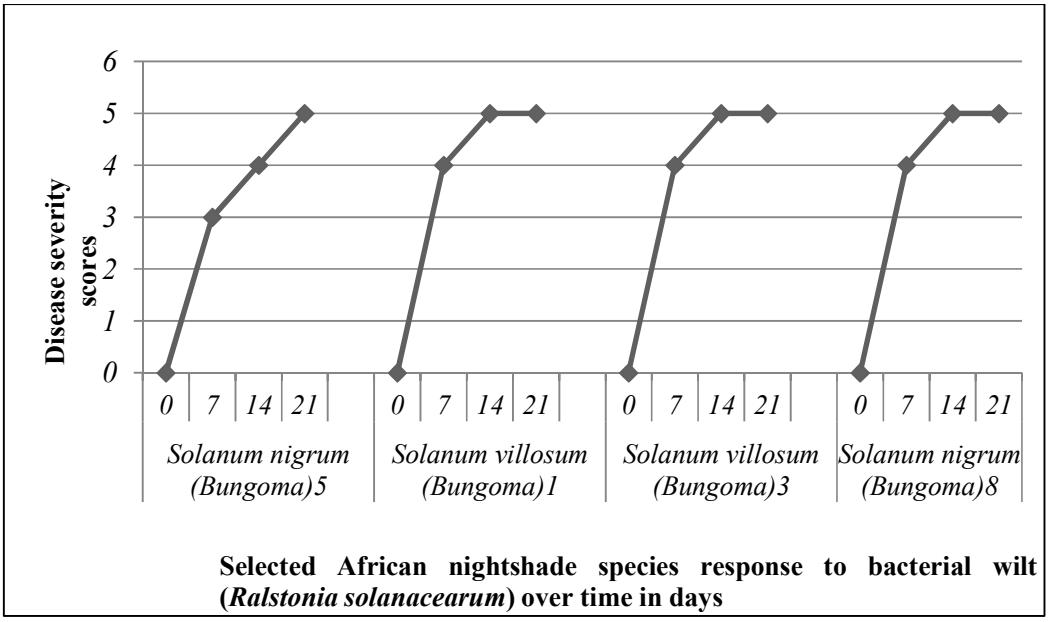


Figure 4.3.1A graph showing disease progression in selected susceptible accessions of African nightshade growing regions in Bungoma County.

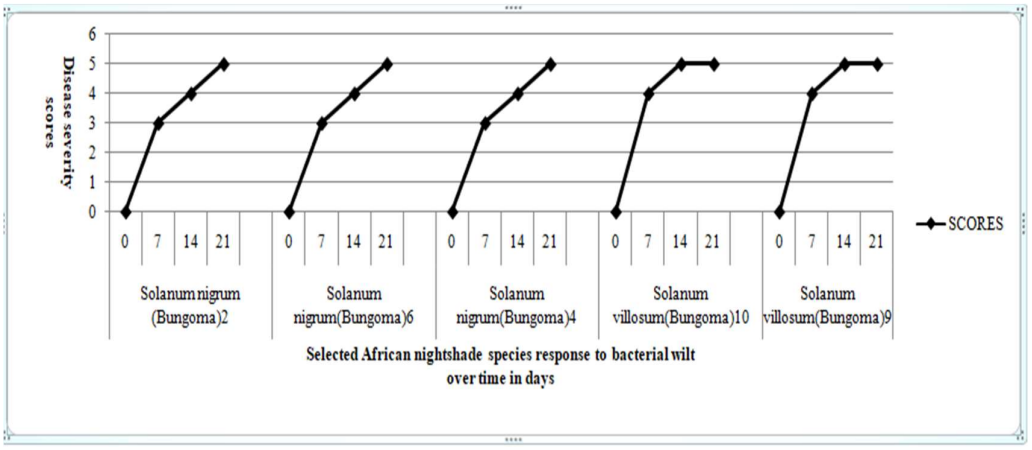


Figure 4.3.2: A graph showing disease progression in selected susceptible accessions of African nightshade growing areas inBungoma county.

Among the resistant accessions were *Solanum scabrum* from the Bungoma 7, Trans Nzoia 4, Kakamega 2, Kakamega 3, Kakamega 9, which did not show any symptoms of wilting up to the end of 21 days (Resistant) as shown on figure 4.3.3, 4.3.6 and 4.3.9 below

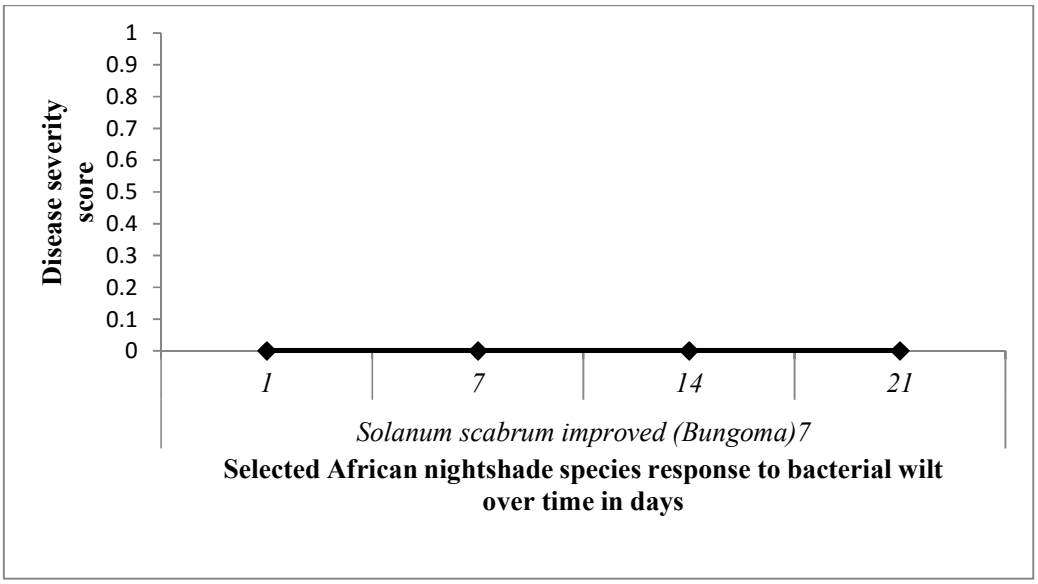


Figure4.3.3 A graph showing disease progression in selected resistant accessions of African nightshade growing areas in Bungoma county.

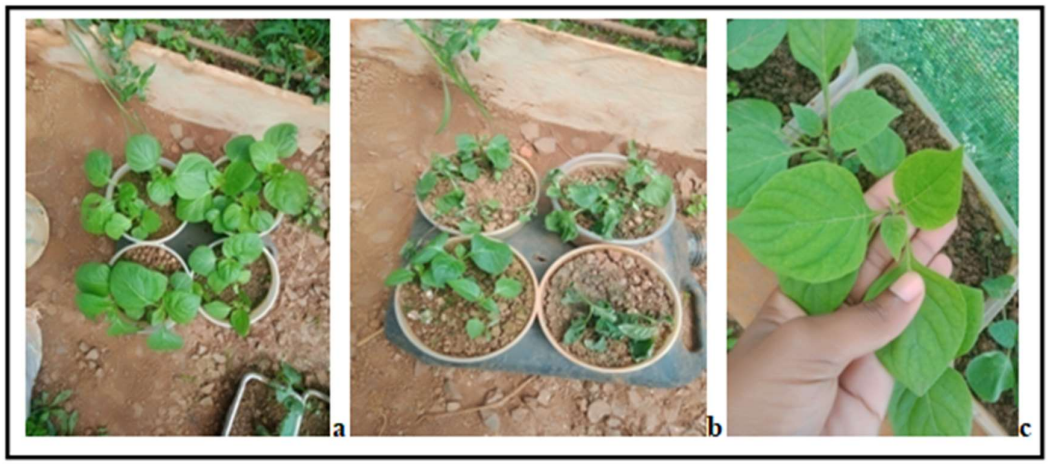


Plate 4.1.3 Photos showing response of African nightshade

accessions to *Ralstonia solanacearum* after inoculation (a) No wilting symptom observed (Resistant) (b) Wilting symptoms observed (c) Chlorate symptoms observed on the leaves of resistant cultivars after inoculation**.**

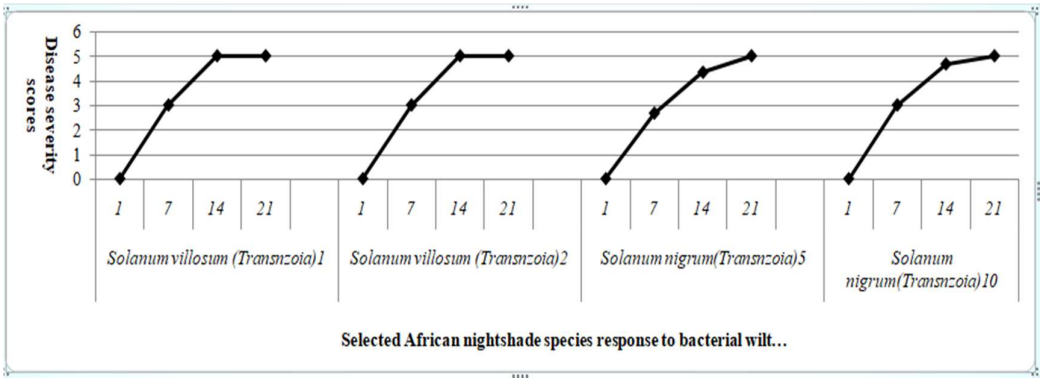


Figure 4.3. 4: Agraph showing disease progression in selected susceptible accessions of African nightshade growing areas in Trans Nzoia county

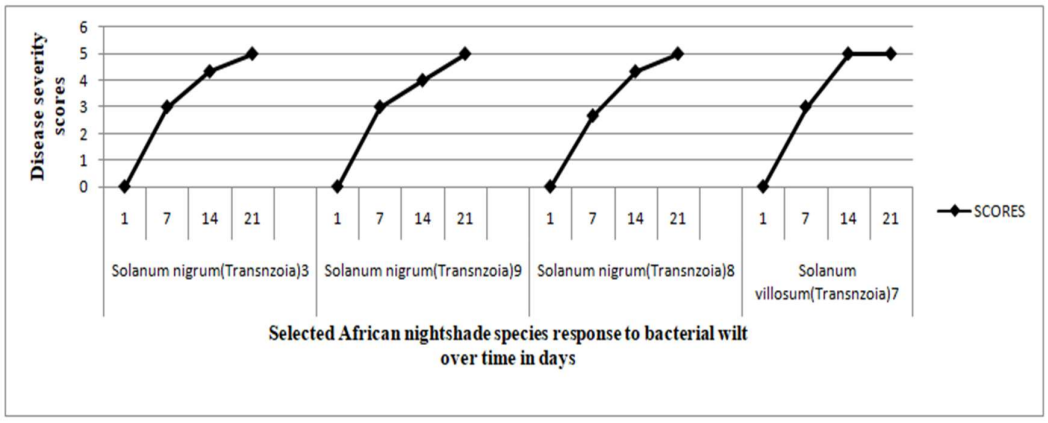


Figure4.3. 5: A graph showing disease progression in selected susceptible accessions of African nightshade growing areas in Trans Nzoia county

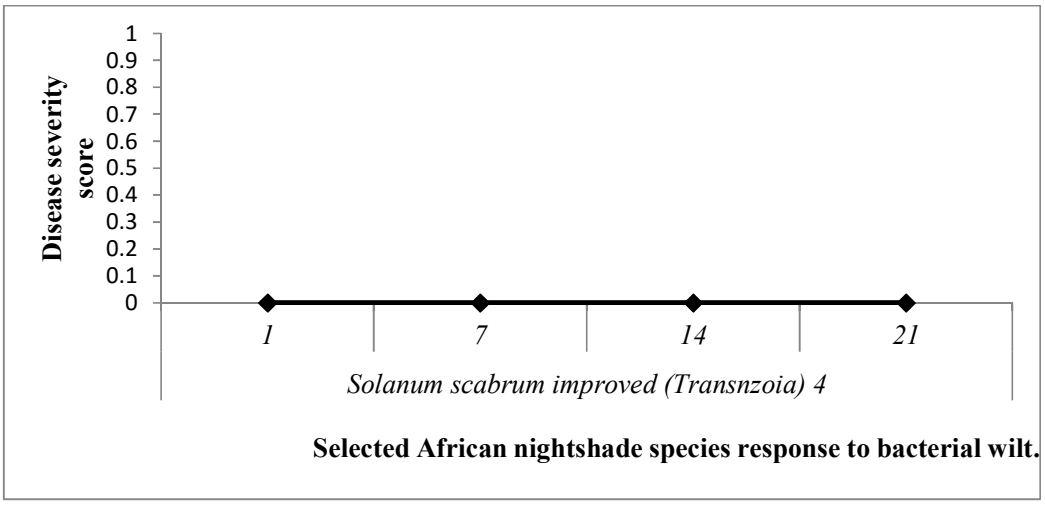


Figure 4.3.6 A graph showing disease progression in selected resistant accessions of African nightshade growing areas in Trans Nzoia county

