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

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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.

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CERTIFICATION

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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.

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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*. Genetic diversity can be utilized in breeding programs to develop improved African nightshade accessions that are high yielding for both leaf and fruit and resistant to biotic and abiotic stresses. The aim of this study was to evaluate the existence of genetic diversity in African nightshade accessions through morphological and genotypic characterization and also existing inherent resistance to bacterial wilt *Ralstonia solanacearum* through screening in the field and greenhouse studies. 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. 6 SSR primers were used and each primer generated 1polymorphic band. Polymorphic Information Content ranged from 0.4215 to 0.8212 with a mean of 0.5881. The average heterozygosity He=0.9111 for SSR markers used. The dendrogram showed that the accessions grouped into three main clusters showing richness in diversity, it also revealed that the coefficient distance that separated most of the accessions was less than 79.56. These findings show that there were possibilities of crossability among the accessions, Variation among regions was not genetically evident. 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.

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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 essential leafy vegetable in Kenya and its origin is Eurasia (Jagatheeswari *et al.,* 2013). The crop occupies an important 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). Kenya's mainly grown African nightshade species include *S. villosum, S. scabrum, S. nigrum, S.americanum, S. sarrachoides* and *S. physalifolium* (Ojiewo *et al*., 2013b; Matasyoh and Bosire Na, 2016).

Even though African nightshade species have been studied broadly, their correct taxonomic identification is yet to be established. This is because of continued inter and intraspecific hybridization which occurs naturally among African nightshade species as well as due to inconsistent genetic variation (Zebish *et al.,* 2016). The susceptibility of morphological traits to phenotypic plasticity and the existence of many ploidy series have also caused problems to their taxonomic identification (Poczai and Hyvonen, 2011). Different communities use different local names to identify African nightshade

species creating further confusion in the differentiation of one species from the other (Poczai and Hyvonen, 2011; Ojiewo *et al.,* 2013a). Also same species are given different names and different species given same name creating confusion in the differentiation of species (Ojiewo *et al.,* 2013a). African nightshade has as well been regarded as a weed hence little studies have been done on the crop and there's also lack of enough personnel assigned the duty of evaluation and preserving of African nightshade germplasm (Zebish *et al.,* 2016).This has led to limited information, to help the scientist breed more improved varieties.

Diversity studies give useful information for scientist to know the genetic relationships and distances between crops requirement for any breeding program. Morphological traits have been used to easily characterise and identify plants but they are affected by the environment and cannot easily differentiate closely related species while molecular markers are stable and present in all tissues not considering growth, differentiation, development, or defense status of the cell, they are also not influenced by environmental, pleiotropic and epistatic effects (Mondini *et al.,* 2009) which corrects the mistakes incurred during phenotypic characterization (Mondini *et al.,* 2009), by which genetic diversity is identified through existence of variation at specific gene loci. African nightshade leaves have an average, 1.4g fiber, 87.2% water, 3660µg of beta carotene, 20mg ascorbic acid, 75mg phosphorus, 442 mg calcium, 0.59mg riboflavin per 100g fresh weight (Ojiewo *et al*., 2013a: Klocke *et al.,* 2016).The vegetable can give the daily nutrients allowance required by an adult for B-carotene, iron, calcium, and ascorbic acid and 40% of protein if 100g of the fresh vegetable consumed (Abukutsa *et al.,* 2005). The leafy African nightshade contains substantial amounts of protein and amino acids such as methionine, minerals like iron, calcium and phosphorus, vitamins A and C, fat and fiber (Zebish *et al.,* 2016). Nutrient composition

of African nightshade however varies according to soil fertility of the site where it is grown, the age of the plant and the plant type (Jagatheeswari *et al.,* 2013).

African nightshade is used worldwide for the treatment of various diseases such as ear pains, as a therapy for convulsions, pain reliever, an anti-helminthes, an antiseptic, ringworm, ulcers, blood pressure and heart diseases(Jagatheeswari *et al.*, 2013; Matasyoh and Mwaura, 2014).

The increase in consumer awareness on the nutritional and medicinal value of African nightshade has concurrently led to increased consumption increasing its market demand (Ojiewo *et al.,* 2013) but the supply is low because researchers have not come up with enough improved varieties to meet the increased demand. There is need that efforts are made to improve on African nightshade production through increased cultivation for commercial purposes so as to try and meet the demand in the market.

Major drawbacks faced during the production of African nightshade include, consumer awareness, human activity, climate change, disease and pest (Ojiewo *et al.,* 2013). In addition, lack of disease and pest resistant, climate resilient varieties and high cost of production which intern interferes with the trade (Ojiewo *et al.,* 2013; Schafer *et al.,* 2006).

Sustainable and cost effective, production of nightshade is endangered by bacterial wilt (Schafer *et al.,* 2006), caused by *Ralstonia solanacearum,* a soil borne pathogen, (Sikoru *et al.,* 2004), which enters and infects the crop (Pradhanang *et al.,*2005) through roots, natural openings, cracks, or wounds causing wilting due to clogging of xylem vessels (Genin, 2010), thus hindering movement of water up the plant (Kelman,1954), the disease is a yield limiting trouble in crops to solanaceae family grown in regions in Kenya.

In addition to its lethality, is the ability of *Ralstonia solanacearum* to survive in soils for many years and to form latent infection of the bacterium (Hayward, 1991; Wenneker *et al*., 1999).The pathogen is found worldwide, mainly in tropical and subtropical regions (Hayward, 1991; Hayward *et al*., 2015) nevertheless, also in Europe and North America where cold tolerant strains were introduced in the 1990s (Janse *et al*., 2004; Swanson *et al*., 2005). The spreading of *Ralstonia solanacearum* is a danger to crops and the pathogen is considered a quarantine bacterium.

The most economic damage has been reported on tobacco, tomato and potato on which it causes up to 90% crop loss (Mallikarjun *et al.,* 2008) and on eggplant causing upto 70% crop loss (Zebish *et al.,* 2016). However, none has been reported on nightshade.

The pathogen survives in soil, plant debris, water for prolonged periods (Muthoni *et al.,* 2010), Small holder farmers are often unaware of severity of the pathogen (Coyne *et al*. 2006a, 2006b) .The home seed selection system does not also ensure clean planting materials. Basically small holder farmers select seed which are healthy with naked eyes without any cornfirmation of being free from bacterial wilt infection for production unaware of the spread and severity of the quarantine bacterium and contagious soil borne pathogen (Coyne *et al.,* 2006a, 2006b) thus difficult to understand the real trouble and hard to plan strategies for intervention to the crop. There is need therefore to evaluate genetic diversity and resistance of African nightshade S*olanum nigrum L* to Bacterial wilt *Ralstonia solanacearum* 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. This is because limited genetic diversity studies of African nightshade have been exploited, however the information already generated has not been consistent among studies since some morphological traits are influenced by environmental conditions and therefore the traits expressed may vary from one environment to another (Dhasmana *et al.,* 2007). African nightshade is a complex consisting of different species but because of the difference, ploidy levels existing between those species and the morphological trait similarity among the different species due to their closely identical genome, many researchers tend to treat different African nightshade species as belonging to one species *S. nigrum L*. (Nandhini and Paramaguru, 2013).Farmers who consume are able to differentiate different Solanum nigrum but this has not presuppose diversity. As much as farmers try to improve on their production levels so as to meet the ever increasing African nightshade demand, the main challenge they are faced with is lack of disease and pest resistant varieties (Ojiewo *et al.,* 2013) and also lack of quality seed, thus reusing saved seed unware of the spread of the quarantine bacterium which is soil borne disease that is a devastating bacterial pathogen of African nightshade (Coyne *et al.*, 2006a, 2006b, Muthoni *et al.,* 2010;Swanson *et al*., 2015) and is genetically diverse, can also stay alive in water and soil for several years (Muthoni *et al.,* 2020).Therefore, there is need to produce more improved varieties to suit the suitable consumer preferences, that are disease and pest resistant and also resilient to climate change.

1.3. General Research objective

The general objective of the study was to Reverage the importance of African nightshade S*olanum 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

African nightshades consist of several species within the section *Solanum* (Shackleton *et al.,* 2009). African nightshades are used mainly as leafy vegetables in sub-Saharan Africa (Berinyuy *et al.,* 2002; Chweya and Eyaguirre, 1999; Edmonds and Chweya, 1997; Ojiewo *et al.,* 2013). They too have medicinal value also (Schippers, 2000) .In spite of the huge variation only the use of little species well known.

The most popular species utilized as vegetables are *Solanum scabrum, S. villosum, S. americanum, S. sarrachodes* and *S. retroflexum* (Mwai *et al.,* 2007; Ojiewo *et al.,* 2013). Some species are specific in distribution and a large number are distributed widely across different geographical locations (Maundu *et al.,* 2009). Traditionally, African nightshades species were conserved and utilized by farmers in garden or harvested from the wild (Dinssa *et al.,* 2014).

The area under cultivation is still small since they are produced at subsistence level (Tuwei *et al.,* 2013). Observed data have revealed that African nightshades are rich in micronutrients (Kamga *et al.,* 2013; Luoh *et al.,* 2014) simple to grow and therefore have a potential to supply nutritional security and a source of livelihood for the

underprivileged/poor rural communities. The awareness of its high nutrient level has led to increase in consumer demand that is more than the production.

Low production in Western Kenya may be due to pest and diseases, environmental factors and poor agronomic techniques. However, the main production challenge still is infection of a quarantine bacterium soil borne pathogen *Ralstonia solanacearum* (Dinssa *et al.,* 2013), and also the insufficient information on the taxonomy of the species that has led to breeders releasing few consumer preferred varieties, Common pinpointing characters which have been used by some scientists to categorize the species belonging to section *Solanum*, are very different, with some species within the section being variable morphologically.

2.3 Taxonomy ofAfrican nightshade species

Genetic variations occur both within cultivars in a given species and among species bringing about genetic diversity. Some of the species might be morphologically similar giving rise to taxonomical confusion.

Despite the fact that African nightshade are morphologically similar, they differ genetically and that is why african nightshade has been revealed to be rich in genetic diversity (Ojiewo *et al.*, 2013 a). Morphological variation among different species can be observed in terms of inflorescent orientation, stem colour, plant growth habits, leaf shape, stem ridging and pubescence among others.

African nightshade varieties grow under wide environmental conditions and this accounts for their spread all over the world. They require an annual rainfall of between 500 to 1200mm and perform well under cool high moisture conditions in medium to high altitude with temperature ranges of 15-30ºC for germination, 20-30ºC for growth. African nightshade genotypes with broad leaves are more prone to water stress as compared to narrow leaved ones (Ojiewo *et al.,* 2013).

Different African nightshade species are thought to originate from diverse parts of the world. The center of origin of diploid species such as *S. americanum* and *S. sarrachoide* is South America, the tetraploid species *S. villosum* and *S. retroflexum* and hexaploid species *S. nigrum* and *S. scabrum* are considered to have originated from Africa, Europe and Asia. African nightshade hexaploid species is thought to have been developed from a cross between the tetraploid *Solanum villosum*Mill and the diploid *Solanum americanum*Mill. (Edmond and Chweya, 1997).Thirty African nightshade accessions collected from Western Kenya were evaluated genetically to determine their morphological and genetic differences and similarities.

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

Kenya faces major food insecurity with 56% of Kenyans living below poverty line.Fifty(50%) of Kenyan population lacks adequate food because of high population growth rate, extreme poverty and prolonged drought (FAO,17). This has led to over reliance on nutritionally poor diets leading to malnutrition and child death in the rural and semi urban areas. Food security and proper nutrition can be achieved in developing countries through increased production, awareness and utilization of indigenous leafy vegetables such as African nightshade which is rich in nutrients (Oniang'o *et al.,* 2005; Ondieki *et al.,* 2011).

African nightshade has been grown in Kenya since the last few centuries and is part of the many indigenous leafy vegetables that continue to be produced by farmers from many Kenyan communities (Ondieki *et al.,* 2011). TheAfrican nightshade is amongst the mainly highly supplied and extensively consumed indegenous leafy vegetables in the country with Kenyan farmers producing yields of 1.5-3.0 tones/ha (Ojiewo *et al.,* 2013). Early flowering and excessive fruiting hinders leaf expansion resulting to lower

yields. Increased utilization of indigenous vegetables over exotic ones have been documented in Eastern Africa since indigenous vegetables need less effort to produce and are cost effective for rural households with low sources of income (Ojiewo *et al.*, 2013).

Species of vegetable African nightshade differ in their growth habits, leaf yield and nutritional value. The most common species grown in Kenya are *S. solanum, S. scabrum and S. sarrachoides. S. villosum* species include *Solanum villosum* subsp. *Villosum* (finely lobed dentate leaf margins and mature berries are orange in colour) and *Solanum villosum*Miller subsp. *Miniatum* (entire, sinuate, sinuate-dented or dentate leaf margins and, mature berries are orange in colour). *Solanum scabrum*Miller, is characterized by entire to sinuate leaf margins with dark purplish black mature berries whereas *Solanum sarrachoides* has mature light green berries with clearly lobed dentate leaf margins which are densely pubescent (Ashilenje *et al.*,2012).

Broad leafed African nightshade cultivar (*Solanum scabrum*) is one of Kenya's most common and promising African nightshade species. It can be differentiated from others by its broad leaves and large purple berries and shows variation in leaf size and plant height. Still, its leaf production remains higher than the other narrow leafed species like *Solanum villosum* and *Solanum eldoretianum. Solanum scabrum* is one of the country's mainly distributed and utilized African nightshade species (Abukutsa *et al.*, 2005).

In Kenya, African nightshade is usually grown in home gardens along with other vegetables or cereals like maize, sorghum or millet. The demand for African nightshades is high particularly in urban areas and the supply is not adequate to satisfy the demands. African nightshade has been reported to be among the ten important

vegetables consumed vegetable and third in terms of quantities sold when a survey was conducted at Kakamega municipal market (Abukutsa *et al.,* 2005).

Increased awareness on the importance of African Indigenous vegetables including African nightshade and allocation of funds for research has led to raise in their production in peri urban areas of Nairobi by ten folds from 1997 to 2007 with farmers increasing their annual net income by US$200 (Biodiversity 2013). Utilization of African indigenous vegetables in Nairobi in 2003 was estimated to be 31 tons of leaf per annum valued at USD. 6000 and this value has continued to increase such that in 2006 it had increased to 600 tons valued at USD.142, 000 (Opiyo *et al.,* 2015).

Production of African nightshade faces major challenges such as neglect and stigmatization. This is mainly because indigenous vegetable was considered old fashioned and a poor man's diet since they sometimes grow naturally. They are also perceived to be weeds that are collected by poor people in the rural areas to supplement their meals (Mwangi and Kimathi, 2006) and also pest and disease especially the quarantine devastating pest, *Ralstonia solanacearum*. These constrains have resulted into low vegetable production of between 1-3 tons per hectare hence bellow optimum production levels requirement of 20-40 tons per hectare (Abukutsa *et al.,* 2005; Mwangi and Kimathi, 2006).Farmers are also still using saved seed unaware of the spread of the quarantine bacterium which is a devastating soil borne pathogen that is yield limiting factor.

2.5 Characterization of the African nightshade

Genetic diversity is the quantitative measure of variation of a given population, indicates the equilbrium between mutation and loss of genetic variation (Carvalho *et al.*,2019). Biodiversity erosion, directly, or indirectly, leads to the loss of plant species. Loss of diversity denies breeders opportunities to develop new cultivars with desired consumer preferences.

Techniques to determine genetic diversity within, and between, plant populations are: morphological, biochemical characterization and molecular marker analysis (Govindaraj *et al.*, 2015).

Diversity evaluation by morphological markers is based on visual traits including leaf shape, fruit colour, growth habit, flower colour, and others, and does not need sophisticated technology although it requires large tracts of land for field experiments.

A molecular marker is a genetic locus that can be tracked and quantified in a population and is normally associated with a certain trait of interest (Hayward *et al.*, 2015). These markers detect variations that arise from either deletions, duplications, insertions, or inversions in chromosomes. They are usually located near, or linked to, genes controlling trait(s) in question; the markers themselves do not have an effect on the phenotype of the trait.

Some of molecular markers used to study genetic diversity in plants include: Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) (Jonah *et al.*, 2011), Start codon targeted (SCoT) markers (Satya *et al.*, 2016). In the present study six SSR primers were used to evaluate the African nightshade accessions that were collected from the three regions in Western Kenya.

2.6. Genetic diversity of African nightshade in Kenya

Variation has been reported to exist between African nightshade species found in Kenya (Matasyoh *et al.,* 2015). Genetic diversity in African nightshade cultivars has been shown to exist both within and between species for example Manoko *et al.,* (2007) found out that cultivars within *S. scabrum* and *S. nigrum* exhibited genetic variation but no studies have reported genetic information concerning African nightshade species grown in Western Kenya.

2.7. Measuring diversity in African nightshade.

Diversity studied are very important in plant breeding as they generate information required for selection of desirable parental lines to cross so as to obtain hybrids with which are better yielding than the parents and it entails analysis of existing cultivars both morphological and genetical characterisation (Matta *et al.,* 2015). The higher the genetic diversity, the wider the genetic distance between parental lines the higher the hybrid vigour observed in the progeny (Khodadadi *et al.,* 2011).

Methods used to measure genetic diversity include hierarchical cluster analysis and clustering based on principle component analysis, principal coordinate analysis (PCoA), and multi-dimensional. Standardization of variable is a requirement before calculation of genetic distance however, it reduces the differences among the groups hence the results obtained from cluster analysis may be different from those obtained from principle component analysis and therefore principle component analysis may be avoided when using hierarchical cluster analysis (Khodadadi *et al.,* 2011).

Cluster analysis is usually preferred over principle component analysis when measuring genetic diversity during evaluation of hierarchical relationships (Ravishanker *et al.,* 2013). Hierarchical analysis depicts the relationship within or between genotypes by

using descriptors. The results are normally presented in a dendrogram which further shows the genetic interaction within the clusters. Genetic diversity can be assessed using pedigree, morphological, biochemical and molecular markers (Osawaru *et al.*, 2015).

Morphological characterization is cost effective though it requires large piece of land for laying out the field experiments, making it more laborious than molecular characterization. Morphological traits are normally prone to environmental interferences, affecting the genetic diversity being evaluated. Biochemical analysis involves the separation of proteins into specific banding patterns and just little amounts of biological reagents are required however, the limited amount of enzymes present for use in biochemical analysis is a major disadvantage to this procedure thereby reducing the degree of diversity observed (Singh *et al.*, 2011).

Morphological characterization allows for a detailed physical sampling and big samples can be used however, morphological traits are prone to interferences and hence don't offer genetic information of a particular genotype neither do they measure the exact genetic diversity present. Unlike morphological traits, molecular markers provide an detailed measure of genetic diversity since it is not affected by environmental interferences.

Molecular analysis is the assessment of genetic variation by the use of various DNA markers which corrects the mistakes incurred during phenotypic characterization (Mondini *et al.*, 2009). Molecular markers may or may not coincide with phenotypic expression of a genomic trait. The use of molecular markers gives more precise results compared to morphological characterization because they are stable and present in all tissues not considering growth, differentiation, development, or defense status of the

cell, they are not also influenced by environmental, pleiotropic and epistatic effects (Mondini *et al.,* 2009).

Phenotypic characterization has traditionally been used to determine genetic diversity and continues to play an important part in the analysis and evaluation of germplasm. Molecular markers also provide a huge number of characters for analysis making it possible to differentiate phenotypically cultivars that were thought to be similar morphologically. Molecular markers are however expensive to purchase hence limiting the size of samples used for analysis (Ojiewo *et al.*, 2013). A combined use of both molecular and morphological method of characterization therefore offers precise genetic diversity studies of high resolution (Tumbilen *et al.*, 2011; Omondi *et al.*, 2016). In the present study, cluster and hierarchical analysis were used to measure morphological and molecular diversity in African nightshade accessions collected from Western Kenya.

2.8. Bacterial Wilt disease

Bacterial wilt, caused by *Ralstonia solanacearum* is a soil borne disease that infects mainly solanaceous crops (Sikoru *et al.,* 2004).*Ralstonia solanacearum* is a quarantine bacterium that is known to infect over 200 plant species and causes extensive crop losses in economically significant crops of solanaceous family. It has been found on all continents with the exception of Antarctica (Swanson *et al.,* 2005, 2007). *R. solanacearum* is genetically diverse and is composed of five different biovars, four phylotypes, and five different races. Races differ in virulence, symptom expression, and host range, whereas biovars differ biochemically in their ability to oxidize various disaccharides and hexose alcohols. Along with difference of races and biovars, *R. solanacearum* can be further divided into phylotypes. Race 1 Biovar 1 of *R. solanacearum* is a common prevalent pest in Southern United States.

Ralstonia solanacearum is a serious hindrance to the cultivation of these crops in both temperate and tropical regions. The greatest economic loss has been reported on tobacco, tomato and potatoes which have been reported to cause up to 90% crop loss (Mallikarjun *et al.,* 2008). Severity of the disease in most cases increases if root nematode occurs in association with the pathogen *R. solanacearum* (Deberdt *et al.,* 1999). It has been reported that Nematode infestation in tobacco may alter its physiology making it susceptible to bacterial wilt (Chen, 1984) and is also reported on *Solanum nigrum*.