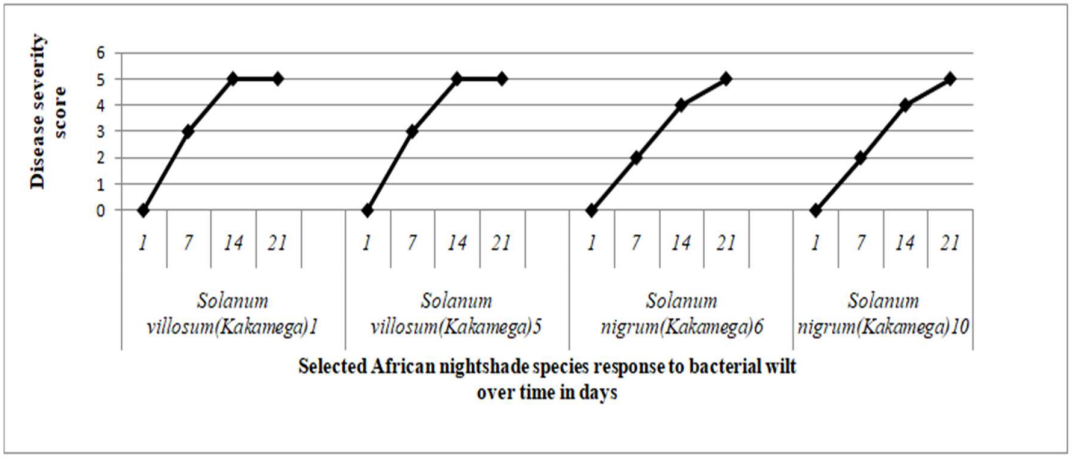


Figure 4.3.7: A graph showing disease progression in selected susceptible accessions of African nightshade growing areas in Kakamega county

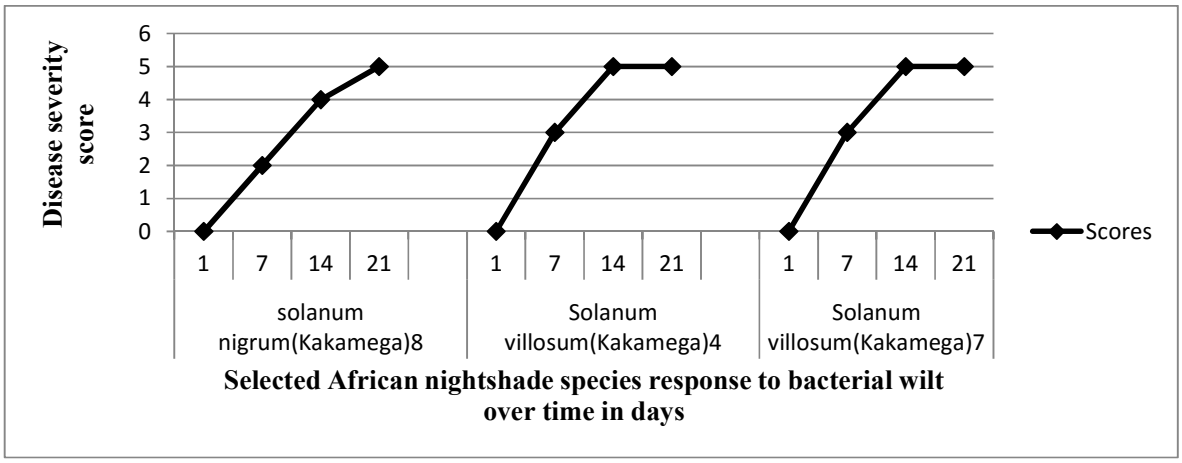


Figure 4.3. 8: A graph showing disease progression in selected susceptible accessions of African nightshade growing areas in Trans Nzoia county

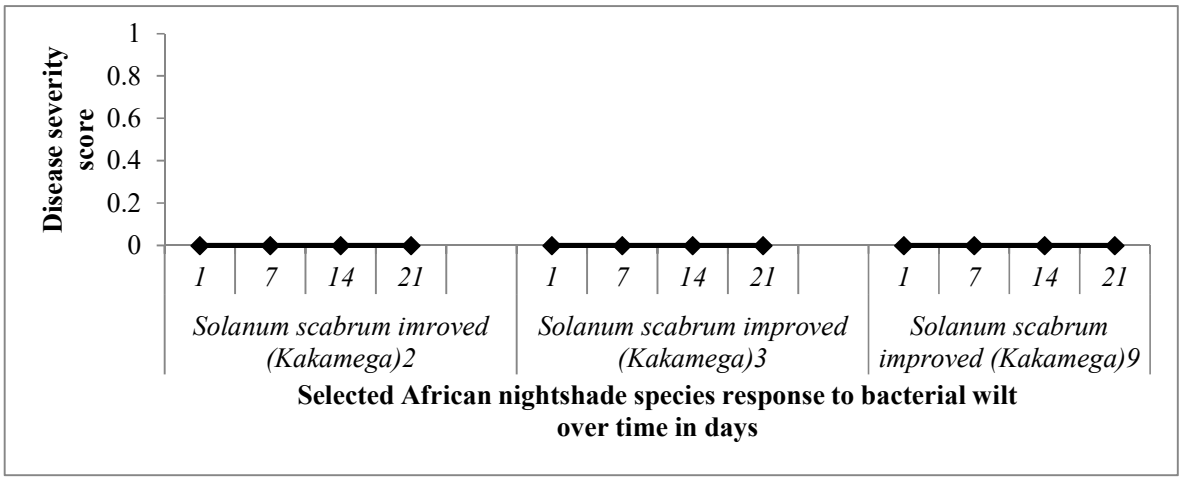


Figure 4.3.9: A graph showing disease progression in selected resistant accessions of African nightshade growing areas in Kakamega county

Discussion

The investigation into identifying a genetic basis of resistance to bacterial wilt in different vegetables has been extensively examined in solanaceous crops. In a study

conducted by In et al. (1996), the researchers examined the resistance of 31 tomato varieties to Ralstonia solanacearum, a bacterial wilt pathogen. The findings revealed that just three of the types exhibited considerable resistance, whereas the remaining varieties were found to be vulnerable to the pathogen. There exists a correlation between resistance, vulnerability, and the manifestation of symptoms. In the present investigation, it was observed that susceptible accessions exhibited symptoms of infection after a period of four days following inoculation. In contrast, resistant accessions displayed chlorotic symptoms after a duration of more than fourteen days, which is consistent with the results reported by Anith et al. (2004). The phenomenon of resistance to bacterial wilt has been discovered to be strain specific and influenced by temperature, as demonstrated in a study conducted on potato by French and De Lindo in 1982. The resistance against bacterial wilt is influenced by environmental circumstances and locations, as observed by Hanson et al. (1996) who documented a diverse response of tomato lines to bacterial wilt across multiple locations in Southeast Asia. The researchers discovered that tomato lines that exhibited resistance to bacterial wilt in Malaysia and Taiwan had susceptibility in the Philippines and Indonesia.

The study identified that the Solanum scabrum improved cultivar exhibited resistance, whereas the Solanum villosum and *Solanum nigrum* cultivars were shown to be vulnerable. Consequently, it is suggested that the cultivation of Solanum scabrum improved be prioritized in integrated production systems, as well as in the development of new African nightshade cultivars with resistance.

Solanum scabrum exhibited resistance to Ralstonia solanacearum across all three regions. This resistance can be attributed to a distinct inherent gene of resistance

possessed by this genotype. This finding aligns with the research conducted by Nguyen and Ranamukhaarachchi (2010), who observed that the ability of a crop to overcome pathogen attacks is frequently influenced by the genetic diversity of the pathogen and the interactions between genotype and environmental factors.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The analysis of clustering based on morphological characteristics reveals that the Solanum scabrum accessions exhibit significant genetic divergence from the Solanum villosum and *Solanum nigrum* accessions. This finding suggests that these accessions have the potential to be utilized in reciprocal improvement efforts.

The presence of notable diversity among African nightshade accessions suggests the existence of variation between cultivars of the same species, as well as within accessions across distinct species. This observed variation may be attributed to either genetic factors or environmental influences.

The leaf shape and leaf pubescence are significant features that can be utilized in the development of African nightshade cultivars with enhanced resistance to drought, pests, and diseases.

The utilization of SSR primers in evaluating African nightshade accessions revealed the presence of genetic variety, as evidenced by the clustering of accessions into three distinct groups (I, II, and III). The dendrogram analysis revealed the presence of distinct clusters and sub-clusters, suggesting a significant level of genetic diversity among the African nightshade accessions obtained from the three counties located in Western Kenya.

Based on the current evaluation, it can be inferred that African nightshade cultivars exhibited variability in their resistance response to R. solanacearum. The enhanced genotype of Solanum scabrum was discovered to exhibit resistance, while the genotypes of Solanum villosum and *Solanum nigrum* were shown to be susceptible.

5.1.1Recommendation

This study suggests that additional morphological characterization, encompassing both qualitative and quantitative traits, be conducted on accession samples from the same species in Western Kenya. This would enable scientists to identify and document superior cultivars within specific African nightshade species.

It is advisable to utilize enhanced Solanum scabrum in integrated production systems and for the development of novel African nightshade cultivars that possess resistance. This suggests that genotypes ought to be assessed under local conditions in relation to specific isolates of the pathogen prevalent in that region.

REFERENCES

Abedayo, O. S**.** and Ekpo, E. J. A. (2009). *Ralstonia Solanacearum* Causing Bacterial Wilt of Tomato in Nigeria. *American Phytopathological Society*, APS Press, St. Paul, 15(12): 1129-1130.

Abukutsa M (2010). African indigenous vegetables in Kenya: *Strategic repositioning in the horticultural sector.*

Abukutsa OMO, Muriithi AN, Anjichi VE, Ngamau K, Agong SG, Fricke A, Hau B & Stutzel H (2005). Proceedings of the third Horticulture Workshop on Sustainable Horticultural Production in the Tropics, 26th -29th November 2003. *ISBN*: 9966-758-11-9. 281.

Abukutsa-Onyango, M.O. (2003)**.** Unexploited potential of Indigenous African Vegetables in Western Kenya. *Maseno Journal of Education Arts and Science* 4(1): 103-122. ISSN:1019-360X.

Adebola P, Morakinyo J. (2006). Evaluation of morpho-agronomic variability of wild and cultivated kola in South Western Nigeria. *Genet Resourc Crop Evol*. 53:687–694.

Akira, M., Kazuhiro, N., Masao, S., Hideki T. and Shigehito, T. 2009. Visualization of *Ralstonia Solanacearum* cells during biocontrol of bacterial wilt disease in tomato with *Pythium oligandrum*. *General Plant Pathology* 75: 281-287.

Akubugwo I. E., Obasi A. N., Ginika S. C., (2007*). Nutritional Potential of the Leaves and Seeds of African nightshade-Solanum nigrum L. Varvirginicum* from Afikpo-Nigeria.

Anith, K. N., Momol, M. T., Kloepper, J. W., Marois, J. J., Olson, S.M., & Jones, J. B. (2004). Efficacy of plant growth-promotingrhizobacteria, acibenzolar-S-methyl, and soil amendment for integrated management of bacterial wilt on tomato*. Plant Disease*, 88, 669–673.

Ashilenje DS, Omunyin ME &Okalebo J R (2012). Potassium Nutrition: Towards sustainable and profitable production of Vegetable African

Nightshades (*Solanum* L. Section *Solanum*) in Western Kenya. *Journal of Chemical and Pharmaceutical Research*, 4(4):2306-2311.

Asian Vegetable Research and Development Corporation (AVRDC). (2003). Progress report, Variations of anti-oxidants and their activity in tomato by Chin H. K. 12: 70-115.

Atanu FO, Ebiloma UG & Ajayi EI **(**2011). A review of the pharmacological aspects of *Solanum nigrum. Biotechnology and Molecular Biology Review*, 6(1): 001-007.

Bagriansky , J.&Ranum , P.(undated manuscript) Oils, fats and margarine*:* Overview of technology . *Vitamin A fortification of PL480 vegetable oil SUSTAIN Washington, DC.*

Berinyuy, J.E., Fontem, D.A., Schippers, R.R. (2002). Morphological diversity of *Solanum scabrum* accessions in Cameroon. *Plant Renetic Resources Newsletter*, 131, 42-48. Wenneker,W, M.S.W.Verdel, R.M.W. Groeneveld, , C.Kempenaar, A.R. Van-Beuningen and J.D. Janse. (1999).*Ralstonia (Pseudomonas) solanacearum* Race 3 (Biovar 2) in Surface Water and Natural Weed Hosts*: First Report on Stinging Nettle* (*Urtica dioica*). *Eur J Plant Pathol; 105(3):307-315.*

Bhat TM &Kudesia R (2011). *Evaluation of Genetic Diversity in five different species of Family Solanaceae using cytological characters and protein profiling. Institute of basic science*, Department of Botany, Bundelkhand University, Jhansi (U.P.)-284128, India.

Black, L. L., Wu, D. L., Wang, J. K., Kalb, T., Abass, D., Chen, J. H. 2003. *Grafting tomatoes for production in hot-wet season: In International cooperators' s guide*. Asian Vegetable Research and Development Center. *The bureau of animal and plant quarantine and health inspection*, *council of agriculture, China.*

Caruso,P. J.L Palomo, E. Bertolini, B.Alvarez, M.M. Lopez, and E.G. Biosca, (2003). Seasonal Variation of *Ralstonia solanacearum* biovar 2 Populations in

a Spanish River: *Recovery of Stressed Cells at Low Temperatures*. *Appl Environ Microbiol*;71:140-148.