2.8.1 Biology and Ecology

R. solanacearum R3 Bvr2 is a gram-negative soil and waterborne pathogen (Wenneker *et al.,* 1999; Swanson *et al.,* 2007; Marco-Noales *et al.,* 2008). *R. solanacearum* can live in the roots of host, in the rhizosphere, in infected plant debris, and in non-hosts (Wenneker *et al.,* 1999; Janse *et al.,* 2004). The bacterium is usually cultured on yeast extract-peptone-glucose agar (YPGA), non-selective media such as triphenyl tetrazolium chloride (TTC/TZC) agar and casamino acid peptone glucose (CPG), or semi-selective media (SMSA) between the temperatures of 28°C and 29°C (82.4°F and 84.2°F) (Cook and Sequeria,1991; Caruso *et al.,* 2003; Ozakman and Schaad, 2003; Marco-Noales *et al.,* 2008).

Since the pathogen is able to stay alive in water and soil for a long period of time, *R. solanacearum* R3 Bvr2 can infect its host in many different ways. The pathogen can enter into the plant's xylem tissue through wounds leading to colonization, wilt and death (Milling *et al.,* 2009). *R. solanacearum* is also able to invade the plant through young root hairs (Swanson *et al.,* 2007). When infected plants decompose, bacteria are released into the environment; bacteria released can also occur through plant wounds,

and in this case bacteria produce a “matrix of protective polysaccharides” that aids in survival (Van Elsas *et al.,* 2000). Once released, millions of bacterial cells can easily spread via irrigation water (Swanson *et al.,* 2007).

Ralstonia solanacearum R3 Bvr2 is also known to be more cold tolerant than race 1 biovar 1 (R1 Bvr1), the native/endemic species to the United States. The cold resistance makes R3 Bvr2 a very troublesome pathogen for areas that grow solanaceous crops (tomato, potato, etc.). *R. solanacearum* R1 Bvr1 is primarily found in Southern United States (warm areas), but R3 Bvr2 may have a chance in surviving above and below the mid-Atlantic line (Swanson *et al.*,2005,2007; Milling *et al.,* 2009). In Australia, *R. solanacearum* R3 Bvr2 can survive in fallow soils with temperatures in the winter as low as 4°C (39.2°F) (Milling *et al.,* 2009).

This pathogen can evade detection in symptomless hosts or latent hosts where symptoms are not expressed under un favourable environmental conditions (Swanson *et al.,* 2005). This characteristic can make it difficult to adequately survey for this pest. In Australia, Kenya, Sweden, and the United Kingdom under moderate conditions, this pathogen survives in deep soil and is known to live up to two years in soil where affected crops have already been removed (Wenneker *et al.,* 1999; Van Elsas *et al.,* 2000). Little information on host resistance is known. Gorissen *et al.*, (2004) found that use of pig waste and solarization can decrease population size and reduce survival of *R. solanacearum* R3 Bvr2 in soil.

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

The control of *R. solanacearum* is hard once the pathogen has infested the soil (Jones,2008) in relationship with a broad range of solanaceous crops such as potato, eggplants, tomato and weeds such as Jimson weed (*Datura* spp). The infection by this disease can be significantly reduced by using resistant crops and crop rotation for 5 - 7years (Smith *et al.,* 1995).

Use of crop rotation has been shown to reduce disease incidence but the management is still insignificant because the pathogen is problematical by the existence of alternate hosts such as Jimson weed (*Datura* spp) and other volunteer crops of solanaceae family (Fajinmi *et al.,* 2010). In combination with crop rotation, weed control can be effective in reducing disease incidence (Allen *et al.,* 2005).

Planting certified disease free seedlings from registered propagators, disinfecting equipments after working in a field, controlled use of flood irrigation and avoiding overhead irrigation on solanaceous crops can reduce spread of the disease (McCarter, 1991).

Bacterial control using chemicals is a challenge because of the pathogen ability to survive in the soil and its location inside the xylem.There is no known chemical control of the bacterial wilt disease (Hartman *et al.,* 1994), it is also difficult to control bacteria with chemicals (Grimault *et al.,* 1994).

2.10 Host resistance

Use of host plant resistance to control *Ralstonia solanacearum* in the field has been hard due to lack of resistance in solanaceous crops and the nature of the pathogen. In tobacco, the presence of a major resistance gene has led to the development of genotypes which have hypersensitive response, characteristic of a gene for gene interaction (Robertson *et al.,* 2004). However, a similar major resistance gene has not been reported in *Solanum nigrum C,* and plant scientist continue to be eluded by the broad variation of this pathogen.

The broad variation of pathogenic *Ralstonia* strains has led to the development of resistant strains that are effective in some growing regions and not in others (Scott *et al.*, 2005).

Early studies of physiological mechanisms involved with bacterial wilt resistance in Solanaceae family suggested that host resistant genotypes physically limit bacteria movement from the soil environment into the collar and mid-stem portions of the plant (Grimault *et al.,* 1994). Host resistance is mainly an economical control option, though it is challenging to develop cultivars that are suitable resistance across locations (Abedayo *et al.,* 2009). Resistance of the crop is overcome often by the genetic diversity of the pathogen as well as genotype by environment interactions (Nguyen and Ranamukhaarachchi, 2010).

Host resistance is an efficient and effective component in integrated management of bacterial wilt disease and some tomato cultivars provide moderate resistance against bacterial disease (Peregrine, 1982). The use of resistant varieties has been reported to be mainly effective and useful method to control bacterial wilt (Black *et al.*,2003; Grimault *et al.*, 1994). *Ralstonia solanacearum* is a diverse species group with a broad

host range (Kelman *et al.,* 1961), posing a challenge in breeding for resistance. Resistance to *Ralstonia solanacearum* has been reported in some solanaceous crops such as tomato genotype (Gomes *et al.,* 1998) but no studies on resistance has been reported on African nightshade.

Ralstonia solanacearum strain type, genetic variability of the plant and reproducibility of the inoculation technique may affect the selection of resistant material (Prior *et al.,* 1990a). Some *Ralstonia solanacearum* resistant cultivars of Solanum spp have been made at the Asian Vegetable Research and Development Center (AVRDC). However, their resistance is restricted to climate, locations, strains of the pathogen and soil characteristics (AVRDC, 2003). Some of tomato varieties have been breed with significant levels of resistance for certain environments (Gomes *et al.,* 1998); in a number of cases the stability in regions with high temperatures and humidity particularly in lowland tropics is difficult to achieve as resistance breaks when variety is transferred to a different region (Hayward *et al.,* 1991; Hanson *et al.,* 1996). No studies have been carried out on African nightshade species to develope significant levels of resistant for certain 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 county lies between latitude $0^{o}26^{'}$ to $0^{0}18^{'}$north, longitude $33^{0}58^{'}$east and $34^{0}33^{'}$ west between altitudes of 1384-2100 meters above sea level (GOK, 2013). It receives a bimodal rainfall pattern with long rains starting from March to June/July a short rain from September to November with a total annual rainfall ranging from 1500mm-2400mm (GOK, 2014). Temperature is about 20-32°C. The area has deep soils ranging from sandy clays to friable loamy clays. The common food crops grown in this county are; maize, common beans, bananas, sweet potatoes, cassava, and also vegetables such as African nightshade (GOK, 2014).

3.2.2 Kakamega County

Kakamega County lies between longitudes 34° and 35° east and latitudes 0° and 1° north of the equator and within altitudes of 1,250-2,000 m above sea level (Barasa *et al.*, 2015a and b). Its climate is predominantly hot and wet most of the year, with mean annual rainfall of between 1,800 and 2,000 mm. It receives a bimodal rainfall partten,

the “long rains” fall between March and May, while the “short rains” fall between October and December (Kabubo-Mariara and Karanja, 2007). The average temperature in the county is 22.5°C. January and February are generally considered dry months (Barasa *et al.,* 2015a). The county has high temperatures all year round, with slight variations in mean maximum and minimum ranging from 28°C to 32°C and 11°C to 13°C, respectively. The mean annual evaporation is high and ranges from 1,600 mm to 2,100 mm with high 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

Trans Nzoia County has a cool and temperate climate with mean maximum (day time) temperatures ranging between 23.4^{oC} and 28.4^{0C} and mean minimum (night time) temperatures ranging between 11.0^{0C} and 13.5^{0C}. The maximum and minimum extreme temperature are recorded in February (about 34.2^{0C}) and January (about 6.5^{0C}) respectively.

The County receives annual rainfall ranging from 1000mm to 1700mm. The annual rainfall is distributed into three major seasons namely; Long rainfall Season-March, April, May (MAM), Intermediate Season- June-July-August (JJA); and short rainfall season- October-November-December (OND). The area has deep soils which ranges from dark red to reddish brown in colour, consists of friable sandy clay to clay.The common crops grown in the county are; maize, common bean, sweet potato, potato, arrow roots and vegetables such as African nightshade.