Carvalho, Y.G.S., L.C. Vitorino, U.J.B. de Souza, and L.A. Bessa. (2019). Recent trends in research on the genetic diversity of plants: Implications for conservation. *Diversity* 11 (4):1–21. doi: 10.3390/d11040062.

Champoiseau, P. G. and Momol, T. M. (2009). Training modules on Bacterial wilt of tomato.*University of Florida IFAS extension. http://plantpath.ifas.ufl.edu/rsol/Trainingmodules/BWTomato_Module.html*

Champoiseau, P. G., Jones, J. B. and Allen, C. (2009). *Ralstonia solanacearum* Race 3 Biovar *2* Causes Tropical Losses and Temperate Anxieties. *Online. Plant Health Progress*. doi:10.1094/PHP-2009-0313-01-RV.

Choudhary SB, Sharma HK, Karmakar PG, Saha AR, HazraP, Mahapatra BS. (2013). Nutritional profile of cultivated and wild jute (Corchorus) species. *Australian J Crop* Sci.7:1973–1982.

Chweya, J.A., Eyaguirre, P.B. (1999). The Biodiversity of Traditional Leafy Vegetable*s*. *International Plant Genetic Resources Institute*, Rome, Italy.

Claudio DG, Pasqua DO, Angela B, Franco C, Concetta L & Lui-gi R (2004). Identification of PCR-based markers (RAPD, AFLP) linked to a novel powdery mildew resistance gene (0l-2) in tomato. *Plant Science*, 166: 41-48.

Coyne, D. L., Nicol, J. M and Claudius-Cole, B. (2006). Practical plant Nematology: a field laboratory guide. *SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Cotonu, Benin.*

Cuppels, D. A, Hanson, R.S., Kelman, A. (1978). Isolation and characterization of a bacteriocin produced by Pseudomonas solanacearum. *Society of General Microbiology1*09:295-303.

D'Arcy W. G., (1991). The Solanaceae since 1976, with a Review of its Biogeography. Pp. 75-137 in Solanaceae III: *Taxonomy, Chemistry and Evolution* (J. G. Hawkes, R. N. Lester, M. Nee and N. Estrada-R., eds.). Academic Press, London.

Das A, Kumar D. (2012). Genetic evaluation and characterization of jute (Corchorus spp L) genotypes using DUS parameters. *SAARC J Agricul.* 10:147–153.

De Vicente MC, Guzma´n FA, Engels J, Ramanatha Rao V (2005) Genetic characterization and its use in decision making for the conservation of crop germplasm. *The Role of Biotechnology*, *Villa Gualino, Turin, Italy*, 5–7 March 2005, pp 121–128

Deberdt, P., Oliver, J., Queneherve, P. and Prior, P. (1999). Evaluation of bacterial wilt resistance in tomato lines nearly isogenic for Mi gene for resistance to root - knot. *Plant Pathology Journal*, 48: 415-424.

Dehmer KJ (2001) Conclusions on the taxonomy of the Solanumnigrum complex by molecular analyses of IPK germplasm accessions. In: van den Berg RG, Barendse GWM, van der Weerden GM, Mariani C (eds) Solanaceae V: *Advances in Taxonomy and Utilization*. *University Press*, Nijmegen, pp 85–96.

Demir K, Bakir M, Sarikamiş G &Acunalp S (2010). Genetic diversity of eggplant (*Solanum melongena*) germplasm from Turkey assessed by SSR and RAPD markers. *Genetics and Molecular Research*, 9(3):1568-1576.

Demir K, Bakir M, Sarikamiş G &Acunalp S (2010). Genetic diversity of eggplant (*Solanum melongena*) germplasm from Turkey assessed by SSR and RAPD markers. Genetics and Molecular Research, 9(3):1568-1576.

Dhasmana M, Simon L, Narayanaswany P, Rathore SKR &Sreeram B (2007). Characterization of *Solanum nigrum* L. *Genotypes by morphological and RAPD Markers*. *Crop Breeding and Applied Biotechnology*, 10: 16-22.

Dhasmana M, Simon L, Narayanaswany P, Rathore SKR &Sreeram B (2007). Characterization of *Solanum nigrum* L. Genotypes by morphological and RAPD Markers. *Crop Breeding and Applied Biotechnology*, 10: 16-22.

Dinssa, F., Ebert, A., Tenkouano, A. (2014). The diversity of African leafy vegetables, agromorphological characterization of germplasm collection. *Acta Horticulturae*, Doi 10.17660/ActaHortic.2015.1102.7.

Dristig, M. C. G. and Dianese, J. C. 1990. Characterization of *Pseudomonas solanacearum* biovars based on membrane protein patterns. *Phytopathology* 80: 641-646.

Dube S. P, D. Marais, S. Mavengahama, C. M. Van Jaarsveld, and A. S. Gerrano (2018), "Characterisation of agro- morphological traits of corchorus accessions," *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, vol. 69, no. 2, pp. 126–134, 2018.

Duke J. A. and Ayensu E. S., (1985). Medicinal Plants of China Reference Publications, *Inc.* ISBN 0-917256-20-4.

Edmond J. M., (1979b). Nomenclatural notes on some species of Solanum L. found in Europe. *Bot. J. Linn. Soc*. 78: 213-233.

Edmonds JM &Chweya JA (1997)a. "Black nightshades. *Solanum nigrum* L. and related species. Promoting the conservation and use of underutilized and neglected crops". Institute of *Plant Genetics and Crop Plant Research*, pp78.

Edmonds JM &Chweya JA (1997)b. Black nightshades International Plant Genetic Resource *Institute, Rome.*

Edmonds, J. M., and Chweya, J. A. (1997)c. Black Nightshades. *Solanum nigrum* L. and related species. Promoting the conservation and use of underutilized and neglected crops, (15). Institute of Plant Genetics and Crop Plant Research, Gatersleben/International *Plant Genetic Resources* Institute, Rome, Italy.

Edmonds, Jennifer M. and James A. Chweya. 1997. Black nightshades. *Solanum nigrum L.* and related species. Promoting the conservation and use of underutilized and neglected crops. 15. Institute of Plant Genetics and Crop Plant Research, Gatersleben / *International Plant Genetic Resources* Institute, Rome, Italy.

Fajinmi, A. A. and Fajinmi, O. B. (2010). An overview of bacterial wilt disease of tomato in Nigeria. Agricultural 5 (4): 242-247.

Favoretto P, Veasey EA & Melo PCT (2011). Molecular characterization of potato cultivars using SSR markers. *HorticulturaBrasileira*29: 542-547.

Food Fortification to End Micronutrient Malnutrition. State of the Art :66–71The Micronutrient Initiative, *International Development Research Centre Ottawa, Canada.*

French, E. R., & De Lindo, L. (1982). Resistance to Pseudomonas solanacearum in potato: *Specificity and temperature sensitivity*. *Phytopathology,* 72, 1408–1412.

Genin S. 2010. Molecular traits controlling host range and adaptation toplants in *Ralstonia solanacearum*. *New Phytologist* 187, 920–928.

Gerrano A. S., W. S. J. Van Rensburg, and P. O. Adebola, "Genetic diversity of amaranthus species in South Africa,"*SouthAfrican Journal of Plant and Soil*, vol. 32, no. 1, pp. 39–46, 2015.

Gomes, A. M., Mariano, R. L., Micherett, and De França, S. J. (1998*).* Selection of processing tomato progenies for resistance to Ralstonia solanacearum. In: Bacterial wilt disease, molecular and ecological aspects.

Govindaraj, M., M. Vetriventhan, M. Srinivasan, M. Govindaraj, M. Vetriventhan, and M. Srinivasan. (2015). Importance of genetic diversity assessment in crop plants and its recent advances: *An overview of its analytical perspectives*. Genet Res Int. 1–14. doi: org/10.1155/ 2015/431487.

Grimault, V. and Prior, P. (1994). Invasiveness of *Pseudomonas solanacearum* in tomato, eggplant and pepper:a comparative study. *European Journal of Plant Pathology* 44: 105-123.

Guo, J. H., Oi, H. O., Guo, Y. H., Ge, H. L., Gong, L.Y., Zhang, L. X. and Sun, P. H. (2004). *Biocontrol of tomato wilt by plant growth-promoting rhizobacteria*. Microbiology Review 29: 66–72.

Gupta, S. K. and Thind, T. S. (2006). Disease Problems in Vegetable Production. Pawan Kumar, Scientific Publishers, India. ISBN: 81-7233-452-3 (H.B); 81-7233-453-2 (P.B), Sub – Title; Diseases of Tomatoes. Pg 65-104. CABI (2005a). Sub-title; *Damage caused by Bacterial wilt of tomato/eggplant*. www.infonet- biovision.org/default/ct/79/pests, Retrieved,2ndMarch,2013. Chen WY. (1984). Influence of the root – knot

nematode on wilt resistance of flu-cured tobacco infested by Pseudomonas solanacearum. Bulletin of the Tobacco Research Institute, 21:41-48.

Haas, D. and De`fago, G. (2005). Biological control of Soil-borne pathogens by *fluorescent Pseudomonas*. *Review of Microbiology* 3: 307–319.

Hammer K, Arrowsmith N, Gladis T (2003) Agrobiodiversity with emphasis on plant genetic resources. *Naturwissenschaften* 90:241–250.

Hanson, P. M., Wang, J. F., Licardo, O., Mah, S. Y., Hartman, G. L., Lin, Y. C., et al., (1996). *Variable reaction of tomato lines to bacterial wilt evaluated at several locations* in Southeast Asia.HortScience, 31, 143–146.

Hashash EFE (2016). Genetic Diversity of Soybean Yield Based on Cluster and Principal Component Analyses. *Journal ofAdvances in Biology & Biotechnology*, 10(3): 1-9, 2016.

Hassan AN, Mostafa S &Twfik A (2013). Assessment of Genetic Diversity of Tomato (*Lycopersicon Esculentum* L.) Germplasm using Molecular Markers (RAPD and ISSR) *Egyptian Journal of Genetics and Cytology*, 42:163-182.

Hayward AC. (1964) Characteristics of *Pseudomonas solanacearum*. *Journal of Applied Bacteriology*. 27(2)265-77.

Hayward, A. C. **(**1991). Biological and epidemiology of bacteria wilt caused by *Pseudomonas solanacearum*. *Annual Phytopathology* 29: 65-87.

Hayward, A.C., R. Tollenaere, J. Dalton-Morgan, and J. Batley. (2015). Molecular marker applications in plants, p. 13–27. In J. Batley (ed.). *Plant genotyping: Methods and protocols*. Vol. 1245. Humana Press, New York. doi: 10.1007/978-1-4939-1966-6.

Idrees M & Irshad M (2014). Molecular Markers in Plants for Analysis of Genetic Diversity. *European Academic Research*, Vol. 2(1).

In, M. S., Choi, E. J., & Choi, J. E. (1996). Resistance of tomato cultivars to bacterial wilt caused by Pseudomonas solanacearum and the effect of soil sterilization. RDA Journal of Agricultural Science. Crop Protection, 38, 473–476.

Islam, T. M. D. and Toyota K., (2004). Suppression of bacterial wilt of tomato by *Ralstonia solanacearum* by incorporation of composts in soil and possible mechanism. *Microbes Environment* 19: 53-60.

Jagatheeswari D, Bharathi T, Sheik H & Ali Jahabar (2013). Black Night Shade (*Solanum nigrum* L.). *International Journal of Pharmaceutical & Biological Archives*, 4(2): 288 – 295.

Jagatheeswari D, Bharathi T, Sheik H & Ali Jahabar (2013). Black Night Shade (*Solanum nigrum* L.). *International Journal of Pharmaceutical & Biological Archives,* 4(2): 288 – 295.

Janse JD, Beld V, Elphinstone HE,Simpkins J, Tjou-Tam-Sin S, Van-Vaerenbergh J. (2004) Introduction to Europe of *Ralstonia solanacearum* biovar 2, race 3 in *Pelargonium zonale*cuttings. *Journal of Plant Pathology*. 86(2):147-155.

Jonah, P.M., L.L. Bello, O. Lucky, A. Midau, and S.M. Moruppa. (2011). *Review: The importance of molecular markers*. Global J Sci Frontier Res 11(5):5–12.

Jones, J.B. 92008): *Tomato plant culture: In the field greenhouse and garden*. Taylor and Francis Group, USA. 55.

Julia CC, Waters DLE, Wood RH, Rose TJ. (2016). Morphological characterisation of Australian ex situ wild rice accessions and potential for identifying novel sources of tolerance to phosphorus deficiency. *Genet Resourc Crop Evol*.63:327–337.

Kalinowski ST (2009). How well do evolutionary trees describe genetic relationship among populations? *Heredity,* 102; 506-513.

Kanga, R.T., Kouame, C., Atangana, A.R., Chagomoka, T., Ndango, R. (2013). Nutritional evaluation of five African indigenous vegetables. *Journal of Horticultural Research*, 21, 99-106.

Kavitha, R. and Umesha, S. (2007). Prevalence of bacterial spot in tomato fields of Karnataka and effect of biological seed treatment on disease incidence. *Crop Protection Journal* 26(7): 991-997.

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

Kelman, A., Person L.H. 1961. Strains of *Pseudomonas solanacearum* differing in pathogenicity to tobacco and peanut. *Phytopathology* 51:158-161.

Khodadadi M, Hussein F &Miransari M (2011). *Genetic Diversity of wheat (Triticum aestivumL) based of cluster and principle component analysis for breeding strategies*. A.J.C.S. 5(1): 17-24.

Kimes KP, Liu Y, Hayes D. N & Marron J. S. (2014*) Statistical Significance for Hierarchical Clustering.* ArXiv: vol, 11411.5259.

Klocke E, Matasyoh GL, Budahn H & Kastner U (2016). BSL 1: Biotechnological tools for improvement of black nightshade (*Solanum nigrum* L. complex), valuable medicinal and vegetable plants in Kenya. *6th International Symposium Breeding Research on Medicinal and Aromatic Plants, BREEDMAP 6*, Quedlinburg, Germany, 19-23.

Lanteri SL, Barcaccia G (2005) Molecular markers based analysis for crop germplasm preservation. *The Role of Biotechnology*, Villa Gualino, Turin, Italy, 5–7 March 2005, pp 55–66.

Lehmann, C., Biela, C., Stefan, T., Jansen, G., Vogel, R. (2007). *Solanum scabrum*—a potential source of a coloring plant extract. *Euphytica*, 158,189–199.

Liao, B. S., Shan, Z. H., Duan, N. X., Tan, Y. J., Lei, Y., Li, D. and Mehan, V. K. (1998). Relationship between latent infection and groundnut bacterial wilt resistance. In: Bacterial wilt disease; Molecular and Ecological aspects. Prior, P., Allen, C. and Elphinstone, J. (Eds.), 294-299. *Reports of the second international bacterial wilt symposium held in Gosier*, Guadaloupe, France, 22-27 June 1997.

Lim SS, Vos T, Flaxman AD, a comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, (1990-2010): AEsystematic analysis for the Global Burden of Disease Study 2010. *Lancet2012*;380;2224-60.*Google Scholar*

Luoh, J.W., Begg, C.B., Symonds, R., Ledesma, D., Young, R. (2014). Nutritional yield of African indigenous vegetables in water-defficient and water-sufficient conditions. *Food and Nutritional Sciences*, 5, 812-822.

Mace ES, Lester RN, Gebhardt CG (1999) AFLP analysis of genetic relationships among the cultivated eggplant, Solanum melongena L., and wild relatives (Solanaceae). *Theor Appl Genet* 99:626–633.

Madic M, Knezevic D, Paunovic A & Durovic D (2016). Plant height and internode length as components of lodging resistance in barley. *Acta Agriculturae Serbica,* Vol. 21, 42 (99-106).

Maduabum FO. Nutritional awareness of bank workers in Lagos State, Nigeria. [master'sthesis], Nsukka, Nigeria: University of Nigeria; 2015.

Malek M. A., M. Y. Rafii, M. S. S. Afroz, U. K. Nath, and M. M. A. Mondal, (2014)"Morphological characterization and assessment of genetic variability, character association, and divergence in soybean mutants," *Scientific World Journal*, vol. 2014, *Article ID 968796*, 12 pages.

Manoko LK M, Berg RG, Feron MCR, Weerden MG & Mariani C (2007). Genetic diversity of the African hexaploid species *Solanum scabrum* Mill. and *Solanum nigrum* L. (*Solanaceae*) *Genetic Resource Crop Evolution* 55:409–418.

Manoko LK M, Berg RG, Feron MCR, Weerden MG &Mariani C (2008). Genetic diversity of the African hexaploid species *Solanum scabrum*Mill. and *Solanum nigrum* L. (*Solanaceae*) *Genetic Resource Crop Evolution* 55:409–418.

Martin AH. Stair design in the United States and obesity: the need for a change. South Med J (2006); 102:610–4.

Matasyoh L G, Abel S &Budahn H &Klocke E (2015). Characterization of the *Solanum Nigrum* Complex of Kenya by AFLP Markers. *International Journal of Agricultural Science and Technology* Vol 3(1).

Matasyoh LG & Mwaura MH (2014). The *Solanum nigrum* complex in western Kenya. *International Journal of Plant, Animal and environmental sciences* Vol 4(2).

Matasyoh LG &Bosire NA. The *Solanum nigrum* Complex (Black Night Shade) Grown in the Rift Valley, Western and Nyanza Provinces of Kenya. Journal of Life Sciences, 10 (2016) 228-232.

Matta L B, Tome LGO, Salgado CC, Cruz CD & Silva LF (2015). *Hierarchical genetic clusters for phenotypic analysis*. Acta Scientiarum, 1679-9275.

Maundu, P. M, G.W. Ngugi and C.H.S. Kabuye, (1999).*Traditional Food Plants of Kenya*. KENRIK National Museums of Kenya, Nairobi, Kenya. 270 pages

Maundu, P., Achigan-Dako, E., Mprimoto, Y. (2009). Biodiversity of African vegetables. In: African Indigenous Vegetables in Urban Agriculture. Eds. Shackleton, C.M., Pasquini, M.W., Drescher, A.W.. London, UK., Earthscan, pp 65-104.

Mbaka, J. N., Gitonga, J. K., Gathambari, C. W., Mwangi, B. G., Githuka, P. and Mwangi, M. (2013). Identification of knowledge and technology gaps in high tunnels tomato production in Kirinyaga and Embu counties.

McCarter, S. M.(1991). Bacterial wilt, p. 28-29.In: Jones, J. B., J. P. Jones, R. E. Stall and T. Zitter (eds*.). Compedium of tomato diseases*. St. Paul. Michigan USA.

McGregor CE, van Treuren R, Hoekstra R, van HintumThJL (2002) Analysis of the wild potato germplasm of the series Acaulia with AFLP: *implications for ex situ conservation. TheorAppl Genet* 104:146–156.

Mekonnen F, F. Mekbib, S. Kumar, S. Ahmed, and T. R. Sharma (2014)"Agromorphological traits variability of theEthiopian lentil and exotic genotypes," *Advances in Agriculture*, vol. 2014, Article ID 870864, 15 pages.

Mihovilovich, E.; Lopes, C.; Gutarra, L.; Lindqvist-Kreuze, H.; Aley, P.;Priou, S.; Bonierbale M. (2017). Protocol for assessing bacterial wilt resistance in greenhouse and field conditions. International Cooperators' Guide. Lima (Peru). International Potato Center. ISBN 978-92-9060-214-9. 35 p.

Milling A, Babujee L, Allen C (2009) *Ralstonia solanacearum extracellular polysaccharide is a specific elicitor of defense responses in wilt-resistant tomato plants. PLoS One* 6(1):e15853.

Miyamoto, N., J.F. Fernández-Manjarrés, M.E. Morand-Prieur, P. Bertolino, and N. Frascaria- Lacoste. (2008). What sampling is needed for reliable estimations of genetic diversity in Fraxinus excelsior L. (Oleaceae)? Ann. For. Sci. 65(4):403p1–403p8. doi: 10.1051/ forest:2008014.

Mnzava, N.A.(1997).Vegetable crop diversification and the place of traditional species. In: Guarino L. editor 1997, *Traditional African vegetables. Promoting the conservation and use of Underutilized and neglected crops*. 16. Proceedings of the IPGRI. *International Workshop On Genetic Resources of Traditional Vegetables in Africa*. Conservation and use, 29-31 August 1995, ICRAF-HQ, Nairobi, Kenya. Institute of Plant Genetic and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome. Italy.pages 1-15.

Momol, J. P., Olson, M. T., Pradhanang, P. M and Jones, J. B (2005). Evaluation of Thymol as biofumigant for control of Bacterial Wilt Of Tomato under field conditions. *Plant disease*, 89: 497 – 500.

Mondini L, Noorani A & Pagnotta M A (2009). Assessing plant genetic diversity by molecular tools. Diversity, vol. 1(1)19–35.

Muluvi GM, Sprent JI, Soranzo N, Provan J, Odee D, Folkard G, McNicol JW, Powell W (1999) *Amplified fragment length polymorphism (AFLP) analysis of genetic variation in Moringa oleifera Lam. Mol Ecol 8:463–470.*

Muthoni,J. J. Kabira, H. Shimelis and R. Melis (2010). Spread of Bacterial Wilt Disease of Potatoes in Kenya: Who is to Blame? *International Journal of Horticulture 2014, Vol.4, No.3, 10-15*

Mwai NG, Onyango JC & Onyango AOM (2007). Taxonomic Identification and Characterization of African Nightshades (*Solanum* L. Section *Solanum*), 7 (4).

Mwangi J.K, A. B. Nyende, P. Demo and V.N. Matiru. (2008).Detection of Latent Infection by *Ralstonia solanacearum* in Potato(*Solanum tuberosum*) Using Stems Instead of Tubers.*African Journal of Biotechnology, 7 (11): 1644-1649.*

Mwangi S & Kimathi M (2006). African leafy vegetable evolves from underutilized species to commercial cash crops. Research Workshop on Collective Action and Market Access for Smallholders. *African journal of ecology, 13:158-161.*

Nandhini T & Paramaguru P (2013). Evaluating the taxonomic status of *Solanum nigrum* L. using flow cytometry and DNA barcoding technique. *Plant Archives, 23(2):233–242*.

Nandhini T and Paramaguru P (2014). Evaluating the taxonomic status of *Solanum nigrum* L. using flow cytometry and DNA barcoding technique. *Plant Archives, 23(2):233–242.*

Nandhini T, Paramaguru P & Vijayakumar RM (2014). Morphological Characterization and genetic variability study on Makoi (*Solanum nigrum* L). Plant Archives, 14 (1): 35-40.

Nei M (1972). Genetic distance between populations. *American Nature* 106, 283-292.

NeiM (2001). Encyclopedia of Genetics. *Biomedical science*.828-823

Ngomuo M, Stoilova T, Feyissa T, Ndakidemi PA. (2017).Characterization of morphological diversity of jute mallow (Corchorus spp). *Int J Agronom. 2017:1–12.*

Nguyen, M. T. and Ranamukhaarachchi, S. L. (2010). Soil-borne antagonists for biological control of Bacterial wilt disease caused by Ralstonia solanacearum in tomato and pepper. *Journal of Plant Pathology, 92 (2): 395 -406. Vol.92, Issue No. 2.*

Nwakanma DC, Pillay M, Okoli B E and Tenkouanoa Theoretical and Applied Genetics, 2003, Vol.107,850-856.

Nyadanu D, Akromah R, Osei KM &Dordoe BM (2014), Agro morphological Characterization of GbomaEggplant, an Indigenous Fruit and Leafy Vegetable in Ghana., *Journal, 22(4):281–289. African Crop Science*.

Ojiewo CO, Abukutsa-Onyango MMO &Womdim NR (2013a). Exploiting the Genetic Diversity of Vegetable African Nightshades. Bioremediation, Biodiversity and bioavailability, 7(3):6-13.

Ojiewo CO, Mbwambo O M, Swai I, Samali S, Mansuet S T, Mnzava RN, Mrosso L, Minja R &Oluoch M (2013b). Selection Evaluation and Release of varieties from Genetically Diverse African Nightshade Germplasm. *International journal of Plant Breeding. Global Science Books.*

Ojiewo CO, Mbwambo O M, Swai I, Samali S, Mansuet S T, Mnzava RN, Mrosso L, Minja R &Oluoch M (2013b). Selection Evaluation and Release of varieties from Genetically Diverse African Nightshade Germplasm. I*nternational journal of Plant Breeding. Global Science Books.*

Okabe, N. and Goto, M. (1963). Bacteriophages of plant pathogens. *Annual Review of Phytopathology, 1: 397-418.*

Olet EA (2004) Taxonomy of Solanum L. section Solanum in Uganda. *PhD thesis, Agricultural University of Norway.*

Omondi EO, Debener T, Linde M, Onyango M A, Dinssa FF & Winkelmann T (2016). Molecular Markers for Genetic Diversity Studies in African Leafy Vegetables. *Advances in Bioscience and Biotechnology, 7, 188-197*.

Ondieki M J, Aguyoh J N & Opiyo A (2011). Variations in growth and yield characteristics of three black nightshade species grown under high altitude conditions. *Agriculture and Biology Journal of North America*: 2151-7517, ISSN Online: 2151-7525, doi:10.5251/abjna.2011.2.3.401.406, Science Huβ,http://www.scihub.org/ABJNA 38.

Oniang'o R, Grum M N E &Obel-Lawson (2005). Developing African Leafy Vegetables for improved nutrition. *RegionalWorkshop*, 6–9 December.

Onyango CM, Ontita EG, Onwong'a RN, Nyamongo D &Gapusi JR (2016). Status and production practices of vegetable African nightshade (*Solanum nigrum*

L.) in selected communities of Kenya. *American Journal of Experimental Agriculture 13(3): 1-12.*

Opiyo AM, Mungai NW, Nakhone LW &Lagat JK (2015). Production, Status and Impact of Traditional Leafy Vegetables in household food security: A case study of Bondo District-Siaya County-Kenya. *ARPN Journal of Agricultural and Biological Science*. VOL. 10, NO. 9.