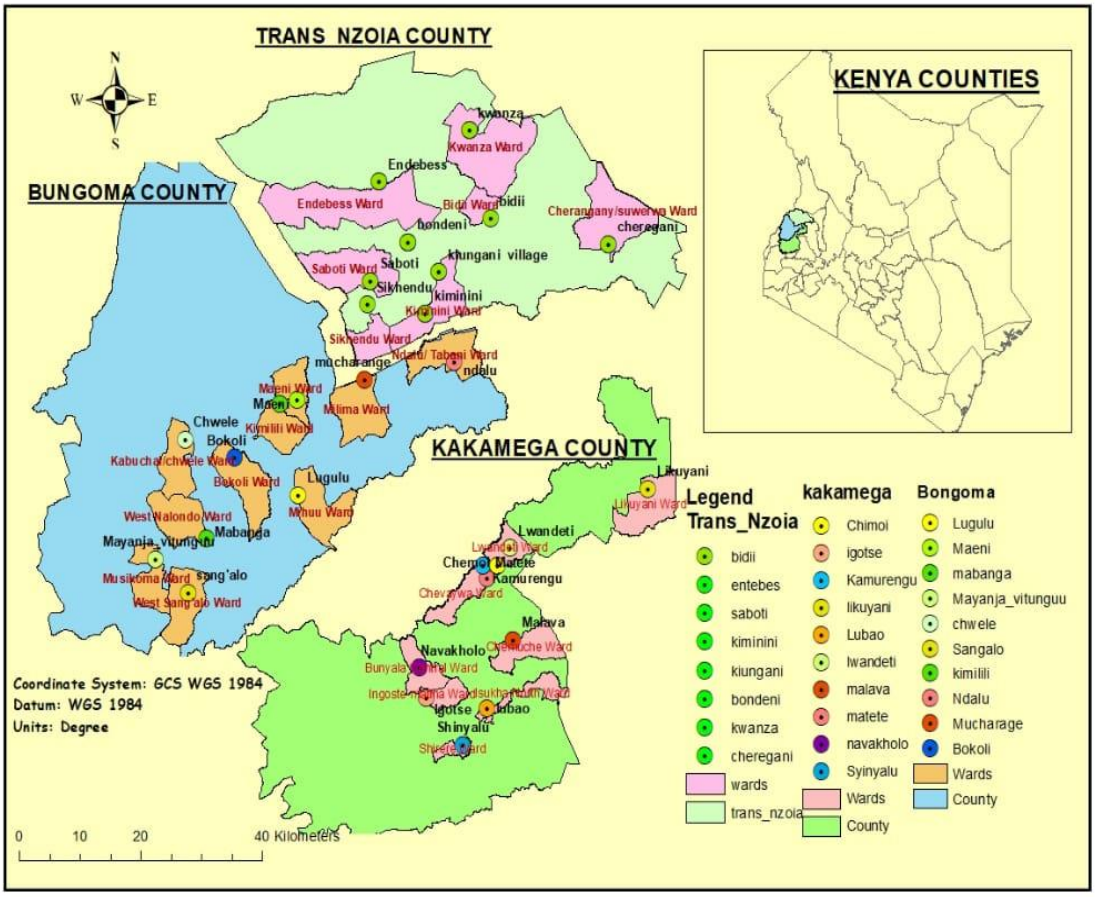

Figure 3. 1: Map showing three counties in Western Kenya where the African nightshade samples were collected. (Source Author, 2021)

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 used to select Bungoma, Kakamega and Trans Nzoia counties because of their high production and consumption of African nightshade. Random sampling was done based on prior information on species grown and sampling partnership with farmers. Consultations were done with area Agricultural officers before collection of materials. Sampling was done in a 0.5km radius in each location. Farms where African nightshade were growing were selected at random. The selected farms ranged in sizes between 0.3ha and 2ha and production of African nightshade done at a small scale. Five mature plants having mature fruits were randomly selected in each farm and also seeds of the same accession in the farm were collected from the farmers. For this study almost all the African nightshade growing farms and farmers from the three regions were included for the study. In addition, a total of 30 seed samples of the accessions were collected from the three selected counties constituting of 10 samples each. These materials were labelled based on the area and farm (farmer) number of collection as Bungoma (B), Kakamega (K) and Trans Nzoia (T). (Appendix B)

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

Land was ploughed and harrowed until fine tilth was achieved. Thirty accessions of African nightshade were then planted using a randomised complete block design with three replications with each accession appearing once per block, at Kibabii University Agricultural farm. Seeds were planted at a depth of 1cm on single row plots of 3 M X

4 M spacing 30 cm between plants, 40cm between rows and 1M between blocks during the short and long rainy seasons. DAP applied at the rate of 0.012kg/ha and thoroughly mixed with the top soil at planting time. The plant stand count (germination percentage) was taken two weeks after planting and thinning was done to ensure a desired plant stand of ten plants per plot was achieved.

3.4.3 Data Collection

Data was collected on four randomly selected plants from the middle of each plot as per the criterion described by Nandhini *et al.,* 2014.The four seedlings were tagged and a phenotypic descriptor list was made and developed using the phenotypical features described by National Bureau of Plant Genetic Resource (NBPGR) (Singh *et al.,* 2003) as follows.

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

Seeds of thirty African nightshade accessions obtained from farmers in African nightshade growing areas in Trans Nzoia, Kakamega and Bungoma (Appendix B), were

used in the study.The African nightshade accessions were planted in pots filled with very fine soil sieved through mesh 60 gauge in a glasshouse.

3.5.1 Leaf harvesting and DNA extraction

Leaves of four week old young, tender and healthy African nightshade plants in each accession were harvested and wrapped in foil paper and then immediately taken to the laboratory and put in refrigerator at -20°C so as to retain its quality. The leaves were then rinsed in distilled water to remove soil particles on their surfaces, as done by Agbagwa *et al.,* (2012). African nightshade accessions leaves were weighed and 200mg of each accession leaves were gently ground into a fine paste in 500µl of CTAB buffer using a motor and pestle. The paste was then transferred into a microfuge tube and incubated for 15 minutes at 55°C in a recirculating water bath. The CTAB/ plant extract paste was then centrifuged at 12000 rpm for five minutes so as to spin down the cell debris. The supernatant was then transferred into clean microfuge tubes, 250 µl of chloroform: Iso-Amyl Alcohol (24:1) was added into each tube and the solution mixed by slow and repeated inversion. The mixture was then centrifuged at 13000rpm for one minute and the upper aqueous phase which contains the DNA was carefully transferred into a clean microfuge tube. 50µl of 7.5M ammonium acetate was added into each tube followed by addition of 500µl of ice cold absolute ethanol. The tubes were then slowly and carefully inverted several times so as to precipitate the DNA. The precipitated DNA accumulated at the bottom of the tubes and the supernatant was carefully removed by slowly pouring it out of the tube while at the same time taking care not to dislodge the DNA pellets. The DNA pellet was then washed twice using ice cold 70% ethanol. The DNA was centrifuged at 13000 rpm for 1 minute after washing and the supernatant removed. The DNA was then dried by inverting the tube containing the DNA on a clean paper towel for 14 minutes and care was taken to make sure the DNA pellet does not

fall out of the tube. The tubes with the DNA pellets were then turned upright and while still covered with paper towel left for 30 minute to ensure that the pellets were completely dry. The extracted DNA was then suspended in 400µl of sterile DNase free water. 10µl/ml (10µl RNase in 10ml H2O) RNase was then added to remove any RNA that might have been present in the preparation. After resuspension, the DNA was incubated at 65°C for 20 minutes to destroy any DNases that might have been present. The DNA was then stored at 4°C for further use in Polymerase chain reaction.

3.5.2DNA Quantification

DNA quantity and quality of each accession was determined by running samples on 1% (w/v) agarose gels for 1 hour at 80 volts diluted in 100 Ml 1 x TAE buffer (0.89 M Tris base, 0.89 M boric acid, 20 Mm EDTA pH8.0) and 900 mL of distilled water. A standard undigested lambda DNA with a range variation of 10, 20, 50, 80 and 100 ng was used as a comparison to determine the DNA concentration of the African nightshade accessions by comparing band sizes and intensities. The gel was stained in ethidium bromide (10mg/ml) for 30 minutes and later destained in distilled water for 20 minutes before viewing under ultraviolet transilluminator. Between 0.5µg and 1µg of high quality DNA was obtained and was diluted to 0.01µg/µl with deionized distilled water for PCR amplification.

The quality of DNA extracted was confirmed through agarose gel electrophoresis where 3% of agarose gel was prepared by weighing 3g of agarose powder and pouring it into a conical flask containing 100ml of 1x TBE buffer and then the mixture was placed into a microwave and heated for 3 minutes for it to melt (until the agarose is completely dissolved and there is a nice rolling boil). The conical flask containing the mixture was then removed and allowed to cool for five minutes. 0.5 µg/ml Ethidium bromide was then carefully added into the gel for visualization and stirred to mix evenly. Gel combs

were then arranged into a gel tray for creation of wells where the DNA samples were to be loaded. The gel was then carefully cast into the tray and allowed to set for 20 minutes at room temperature on a flat surface until it completely solidified. During gel casting care was taken to ensure no bubbles were formed in the gel since the bubbles could interfere with DNA movement during electrophoresis. The type of combs used were to create a minimum of 31 wells, one well for loading the ladder and the remaining wells for loading the 30 samples. The combs were then carefully removed after the gel had hardened and the gel was transferred into a gel electrophoretic tank filled with 0.5x TBE buffer for loading of the DNA samples.

10µl of 1kb ladder was loaded into the first well followed by a mixture of 5µl sample (the DNA extracted for each ANS genotype), 5µl water and 2µl 6x loading buffer which were loaded in theremaining wells. The loading enabled the DNA samples to settle at the bottom of the gel wells and not to diffuse into the buffer. Gel electrophoresis was then conducted for 30minutes at 100 voltages and thereafter carefully removed from the gel tanks and exposed to ultra violet light after which it was photographed. The presence of high resolution molecular weight bands confirmed that the quality of DNA extracted was good.

3.5.3 PCR Amplification

PCR amplification was carried out in 25 µl volume of reaction mixture consisting of 2µl DNA sample template, 5µl of 5x PCR buffer, 0.1 µl Taq polymerase, 0.5 µl reverse primer, 0.5 µl forward primer and 17 µl double distilled water. A total of six different primers were used for polymerase chain reaction with each primer pair (reverse and forward) being used per reaction. After an initial denaturation of 4 min at 94°C, 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 49–55 °C and 1 min of

extension at 72 °C were performed, followed by a final extension of 10 min at 72°C. The amplifications were carried out using Applied Bio systems 2720 Thermo cycler.

3.5.4 DNA Data analysis

For each reproducible band visualized, the Allele sizes were scored manually using molecular size ladder and scored as 1 for each present band and 0 for absence of the band for each primer. The size of the band was determined by comparing the band with 100 base pair molecular size ladder. The matrix data generated was used for statistical analysis. Cluster analysis was performed to establish the genetic associations among accessions and Genetic distance dendrogram drawn using Numerical Taxonomy Multivariate Analysis System package (NTSYS-pc) software, version 2.1. The genetic associations were evaluated by calculating the genetic similarity matrix using Euclidean and subjected to Un weighted Pair-Group method (UPGMA) clustering using the sequential agglomerative hierarchical nested (SAHN) programme and tree plot analysis generated.Matrix data was also subjected to analysis using Power Marker (ver 3.0) to determine 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

Samples of diseased African nightshade plants and soil were collected by observing the bacterial wilt symptoms in the field and distinguishing it from vascular wilts caused by fungal pathogens by visual observation of primary symptoms such as wilting, vascular discoloration and also through bacterial streaming test in a glass of water.

Plants which oozed milky white strands from the cut ends of xylem while in a glass of water were picked. At least 10 samples of the diseased plants were collected from each

of the surveyed farms in the African nightshade growing areas of Western Kenya in Bungoma, Kakamega and Trans Nzoia county.

The diseased plants were brought to the Microbiology laboratory at Masinde Muliro University of Science and Technology (MMUST) and to the Microbiology laboratory at Kibabii University.