OsawaruM E, Ogwu M C &Aiwansoba R O (2015). Hierarchical Approaches to the Analysis of Genetic Diversity in Plants: *A Systematic Overview. University of Mauritius Research Journal, Vol 21.*

Osei MK, Bonsu KO, Agyeman A & Choi HS (2013). Diversity studies on tomato germplasm in Ghana. *African Crop Science Conference Proceedings, Vol. 11:121 – 126.*

Owuor KO, Darius AO &Muriithi NA (2016). Assessment of socio-economic factors affecting production of African nightshades vegetable for enhanced livelihoods in Siaya County, Kenya. *International Journal of Agronomy and Agricultural, Vol. 9(6), 126-134.*

Ozakman M, Schaad NW.(2003) A real-time BIOPCR assay for detection of *Ralstonia solanacearum* race 3, biovar 2, in asymptomatic potato tubers. Canadian Journal of Plant Pathology. 2003;25(3):232-239.*Pakistan Journal of Nutrition* 6(4): 323-326.

Papa R and Gepts P. *Theoretical and Applied Genetics*, (2003), Vol. 106, pp.239-250.

Paramaguru, P. Tilahun, S., and Bapu, J.R.K. (2013) Genetic Diversity in Certain Genotypes of Chilli and Paprika as Revealed by RAPD and *SSR Analysis. Asian Journal of Agricultural Sciences, 5, 25-31.*

Parmar P, Sudhir A, Preethi R, Bhaumik D, Panchal K, Subramanian1 RB, Patel A &Kathiria K B (2013). Identification of a SSR marker (TOM-144) linked to Fusarium wilt resistance in *Solanum lycopersicum*. American Journal ofMolecular Biology, 3: 241-247.

Peratoner G, Seling S, Klotz C, Florian C, Figl U, Schmitt A. (2016).Variation of agronomic and qualitative traits and local adaptation of mountain landraces

of winter rye (Secale cereale L) from Val Venosta/Vinscgau (South Tyrol). *Genet Resourc Crop Evol*. 63:261–273.

Peregrine, W. (1982). Grafting - A simple technique for overcoming bacterial wilt in tomato. Tropical Pest Management 28: 71-76.

Perera L, Russell JR, Provan J, McNicol JW, Powell W (1998) Evaluating genetic relationships between indigenous coconut (Cocos nucifera L.) accessions from Sri Lanka by means of AFLP profiling. *TheorAppl Genet* 96:545–550.

Perrier X, Flori A &Bonnot F (2003). Data analysis methods. In: Hamon P, Seguin M, Perrier X &Glaszmann J C. Genetic diversity of cultivated tropical plants. Enfield, Science Publishers. Montpellier, 43 – 76.

Peterson, R. A., Inch, A.J., Herrington, M.E. and Saranah, J. 1983. A tomato resistant to bacterial wilt biovar 3. *Australasian Plant Pathology* 12: 8-10.

Poczai P &Hyvonen J (2011). The origin of *Solanum nigrum*: can networks help? Springer Science Business Media B.V., 1171–1185.

Potokina E, Blattner F, Alexandrova T, Bachmann K (2002) AFLP diversity in the common vetch (Vicia sativa L.) on the world scale. *TheorAppl Genet 105:58–67.*

Pradhanang, P. M., Elphinstone, J. G. and Fox, R. T. (2005). Identification of crop and weed hosts of *Ralstonia solanacearum* biovar 2 in the hills of Nepal. *Plant Pathology* 49: 403-413.

Pradhang, P. M., Elphinstone, J. G. and Fox, R. T. (2005). Identification of crop and weed hosts of *Ralstonia solanacearum* biovar 2 in the hills of Nepal. *Plant Pathology* 49: 403-413.

Prior, P. and Beramis, M. (1990)a: Introduction de la resistance au fletrissement bacterrien du a *Pseudomonas solanacearum*. In: Persley, G.J., ed., Bacterial wilt disease in Asia and South Pacific. *ACIAR Proceedings* 13: 105-111.

Rao KN (20(04). Plant genetic resources: Advancing conservation and use through biotechnology**.** African Journal of Biotechnology, 3(2), pp. 136-145.

Ravishanker, Kumar S, Baranwal DK, Chatterjee A &Solankey SS (2013). Genetic Diversity Based on Cluster and Principal Component Analyses for Yield and Quality Attributes in Ginger (*Zingiber officinale* Roscoe). *International Journal of Plant Breeding and Genetics, 7: 159-168.*

Regina Ronoh, Noella A. Ekhuya, Marcus Linde,Traud Winkelmann, Mary Abukutsa- Onyango, FekaduFufaDinssa& Thomas Debener (2017): African nightshades: genetic, biochemical and metabolite diversity of an underutilised indigenous leafy vegetable and its potential for plant breeding, *The Journal of Horticultural Science and Biotechnology,* DOI:10.1080/14620316.2017.1358112

Remi K., Ludovic T., Pierre B., & Hubert D. B. (2005). Les légumesfeuilles des pays tropicaux: Diversité, richesse économique et valeursante dans un contextetrès fragile (Leafy vegetables from tropical countries: *Diversity, economic wealth and health value in a very fragile context). Montpellier, France: CIRAD DépartementFlhor.*

Robertson, S. J., Eden-Green, S. J, Jones, P., Ambler, D. J, (2004). *Pseudomonas syzygii*sp. Nov., the cause of Sumatra disease of cloves. *Systematic and Applied Microbiology* 13: 34–43

Rocha EA, Paiva VL, Henrique de Carvalho E & Guimarães4 CT (2010). Molecular characterization and genetic diversity of potato cultivars using SSR and RAPD markers.

Saddler, G. S**. (**2005). Management of bacterial wilt disease. APS Press ST.Paul, MN. 121-132.

Satya, P., R. Banerjee, M. Karan, E. Mukhopadhyay, B. Chaudhary, A. Bera, R.T. Maruthi, and S.K. Sarkar. 2016. Insight into genetic relation and diversity of cultivated and semi-domesticated under- utilized Crotalaria species gained using start codon targeted (SCoT) markers. Biochem. Syst. Ecol. 66: 24–32.

Schaad, N. W., Takatsu, A. and Dianese, J. C. 1978. Serological identification of strains of *Pseudomonas solanacearum* in Brazil. In: Proceedings of

Fourth International Conference on Plant Pathogenic Bacteria, Angers, France, 295-300.

Schippers, R.R. 2000. African Indigenous Vegetables: an overview of the Cultivated Species Chatham, UK: Natural Resources Institute/ACP-EU Technical *Centre for Agricultural and Rural Cooperation*. 214 pages.

Scott JW, Wang JF, Hanson PM. (2005). Breeding tomatoes for resistance to bacterial wilt, a global view. *Acta Horticulturae* 695, 161–172.

Scott, J. W. (1996). Tomato improvement for bacterial disease resistance for the tropics: A contemprary basis and future prospects. *1st International Symposium on Tropical Tomato Diseases. November, 21-22, 1996, at Recife, Pemambuco, Brazil*.

Shackleton, C.M., Pasquini, M.W., Drescher, A.W. (2009). African Indigenous Vegetables in Urban Agriculture. Sterling VA, London.

Shan F, Clarke HC, Plummer JA, Yan G, Siddique KHM (2005) Geographic patterns of genetic variation in the world collections of wild annual Cicer characterized by amplified fragment length polymorphisms. *TheorAppl Genet 110:381–391.*

Sharifova S, Mehdiyeva S, Theodorikas K &Roubos K (2013). Assessment of Genetic Diversity in Cultivated Tomato (*Solanum Lycopersicum*L.) Genotypes using RAPD Primers. *Journal of Horticultural Research, vol. 21(1): 83-89.*

Sharma, M. P., Gaur, A. Tanu, U. Sharma, O. P. (2004). Prospects of arbuscular mycorrhiza in sustainable management of root and soil-borne diseases of vegetable crops. In: Mukerji, K. G. (Ed.). *Disease management of fruits and vegetables. Vol. I. Fruit and vegetable diseases*. Kluwer Academic Publishers, The Netherlands 501-539.

Sikoru, R., Beed, F., Ezin, V., Gbèhounou, G., Miller, S. A. and Wydra, K. (2004). First report of bacterial wilt of tomato (Solanum lycopersicum L) caused by Ralstonia solanacearum in Benin.

Singh M, Tomar A, Mishra C.N. & Srivastava S.B.L. (2011). *Genetic parameters and character association studies* in Indian mustard. J. oilseed Brassica. 2(1): 35-38.

Singh S. P., Raghavendra K., Singh R. K. and Subbarao S. K., 2001. Studies on larvicidal properties of leaf extract of *Solanum nigrum* Linn. (family Solanaceae). Cur.Sci.81(12): 1529-1530.

Singh, S.P., H.K.Yadav, S. Shukla and A. Chatterjee. (2003). Studies on different selection parameters in opium poppy (*Papaver somniferum*). J. Med. Arom. Pl. Sci., 25: 8-12.

Smith, J. J., Offord, L.C., Holderness, M. and Saddler, G. S. 1995. Genetic diversity of *Burkholderia solanacearum* (Synonym *Pseudomonas solanacearum*) race 3 in Kenya. *Applied and Environmental Microbiology* 61: 4263-4268.

Solon, F. S. (1997) The progress of wheat flour fortification with vitamin A in the Philippines.

Steinite I &Ievinsh G (2003). Possible role of trachomes in resistance of strawberry cultivars against spider mite. Acta Universitatis Latviensis, 662:59–65.

Stoilova T & Pereira G (2013). Assessment of the genetic diversity in a germplasm collection of cowpea (*Vigna unguiculata* (L.) Walp) using morphological traits, *African Journal of Agricultural Research Vol. 8(2), pp. 208-215*.

Suvakanta-Barik, Senapati S K, Subhashree-Apa-rajita, Anuradha-Mohapatra Rout G. *Bioscience, (2006), Vol.60, No.1, pp. 123-128.*

Swanson,J.K.,Yao,J.,Tans-Kersten,J.,andAllen,C.(2005).Behaviorof *Ralstonia solanacearum* race3biovar2duringlatentandactiveinfectionofgeranium. *Phytopathology* 95, 136– 143.doi:10.1094/PHYTO-95-0136.

Swanson,J.K.,Yao,J.,Tans-Kersten,J.,andAllen,C.(2007).Behaviorof *Ralstonia solanacearum* race3biovar2duringlatentandactiveinfectionofgeranium. *Phytopathology* 95, 136– 143.doi:10.1094/PHYTO-87-0123.

Tahat, M. M**.** and Kamaruzaman S., (2010). *Ralstonia solanacearum*: The Bacterial Wilt Causal Agent. Asian Plant Sciences 9: 385-393.

Tahat, M. M., Kamaruzaman, S. Radziah, O. (2011). Bio-compartmental *In Vitro* System for *Glomus mosseae* and *Ralstonia solanacraum Interaction. International Botany 7: 295-299.*

Teixeira da Silva JA, Rana TS, Narzary D, Verma N, Meshram DT & Ranade SA (2013). Pomegranate biology and biotechnology: a review. *Science and Horticulture 160:85–107*

Tumbilen Y, Farary A, DaunayMC, Mutlu S &Doganlar S (2011). Genetic diversity in Turkish eggplant as determined by morphological and molecular analysis*. International research journal of Biotechnology, 2:16-25.*

Tuwei, C., Opala, P.A., Omami, E.N., Opile, W.R. (2013). Response of the African nightshade to phosphate fertilizer application in Western Kenya. *Archives of Applied Science Research*, 5, 195-201.

van Elsas, J. D., Kastelein, P., de Vries, P. M., and van Overbeek, L. S. 2001. Effects of ecological factors on the survival and physiology of *Ralstonia solanacearum* bv. 2 in irrigation water. Can. J. Microbiol. 47:842-854.

Vergara GV, Bughrara SS (2003) AFLP analysis of genetic diversity in bentgrass. Crop Sci 43(66):2162–2171.

War AB, Paulraj GM, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S & Sharma CH (2012). Mechanisms of plant defense against insect herbivores. *Plant Signaling& Behavior, 7:10, 1306-1320, DOI: 10.4161/psb.2166.*

World Health Organization. WHA global nutrition targets 2025: wasting policy brief. 2014.www.who.int/nutrition/topics/globaltargets_wasting_policybrief.pdf.*G oogle Scholar.*

Wu Q., Leung J. Y. S., Huang X., Yao B., Yuan X., Ma J., Guo S. (2015). Evaluation of the ability of African nightshade *Solanum nigrum* L. for phytoremediation of thallium- contaminated soil. Environ Sci Pollut Res (2015) 22: 11478. doi:10.1007

Yim O &Ramdeen TK (2015). Hierarchical Cluster Analysis: Comparison of Three Linkage Measures and Application to Psychological Data. The Quantitative Methods for Psychology Journal, 11(1).

Zebish A, Virginia P, Pallavi S & Pandey M (2016). Assessment of Nutritional, Anti Nutritional and Antioxidant Properties of Underutilised Leaves of Moringa Oliefera and *Solanum nigrum*. *World Journal of Pharmacy and Pharmaceutical Sciences, Vol 5 (8):1285-1292.*

Zhang C, A. S. Pratap, S. Natarajan (2012). "Evaluation of morphological and molecular diversity among south Asian germplasms of *Cucumis sativs*and*Cucumis melo*," *International Scholarly Research Notices*, vol. 2012, Article ID 134134,11 pages.

Zhou, Y., B. Liu, Y. Mbuni, X. Yan, G. Mwachala, G. Hu, and Q. Wang. (2017). Vascular flora of Kenya, based on the Flora of Tropical East Africa. PhytoKeys 126(90):113– 126. doi: 10.3897/ phytokeys.90.20531.

Zhu, G. (2012). Rewiring of the fruit metabolome in tomato breeding. Cell 172,

APENDICES

Appendix A: African nightshade accession used in the study

(Accessions) Name	Local Name	Source	GPS Coordinates	
			longitude	Latitude
Bungoma 1	Namasaka	Lugulu	34.751	0.665
Kakamega1	Esucha	Ingotse	34.696	0.356
Trans Nzoia 1	Kisoyet	Kwanza	35.003	1.163
Bungoma 2	Namasaka	Maeni	34.750	0.792
Kakamega 2	Irisuza	Lukuyani	35.103	0.711
Bungoma 3	Namasaka	Mabanga	34.619	0.601
Kakamega 3	Liisucha	Chimoi	34.826	0.580
Kakamega 4	Esucha	Navakholo	34.681	0.407
Kakamega 5	Lisutsa	Shinyalu	34.766	0.274
Bungoma 4	Namasaka	Mayanja Vitunguu	34.544	0.528
Bungoma 5	Namasaka	Chwele	34.581	0.737
Kakamega 6	Liisucha	Lubao	34.807	0.332
Bungoma 6	Namasaka	Bokoli	34.660	0.712
Trans Nzoia 2	Managu	Kiminini	34.927	0.884
Trans Nzoia 3	Namasaka	Sikhendu	34.830	0.884
Bungoma 7	Namasaka	Sang'alo	34.593	0.528
Bungoma 8	Namasaka	Kimilili	34.727	0.792
Trans Nzoia 4	Managu	Kiungani	34.951	0.95
Bungoma 9	Namasaka	Ndalu	34.987	0.818
Kakamega 7	Liisucha	Malava	34.855	0.454
Kakamega 8	Liisucha	Kaburengo	34.801	0.578
Trans Nzoia 5	Kisocheet	Saboti	34.838	0.931

Trans Nzoia 6	Osoig	Endebesi	34.852	1.086
Kakamega 9	Liisucha	Lwandeti	34.849	0.607
Bungoma 10	Namasaka	Kamukuywa	34.784	0.799
Trans Nzoia 7	Managu	Mucharage	34.856	0.818
Trans Nzoia 8	Namasaka	Bidii	35.035	1.033
Kakamega 10	Liisucha	Matete	34.805	0.555
Trans Nzoia 9	Managu	Bondeni	34.902	0.991
Trans Nzoia 10	Ksoyo	Cherang'ani	35.234	0.988

Appendix B: Qualitative traits and their scores according to the NBPGR descriptors

Accession	Colour of ripe fruit	Stem ridge	Leaf shape	Leaf margin	Inflorescence Type	Leaf surface	Plant type
Bungoma (*Solanum villosum* 1)	1	1	1	1	1	1	1
Kakamega 1(*Solanum villosum)*	1	2	1	1	1	1	1
Trans Nzoia 1*Solanum nigrum*	2	1	2	2	1	1	1
Bungoma 2(*Solanum nigrum*	2	2	2	2	1	1	1
Kakamega 2(*Solanum scabrum* improved)	3	2	1	1	2	2	2
Bungoma 3(*Solanum villosum)*	1	1	1	1	1	1	1
Kakamega 3(*Solanum scabrum* improved)	3	2	1	1	2	2	2
Kakamega 4(*Solanum villosum)*	1	2	1	1	1	1	1
Kakamega 5(*Solanum villosum*)	1	1	1	1	1	1	1
Bungoma 4(*Solanum nigrum)*	2	2	2	2	1	1	1
Bungoma 5(*Solanum nigrum*)	2	2	2	2	1	1	1
Kakamega 6(*Solanum nigrum)*	2	2	2	2	1	1	1
Bungoma 6(*Solanum nigrum*)	2	2	2	2	1	1	1
Trans Nzoia 2(*Solanum villosum)*	1	2	1	1	1	1	1
Trans Nzoia 3(*Solanum nigrum)*	2	1	2	2	1	1	1
Bungoma 7(*Solanum*	3	2	1	1	2	2	2

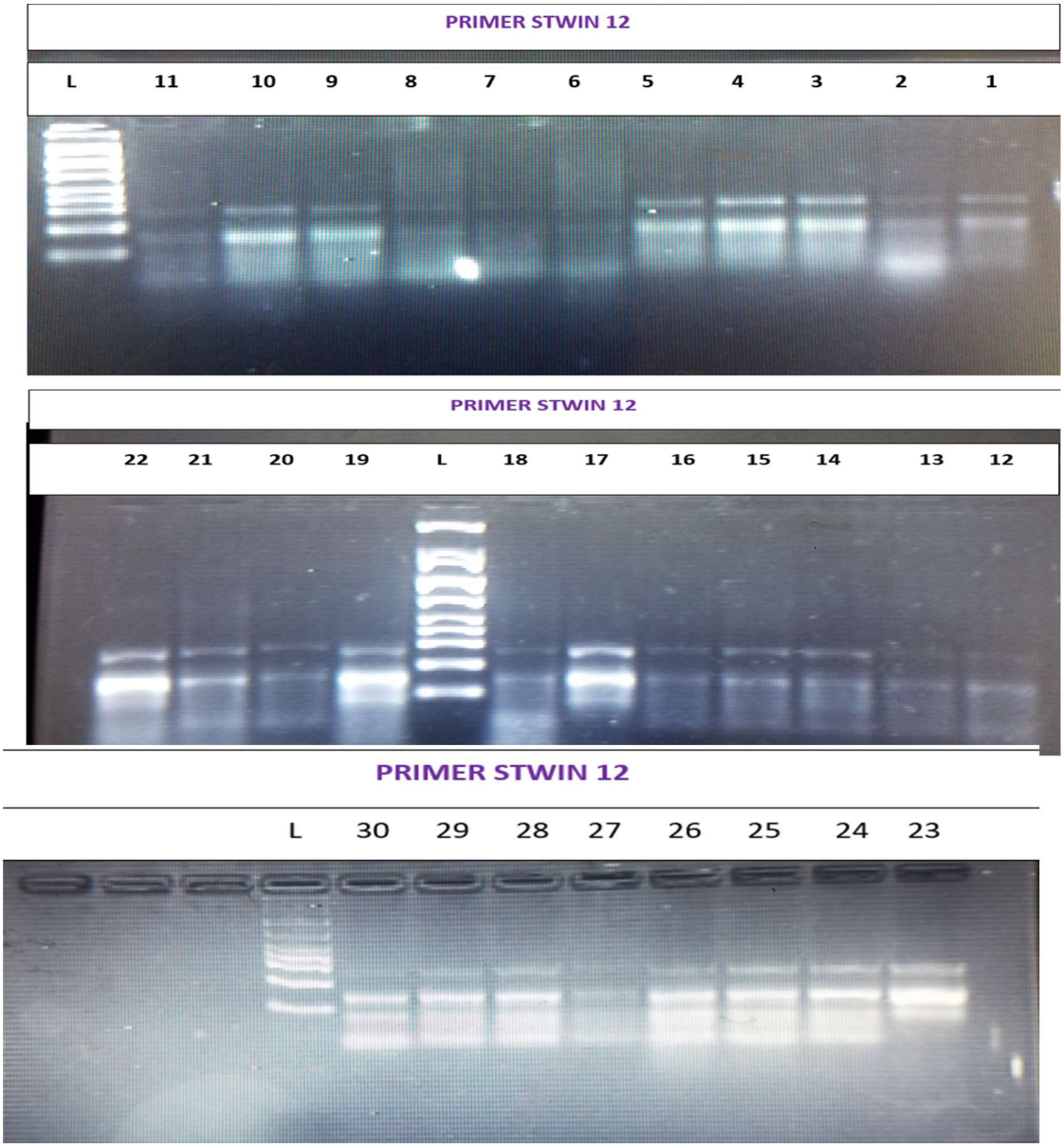
scabrum improved)							
Bungoma 8(*Solanum nigrum)*	2	2	2	2	1	1	1
Trans Nzoia 4(*Solanum scabrum* improved)	3	2	1	1	2	2	2
Bungoma 9(*Solanum villosum)*	1	2	1	1	1	1	1
Kakamega 7(*Solanum nigrum)*	2	2	2	2	1	1	1
Kakamega 8(*Solanum nigrum)*	2	1	2	2	1	1	1
Trans Nzoia 5 (*Solanum nigrum)*	2	2	2	2	1	1	1
Trans Nzoia 6 (*Solnum nigrum)*	2	1	2	2	1	1	1
Kakamega 9 (*Solanum scabrum* improved)	3	2	1	1	2	2	2
Bungoma 10(*Solanum villosum)*	1	1	1	1	1	1	1
Trans Nzoia 7(*Solanum villosum)*	1	1	1	1	1	1	1
Trans Nzoia 8(*Solanum villosum)*	2	2	1	2	1	1	1
Kakamega 10(*Solanum nigrum)*	2	1	2	2	1	1	1
Trans Nzoia 9(*Solanum nigrum)*	2	1	2	2	1	1	1
Trans Nzoia 10(*Solanum nigrum)*	2	1	2	2	1	1	1

NBPGR Descriptors

Plant type: 1=Semi erect2=Erect; Leaf surface: 1=Glaborous (sparsely), 2=pubescent (Densely). Leaf margin: 1 =Sinuate –dentate, 2 =Entire, 3 = Lobed; Leaf shape: 1 = Lanceolate, 2 = Ovate, 3 = Rhomboid; Stem ridge: 1= Smooth ridges, 2= Dented; Fruit colour: 1= Orange, 2= Dark purple, 3=Black; Inflorescence type:1=Simple,2=Forked.

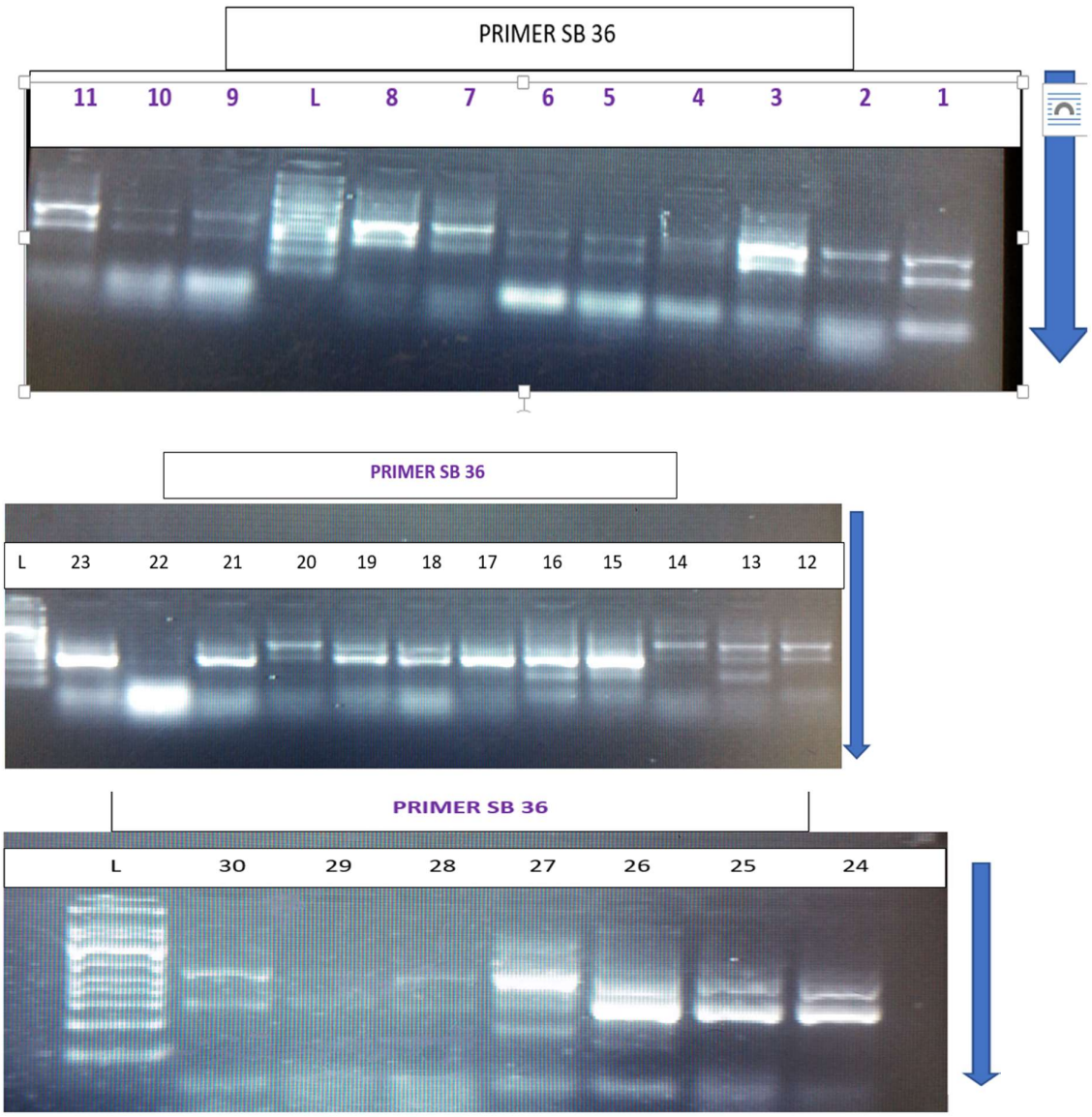
Source: Singh 2003.