At the Laboratory the samples collected from the fields were washed under running tap water to remove sand and soil, the stem were surface sterilized with 70% alcohol, crushed and the bacteria cultured on Casamino acid Peptone Glucose media .Separately growing colonies were then picked and sub-cultured onto fresh media to obtain pure cultures on triphenyl tetrazolium chloride (TTC) agar medium using 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 nurseries of all the 30 accessions were raised separately in sterilized potting mixture in germination trays containing sand, silt and compost at the ratio 3:1:1, respectively in the green house. The trays were watered daily.When the seedlings were four-week-old, three seedlings which had four to five leaves of each accessions from the tray were transferred to the pots. Each treatment was replicated thrice. The pots were arranged randomly in a completely randomized design in a greenhouse and were properly moistened in alternative days. One week after transplantation, Inoculations was performed by pouring 30 ml of a 107 CFUml^{-1} bacterial suspension on every pot through soil drenching. After inoculation, the plants were watered at alternative days.

Infected plants were scored for wilting symptoms and pathogenicity was also confirmed on susceptible cultivars of African nightshade plants using the protocol of Elphinstone *et al.,* 1996.

3.6.5 Data collection

The status of bacterial wilt was recorded using severity scale as described previously by Horita and Tsuchiya (2001) and disease index, briefly,1= No symptom (Highly Resistant), 2 = Top young leaves wilted (Resistant 25%), 3 = Two leaves wilted (Moderately susceptible 50%), 4 =Four or more leaves wilted (Susceptible 75%) and 5 = Plant dies (Highly susceptible 100%) wilted canopy.

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 observed complex clustering with the dendrogram was suggestive of a rich diversity within the African nightshade cultivars assessed since the shorter the Euclidean distance of the branches of a dendrogram the more similar the cultivars are while the longer the branches the more genetically diverse the cultivars are (Kalinowski, 2009).These indicates that the cultivars in a given cluster are more genetically similar than cultivars across cluster groups. These results are in line with those of Nyadanu *et al.,* (2014) worked on 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; **$A1_1$** comprises of *Solanum nigru*m (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)

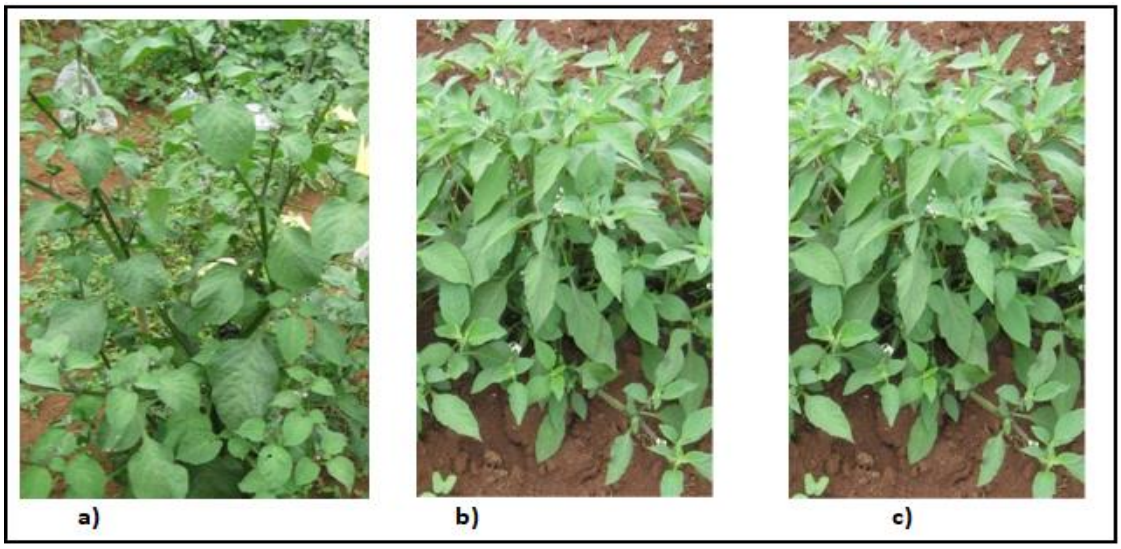

Plate 4.1. 1: African nightshade accessions exhibiting diversity in plant type;

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

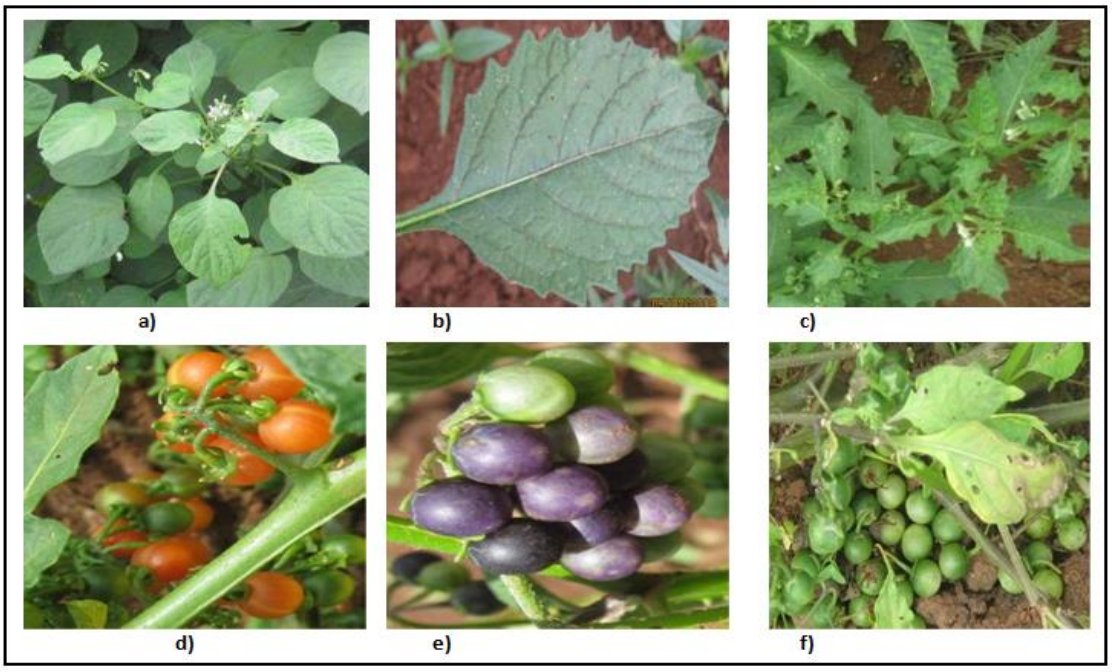

Plate 4.1. 2: African nightshade accessions exhibiting diversity in leaf.

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

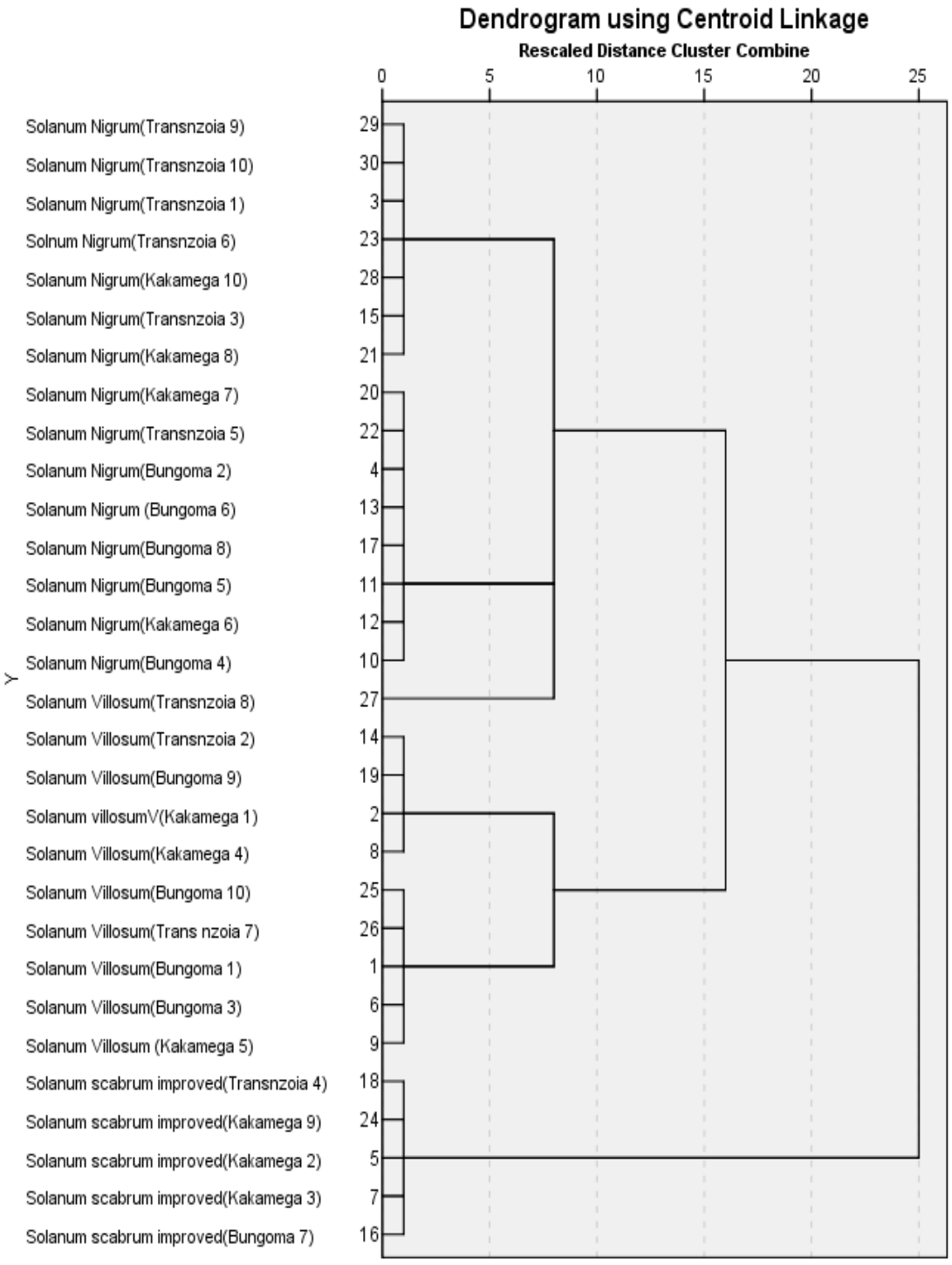

Figure 4.1.1 Hierarchical cluster analysis dendrogram displaying relationship among 30African nightshade accessions using qualitative traits.