Appendix C: PCR products amplified with primer STWIN 12 and visualized under UV light.



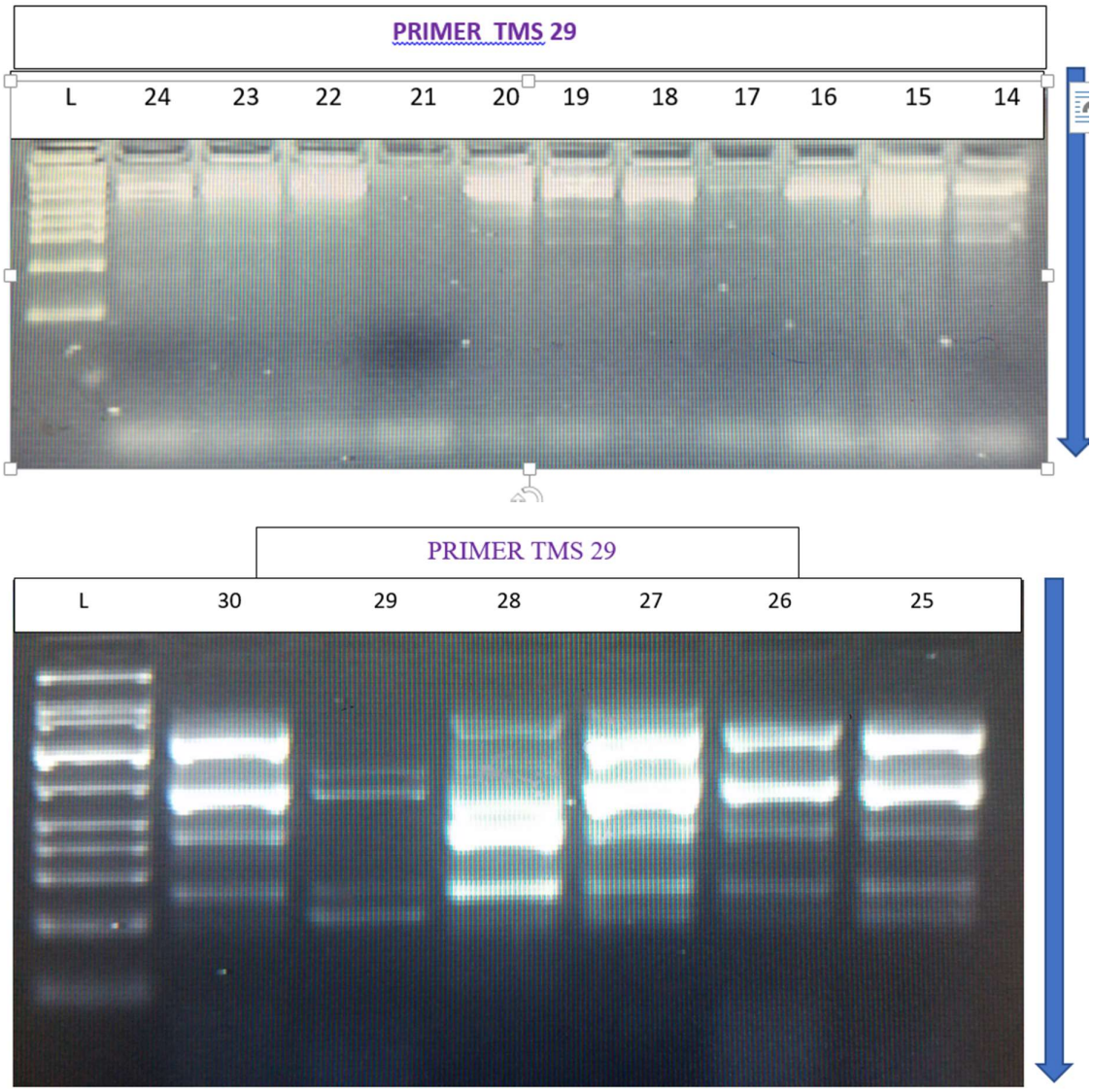
1= Bungoma 1 (S.N), 2= Kakamega 1(S.V), 3= Trans Nzoia 1 (SN), 4= Bungoma 2(SN), 5= Kakamega 2(SS), 6= Bungoma 3 (SS), 7=Kakamega 3 (SS),8= Kakamega 4(SV), 9= Kakamega 5(SV), 10= Bungoma 4 (SN), 11= Bungoma 5(SN), 12= Kakamega 6 (SN), 13= Bungoma 6(SN), 14= Trans Nzoia 2(SV), 15= Trans Nzoia 3(SN), 16= 2 Bungoma 7 (SS), 17= Bungoma 8 (SN),18= Trans Nzoia 4 (SS), 19=Bungoma 9(SV),20= Kakamega 7 (SN), 21=Kakamega 8 (SN)22= Trans Nzoia 5 (SN), 23= Trans Nzoia 6 (SN), 24= Kakamega 9(SS), 25= Bungoma 10 (SV), 26= Trans Nzoia 7(SV), 27= Trans Nzoia 8(SV), 28= Kakamega 10(SN0, 29= Trans Nzoia 9 (SN), 30=Trans Nzoia 10(SN).(SN-*Solanumnigrum*, SV-*Solanum villosum*, SS-*Solanumscabrum*)

Appendix D: PCRproducts amplified with primer SB 36 and visualized under UVlight



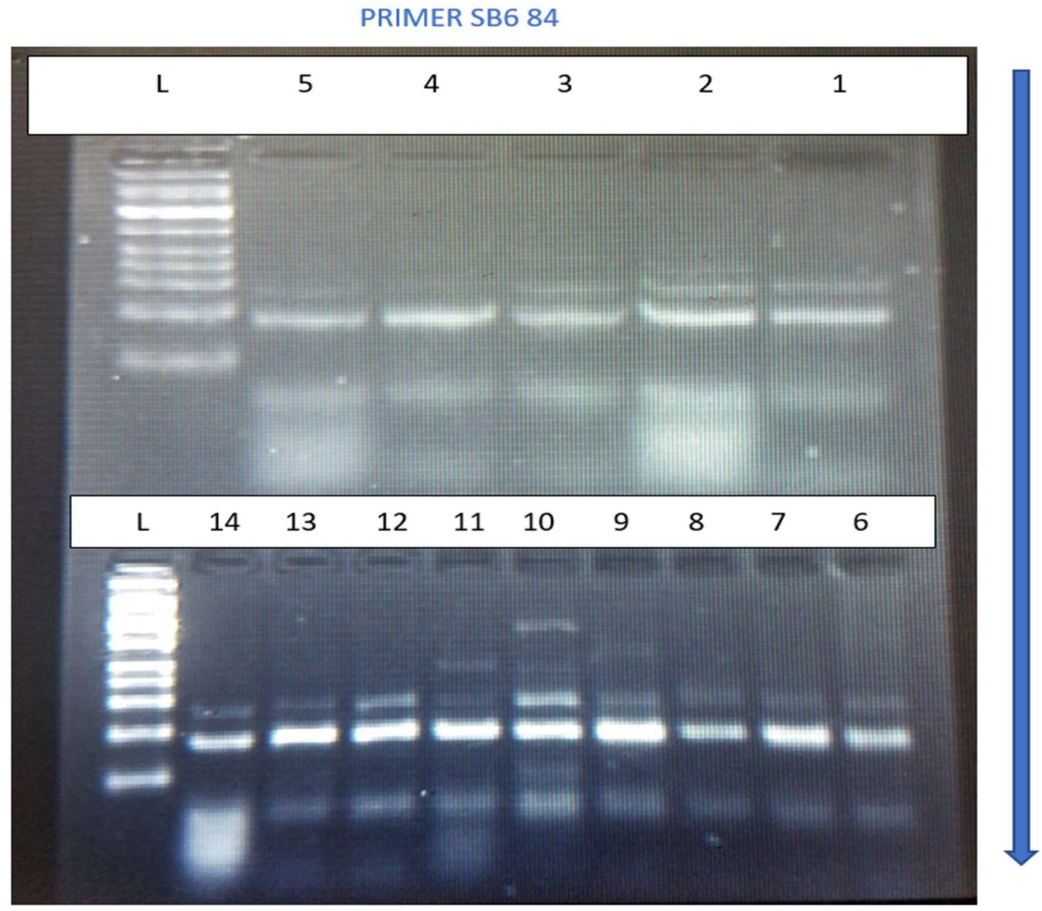
1= Bungoma 1 (S.N), 2= Kakamega 1(S.V), 3= Trans Nzoia 1 (SN), 4= Bungoma 2(SN), 5= Kakamega 2(SS), 6= Bungoma 3 (SS), 7=Kakamega 3 (SS),8= Kakamega 4(SV), 9= Kakamega 5(SV), 10= Bungoma 4 (SN), 11= Bungoma 5(SN), 12= Kakamega 6 (SN), 13= Bungoma 6(SN), 14= Trans Nzoia 2(SV), 15= Trans Nzoia 3(SN), 16= 2 Bungoma 7 (SS), 17= Bungoma 8 (SN),18= Trans Nzoia 4 (SS), 19=Bungoma 9(SV),20= Kakamega 7 (SN), 21=Kakamega 8 (SN)22= Trans Nzoia 5 (SN), 23= Trans Nzoia 6 (SN), 24= Kakamega 9(SS), 25= Bungoma 10 (SV), 26= Trans Nzoia 7(SV), 27= Trans Nzoia 8(SV), 28= Kakamega 10(SN0, 29= Trans Nzoia 9 (SN), 30=Trans Nzoia 10(SN).(SN-*Solanumnigrum*, SV-*Solanumvillosum*, SS-*Solanumscabrum*)

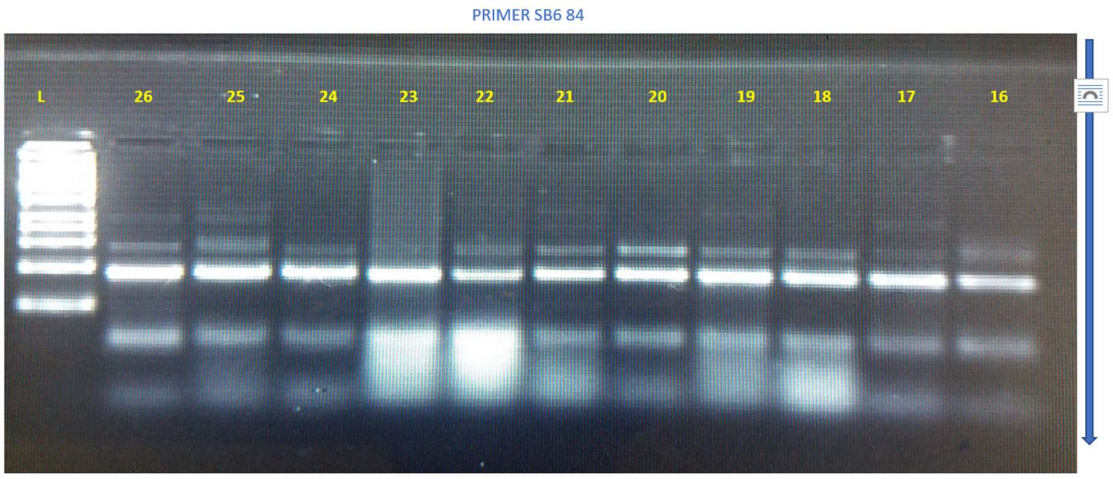
Appendix E PCR products amplified with primer TMS 29 and visualized under UV light.