Discussion

Morphological characterisation of plant species is an important step in crop breeding programmes as it helps scientists to identify and select superior lines for further crop

development and improvement (Adebola and Morakinyo 2006; Das and Kumar 2012; Julia *et al.,* 2016; Peratoner *et al.,* 2016; Ngomuo *et al.,* 2017) as it allows for the study of plant diversity using observable attributes. Significant variation was observed for all the qualitative traits among the 30 African nightshade accessions in this study and this implies that qualitative traits can be used as a measure of diversity among African nightshade cultivars. These results are in line with those of Olet (2004) who stated that qualitative traits are more reliable in the identification of genetic relationship among African nightshade than quantitative traits.

Nandhini *et al.,* (2014) also observed considerable differences in qualitative traits among African nightshade cultivars. This variation observed could either be genetic or as a result of the effect of the environment of the genes of the cultivars. For instance, the different fruit colours expressed by different African nightshade accession could be as a result of anthocyanin concentration in the plants and could be influenced by environmental factors (Manoko, 2008) which has a great effect on phenotype, that is why the different locations show similarities in genetics

A cluster dendrogram is a good measure of diversity among and within species as it groups similar entries under one cluster (Malek *et al.,* 2014). The cluster analysis demonstrated the existence of variability among the 30 African nightshade accessions for the morphological traits studied (Figure 4.1.1). A similar strategy was applied by Zhang *et al.,* (2012) and Mekonnen *et al.,* (2014) on the morphological characterization of *Cucumis melo* and lentils accessions, respectively.

The clustering pattern shows that *Solanum scabrum* accessions were genetically distant from the *Solanum villosum* and *Solanum nigrum* accessions and can be used to improve one another. Accessions from the same counties were grouped together but there was also sub-clustering of the major clusters, suggesting that there was still variation within

clusters. The clustering also revealed some singletons (*Solanum villosum)* Trans Nzoia 8. Singletons are those accessions that are placed separately from the rest of the accessions in a cluster. They are more diverse and are to be given special attention during selection because of their superiority over other accessions as suggested by Choudhary *et al.,* (2013).

Singletons were also observed in other genetic characterization studies Corchurus by Dube *et al.,*2018, Chickpea by Chowdhury *et al.,*2015,Amaranthus by Gerrano *et al.,*2015).The observed complex clustering with the dendrogram was suggestive of a rich diversity within the African nightshade cultivars assessed since the shorter the Euclidean distance of the branches of a dendrogram the more similar the cultivars are while the longer the branches the more genetically diverse the cultivars are (Kalinowski, 2009).These indicates that the cultivars in a given cluster are more genetically similar than cultivars across cluster groups. These results are in agreement with those of Nyadanu *et al.,* (2014) working on agro morphological characterization of eggplant.

All accessions belonging to African nightshade species *Solanum scabrum,* had leaves with hairs (pubescent surfaces). Cultivars with pubescence have been shown to be tolerant to pests and insects (War *et al.,* 2012) since hairs hinder insects and pests from laying eggs, feeding and also interferes with their larval feeding (Steinite and Ievinsh, 2003). The hairs also interfere with the movement of insects and pest on the plant surface thereby decreasing their contact with the leaf epidermis hence preventing leaf damage (War *et al.,* 2012). Pest and diseases have been indicated to be the main challenge encountered by farmer during production of African nightshade (Onyango *et al.,* 2016).

These two traits leaf shape and leaf pubescence are important traits that can be exploited in developing African nightshade cultivars that are resistant to drought and pest and also disease tolerant.

The existing intra specific and inter specific dismillarity between cultivars is the key to crop improvement (Nyadanu *et al.,*2014;Ojiewo *et al.,* 2013) and this is because cultivars with superior yield traits can be developed through breeding for improved vegetable productivity. Different communities prefer different African nightshade species for instant the Abagusii community prefer genotypes with spreading plant type, producing small leaves lanceolate with mild bitterness such as *S. sarrachoides* species while the Abaluhya prefer genotypes that have an erect plant type producing broad leaves with bitter taste such as the *S scabrum* species (Onyango *et al.,* 2016). This is an indication that there are variations in terms of the preferred African nightshade species from one community to the next hence when breeding African nightshade for improved productivity, specific community interests should also be put into consideration.

For qualitative traits, most variations observed were across species and not within species for instance all cultivars belonging to *S. villosum* had semi erect plant type, and produces mature orange berries *S. scabrum*s producing mature purple berries. This may imply that the variation seen are genetical and not environmental and hence do not change from one location to another since the same qualitative traits observed on the accessions within a certain species long rain were the very same ones observed in the short rain for example, the leaf shape of cultivars within *S. scabrum* species was ovate both in the short and long rain which contradicts with Madic *et al.,* 2016).

4.3 Molecular characteristics of African nightshade accessions in Western Kenya

The clustering and sub clustering seen in the dendrogram indicated that there were possibilities of crossability among African nightshade accessions being studied. The

dendrogram (Figure 4.2.1) revealed 3 main clusters joined at a Euclidean distance of 198.91. The first cluster comprises of Trans Nzoia 5 (*Solanum nigrum*). The second cluster comprised of Kakamega 10 (*Solanum nigrum*). The third cluster comprised of all the other 28 African nightshade accessions. The third cluster is further subdivided into two other clusters at a distance of 115.49. The third cluster comprised 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*), Kakamega1 (*Solanum villosum*), Bungoma 1 (*Solanum villosum*). The third cluster comprises of two clusters linked at a Euclidean distance of 58.02 with eleven sub clusters linked at a Euclidean distance of 39.78.

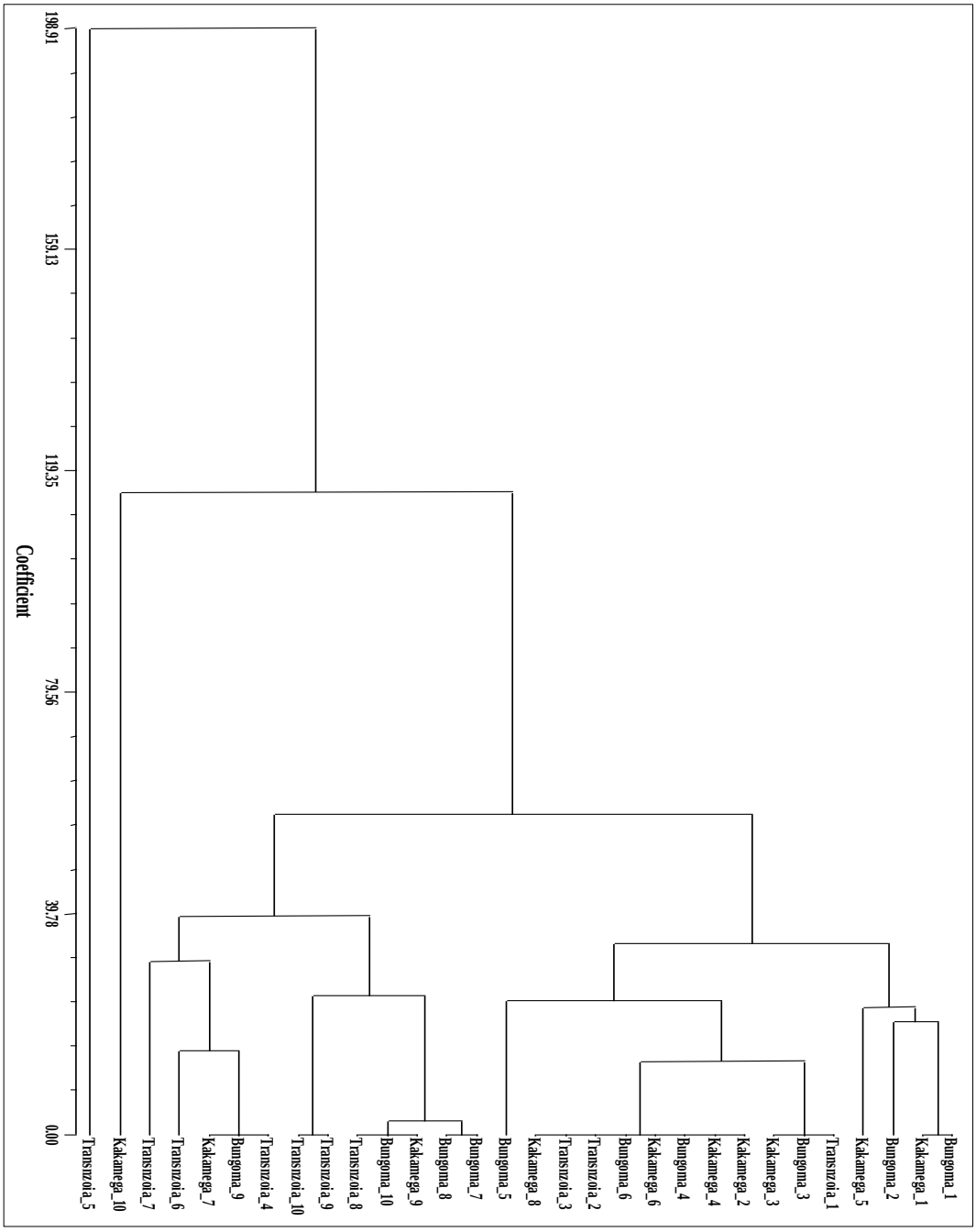

Figure 4.2. 1Genetic distance among accessions

This was estimated with using Numerical Taxonomy Multivariate Analysis System package (NTSYS-pc) software, version 2.1.

The genetic associations were evaluated by calculating the genetic similarity matrix using Euclidean algorithm calculator and subjected to Unweighted Pair-Group method (UPGMA) clustering using the sequential agglomerative hierarchical nested (SAHN) programme and tree plot analysis generated.

Discussion

Molecular markers (SSR) have been used successfully to clarify genetic diversity within crops and their wild relatives, and between accessions of cultivated or semi-cultivated plants from different geographical or ecological areas, and as a source for selection and for conservation of genetic diversity (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; de Vicente *et al.,* 2005).

The present study applies SSR to study the genetic diversity in African nightshade accessions of Western Kenya. The clustering pattern exhibited by the African nightshade accessions in this study indicates that the genetic variation between accessions is high. The lack of clustering according to region provenance is an indication that accessions from different regions (Bungoma, Kakamega and Trans Nzoia counties) are not significantly different genetically either. A similar clustering pattern was reported between Ugandan, Indonesian and European material (Olet 2004). In the present study, the clustering pattern also did not reflect the reported morphological differences. The lack of congruency between morphological and genetic differences suggests that the morphological differences cannot be explained by selection for different plant types, these differences must therefore be caused by

environmental factors and the interaction between the genetics of the plant and the environment this is in line with (Onyango *et al.,* 2016).