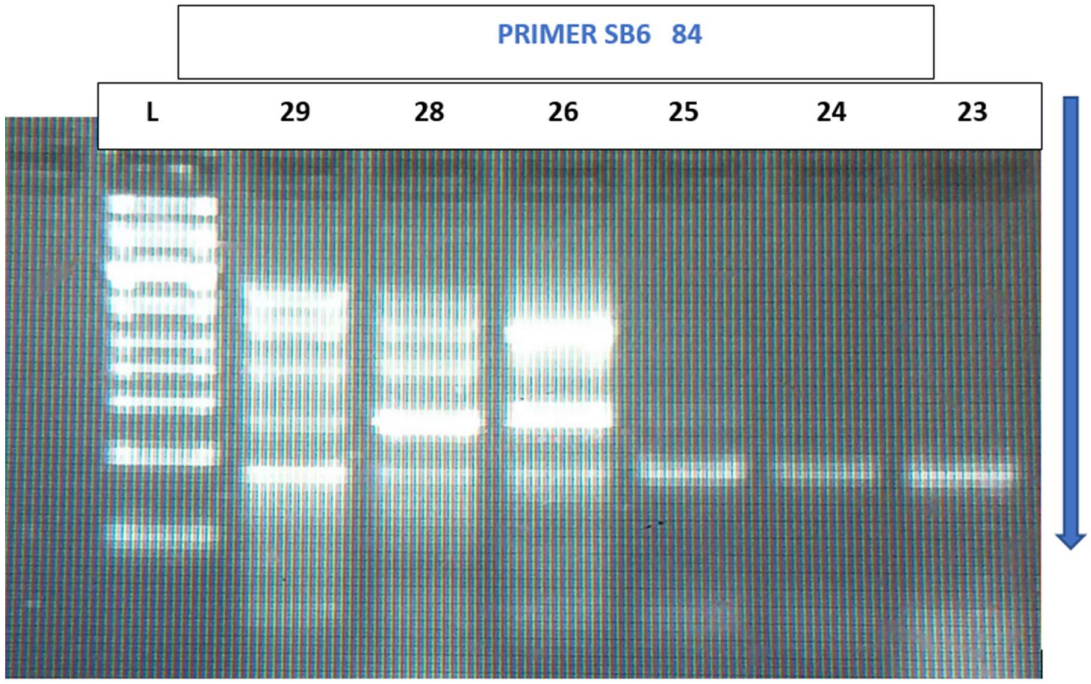


1= Bungoma 1 (S.N), 2= Kakamega 1(S.V), 3= Trans Nzoia 1 (SN), 4= Bungoma 2(SN), 5= Kakamega 2(SS), 6= Bungoma 3 (SS), 7=Kakamega 3 (SS),8= Kakamega 4(SV), 9= Kakamega 5(SV), 10= Bungoma 4 (SN), 11= Bungoma 5(SN), 12= Kakamega 6 (SN), 13= Bungoma 6(SN), 14= Trans Nzoia 2(SV), 15= Trans Nzoia 3(SN), 16= 2 Bungoma 7 (SS), 17= Bungoma 8 (SN),18= Trans Nzoia 4 (SS), 19=Bungoma 9(SV),20= Kakamega 7 (SN), 21=Kakamega 8 (SN)22= Trans Nzoia 5 (SN), 23= Trans Nzoia 6 (SN), 24= Kakamega 9(SS), 25= Bungoma 10 (SV), 26= Trans Nzoia 7(SV), 27= Trans Nzoia 8(SV), 28= Kakamega 10(SN0, 29= Trans Nzoia 9 (SN), 30=Trans Nzoia 10(SN).(SN-*Solanumnigrum*, SV-*Solanumvillosum*, SS-*Solanumscabrum*)

Appendix F: PCR products amplified with primer SB6 84 and visualized under UV light

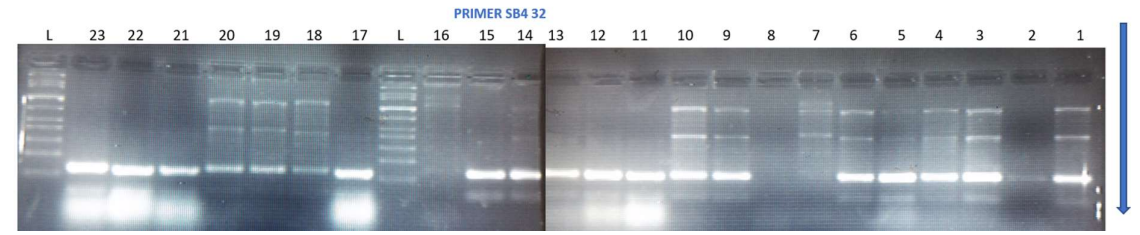


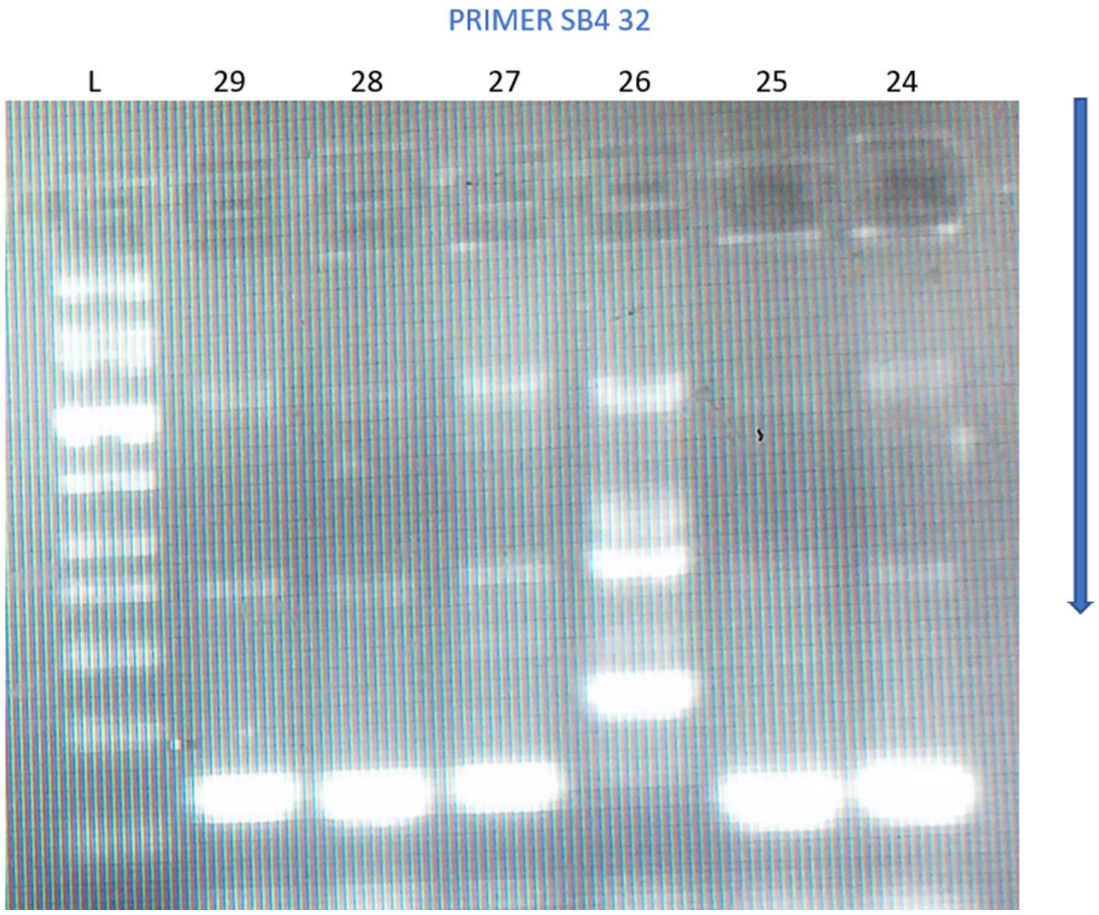




1= Bungoma 1 (S.N), 2= Kakamega 1(S.V), 3= Trans Nzoia 1 (SN), 4= Bungoma 2(SN), 5= Kakamega 2(SS), 6= Bungoma 3 (SS), 7=Kakamega 3 (SS),8= Kakamega 4(SV), 9= Kakamega 5(SV), 10= Bungoma 4 (SN), 11= Bungoma 5(SN), 12= Kakamega 6 (SN), 13= Bungoma 6(SN), 14= Trans Nzoia 2(SV), 15= Trans Nzoia 3(SN), 16= 2 Bungoma 7 (SS), 17= Bungoma 8 (SN),18= Trans Nzoia 4 (SS), 19=Bungoma 9(SV),20= Kakamega 7 (SN), 21=Kakamega 8 (SN)22= Trans Nzoia 5 (SN), 23= Trans Nzoia 6 (SN), 24= Kakamega 9(SS), 25= Bungoma 10 (SV), 26= Trans Nzoia 7(SV), 27= Trans Nzoia 8(SV), 28= Kakamega 10(SN0, 29= Trans Nzoia 9 (SN), 30=Trans Nzoia 10(SN).(SN-*Solanumnigrum*, SV-*Solanumvillosum*, SS-*Solanumscabrum*)

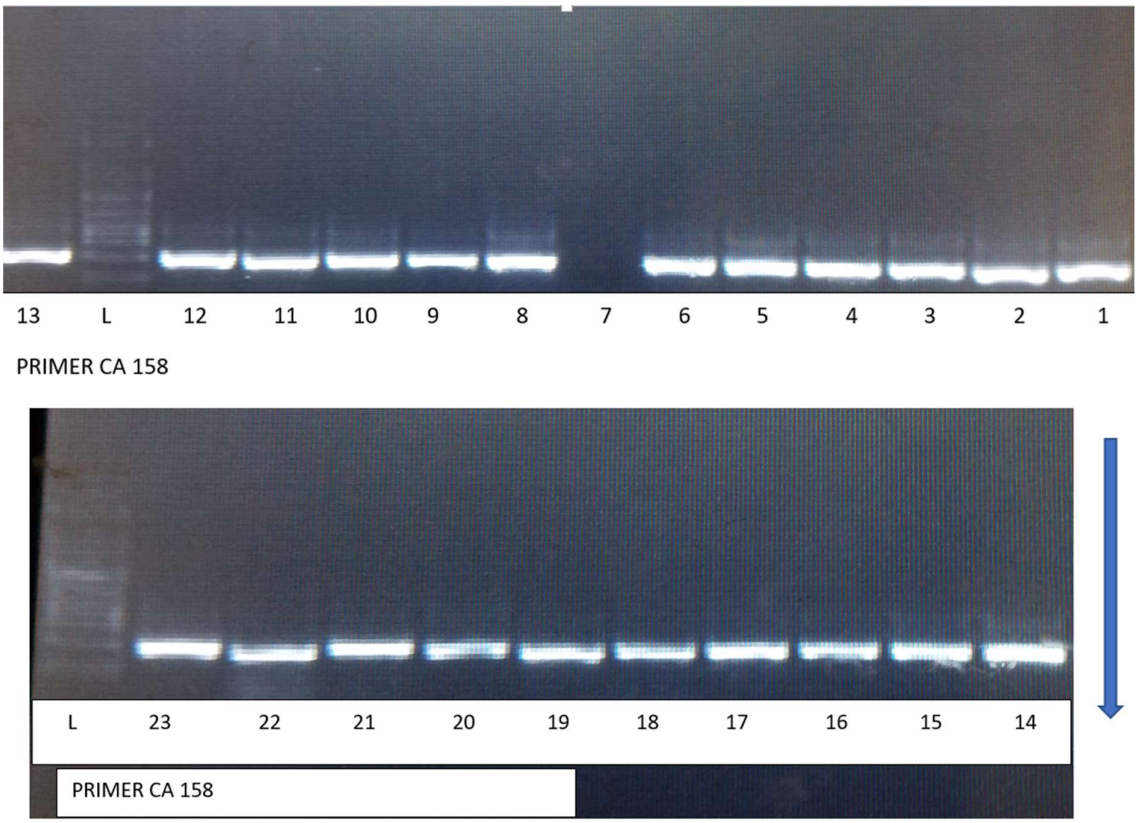
Appendix G: PCR products amplified with primer SB4 32 and visualized under UV light.





1= Bungoma 1 (S.N), 2= Kakamega 1(S.V), 3= Trans Nzoia 1 (SN), 4= Bungoma 2(SN), 5= Kakamega 2(SS), 6= Bungoma 3 (SS), 7=Kakamega 3 (SS),8= Kakamega 4(SV), 9= Kakamega 5(SV), 10= Bungoma 4 (SN), 11= Bungoma 5(SN), 12= Kakamega 6 (SN), 13= Bungoma 6(SN), 14= Trans Nzoia 2(SV), 15= Trans Nzoia 3(SN), 16= 2 Bungoma 7 (SS), 17= Bungoma 8 (SN),18= Trans Nzoia 4 (SS), 19=Bungoma 9(SV),20= Kakamega 7 (SN), 21=Kakamega 8 (SN)22= Trans Nzoia 5 (SN), 23= Trans Nzoia 6 (SN), 24= Kakamega 9(SS), 25= Bungoma 10 (SV), 26= Trans Nzoia 7(SV), 27= Trans Nzoia 8(SV), 28= Kakamega 10(SN0, 29= Trans Nzoia 9 (SN), 30=Trans Nzoia 10(SN).(SN-*Solanumnigrum*, SV-*Solanumvillosum*, SS-*Solanumscabrum*)

Apendix H: PCR products amplified with primer CA 158 and visualized under UV light.



1= Bungoma 1 (S.N), 2= Kakamega 1(S.V), 3= Trans Nzoia 1 (SN), 4= Bungoma 2(SN), 5= Kakamega 2(SS), 6= Bungoma 3 (SS), 7=Kakamega 3 (SS),8= Kakamega 4(SV), 9= Kakamega 5(SV), 10= Bungoma 4 (SN), 11= Bungoma 5(SN), 12= Kakamega 6 (SN), 13= Bungoma 6(SN), 14= Trans Nzoia 2(SV), 15= Trans Nzoia 3(SN), 16= 2 Bungoma 7 (SS), 17= Bungoma 8 (SN),18= Trans Nzoia 4 (SS), 19=Bungoma 9(SV),20= Kakamega 7 (SN), 21=Kakamega 8 (SN)22= Trans Nzoia 5 (SN), 23= Trans Nzoia 6 (SN), 24= Kakamega 9(SS), 25= Bungoma 10 (SV), 26= Trans Nzoia 7(SV), 27= Trans Nzoia 8(SV), 28= Kakamega 10(SN0, 29= Trans Nzoia 9 (SN), 30=Trans Nzoia 10(SN).(SN-*Solanumnigrum*, SV-*Solanumvillosum*, SS-*Solanumscabrum*)