African nightshade accessions within a cluster consisted of more genetically similar accessions than those among different clusters similar results were obtained by (Osei *et al.*, 2013).

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

Susceptible *Solanum nigrum from (*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) had all leaves wilted a part from the top 2 to 3 leaves by day 7thafter inoculation.The plants continued wilting until all the leaves on the whole plant wilted by 14th day. The plant continued wilting until the whole plant died by the end of 21days with the highly susceptible accessions while *Solanum nigrum* (Trans Nzoia 10) plants died after the 14th day of inoculation (fig 4.1.3) . *Solanum villosum* from the (Bungoma1, Bungoma 3, Bungoma 9, Bungoma 10, Trans Nzoia 1, Trans Nzoia 7, Kakamega 1, Kakamega 5, Kakamega 4, Kakamega 7, Kakamega 8) had all leaves wilted by the 7th day and the plants continued wilting and by the end of 14 days were dead showing that they are highly susceptible as shown in Figure 4.3.1, 4.3.2, 4.3.4, 4.3.5, 4.3.7 and 4.38 respectively.

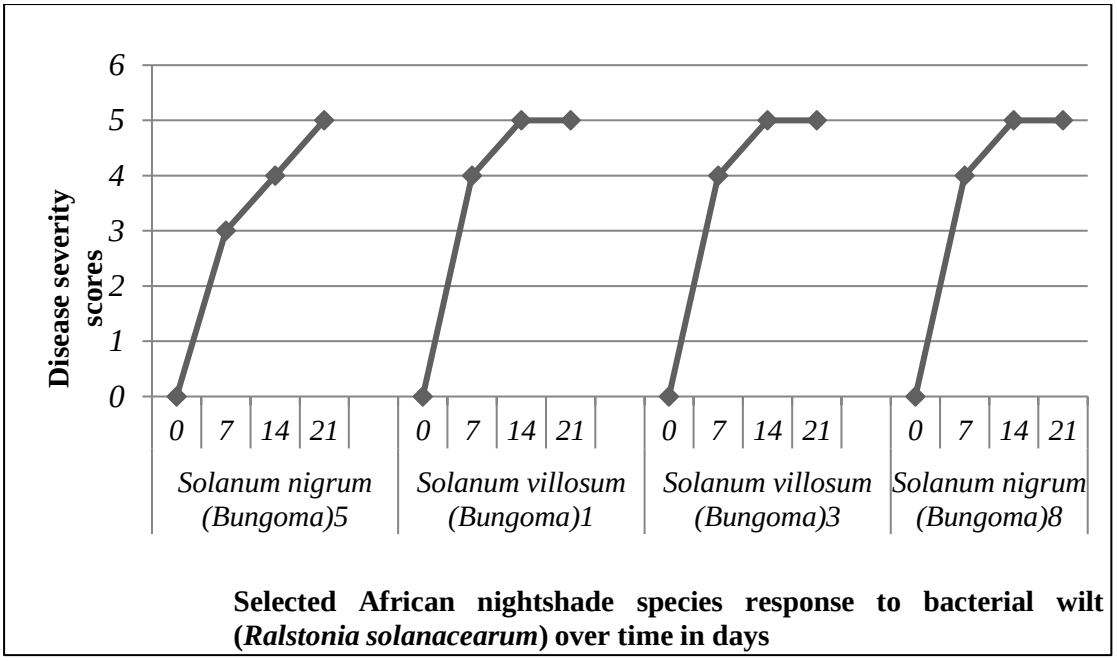

Figure 4.3.1A graph showing disease progression in selected susceptible accessions of African nightshade growing regions in Bungoma County.

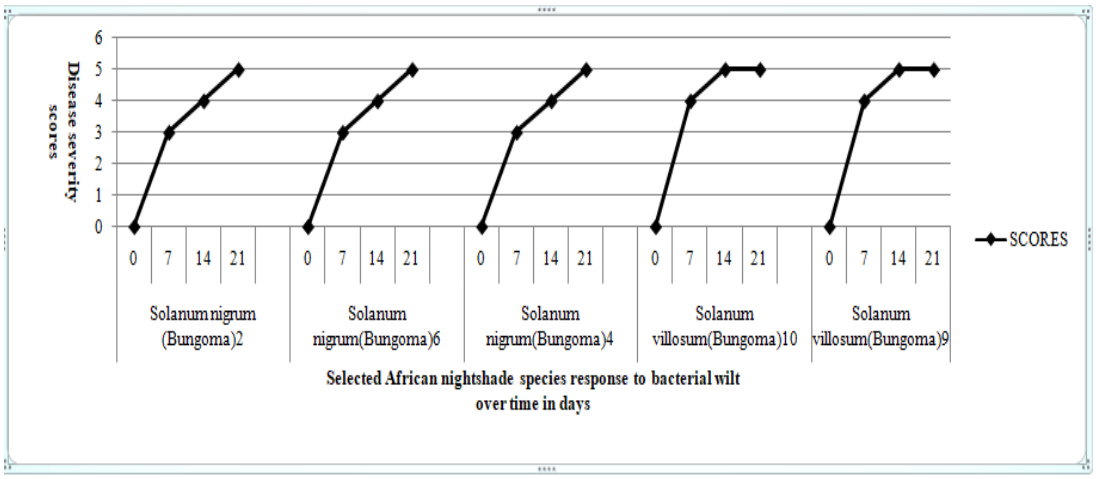

Figure 4.3.2: A graph showing disease progression in selected susceptible accessions of African nightshade growing areas inBungoma county.

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

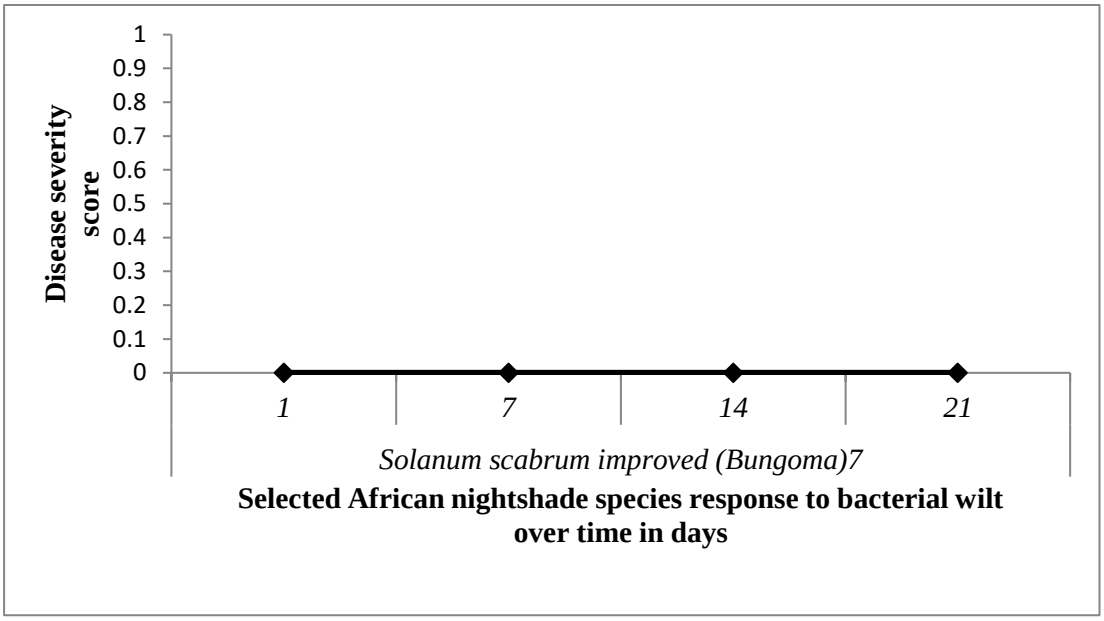

Figure4.3.3 A graph showing disease progression in selected resistant accessions of African nightshade growing areas in Bungoma county.

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**.**

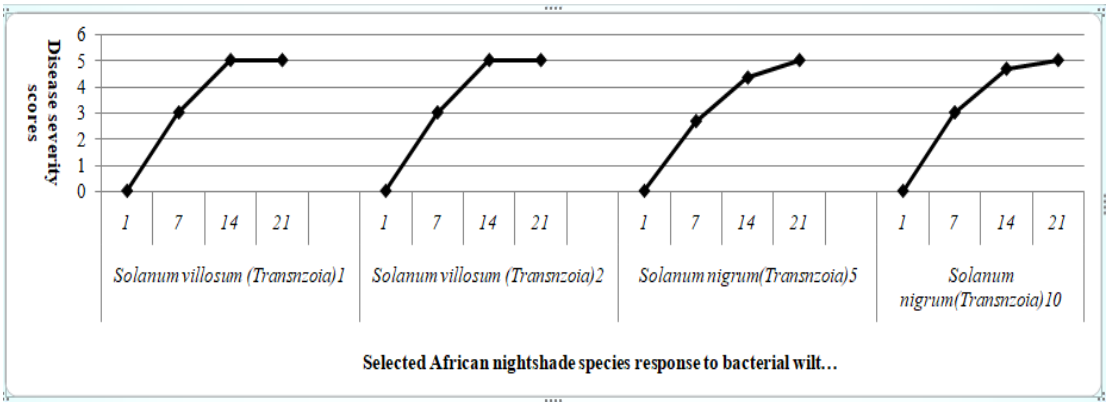

Figure 4.3. 4: Agraph showing disease progression in selected susceptible accessions of African nightshade growing areas in Trans Nzoia county

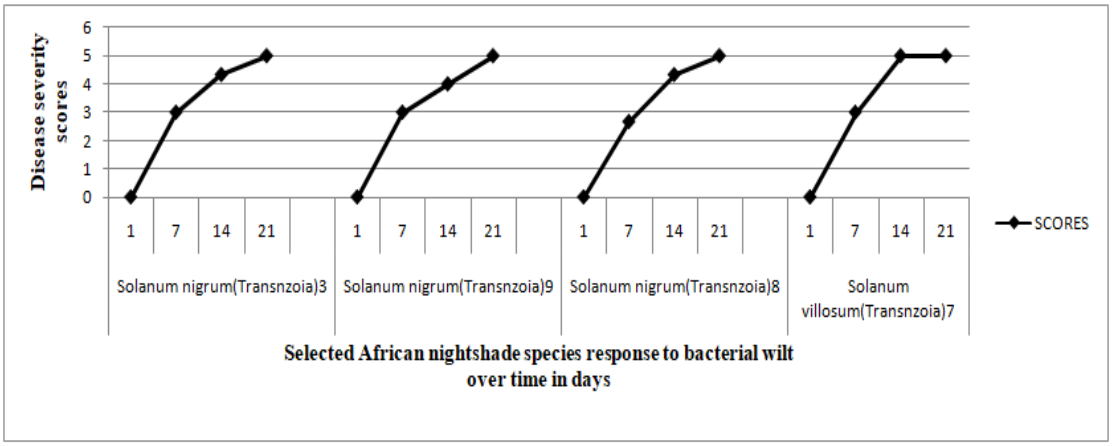

Figure4.3. 5: A graph showing disease progression in selected susceptible accessions of African nightshade growing areas in Trans Nzoia county

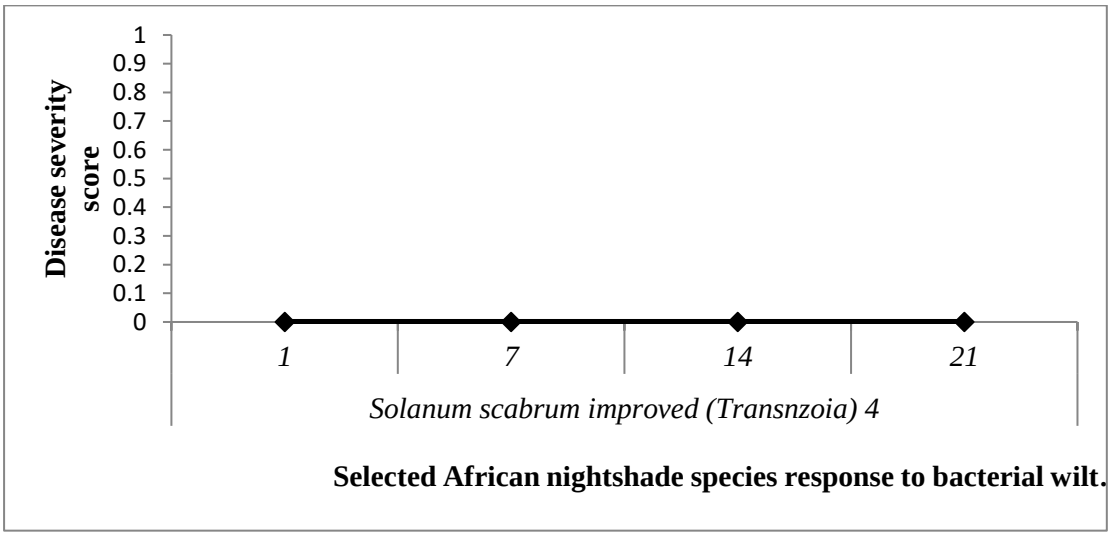

Figure 4.3.6 A graph showing disease progression in selected resistant accessions of African nightshade growing areas in Trans Nzoia county

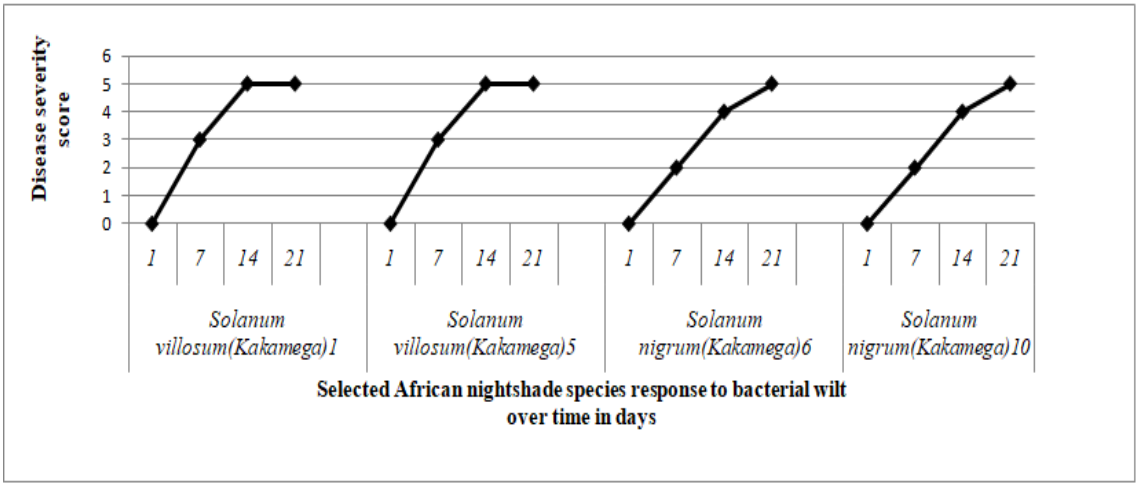

Figure 4.3.7: A graph showing disease progression in selected susceptible accessions of African nightshade growing areas in Kakamega county

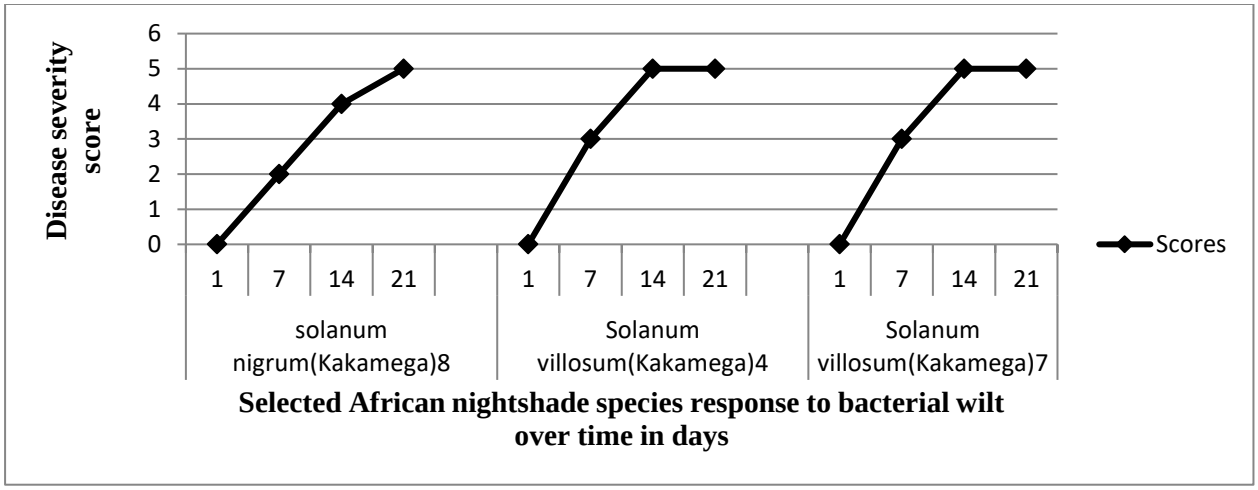

Figure 4.3. 8: A graph showing disease progression in selected susceptible accessions of African nightshade growing areas in Trans Nzoia county

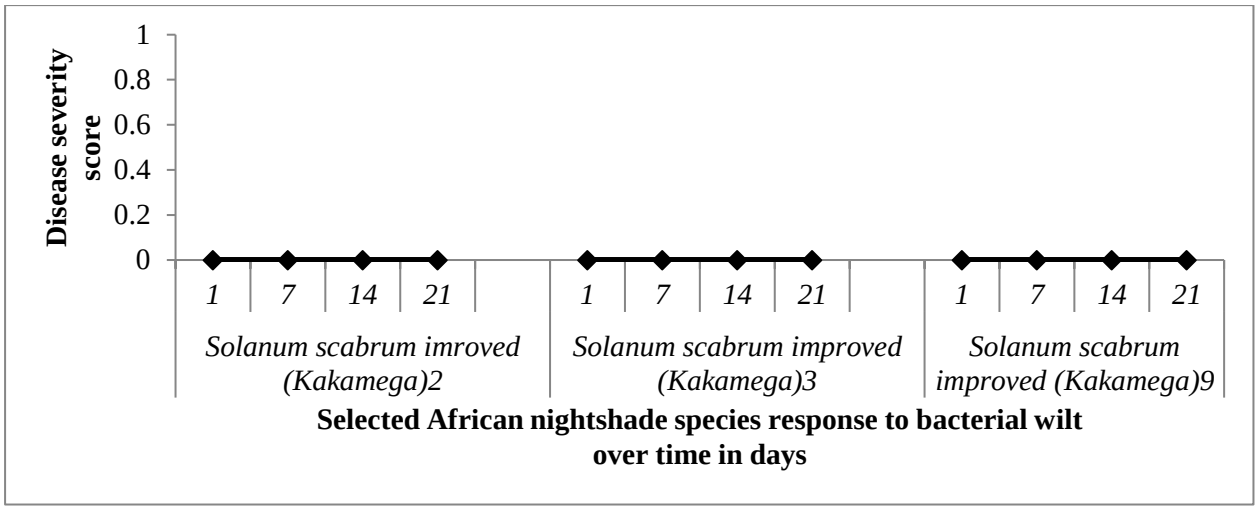

Figure 4.3.9: A graph showing disease progression in selected resistant accessions of African nightshade growing areas in Kakamega county

Discussion

The search for a source of genetic resistance to bacterial wilt of various vegetables has been studied in solanaceous crops. Resistance to bacterial wilt has been reported by In

et al., (1996) who screened 31 tomato varieties to *Ralstonia solanacearum* and determined only three as moderately resistant, while the rest were susceptible. The resistance and susceptibility have also been related with symptom development. In the current study, in susceptible accessions symptoms appeared after 4 days of inoculation, while resistant ones took more than 14 days to show chlorotic symptoms and is in line with the findings of Anith *et al.*, (2004). The resistance to bacterial wilt is strain specific and temperature dependent as was observed in potato (French and De Lindo 1982). Environmental conditions and locations also influence resistance against bacterial wilt, Hanson *et al.*, (1996) reported variable reaction of tomato lines to bacterial wilt evaluated at several locations in Southeast Asia. They found that tomato lines which were resistant to bacterial wilt in Malaysia and Taiwan showed susceptible reactions in Philippine and Indonesia.

Solanum scabrum improved cultivar was found resistant, and two cultivars *Solanum villosum* and *Solanum nigrum* as susceptible and therefore *Solanum scabrum* improved is recommended for cultivation under integrated production systems and in developing new resistant African nightshade cultivars.

Solanum scabrum from all the three regions was resistant to the *Ralstonia solanacearum* this genotype was able to overcome the pathogen because of its unique inherent gene of resistance which is in line with (Nguyen and Ranamukhaarachchi, 2010) who reported that resistance of the crop is overcome often by the genetic diversity of the pathogen as well as genotype by environment interactions.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The clustering pattern using morphological characteristics shows that *Solanum scabrum* accessions were genetically distant from the *Solanum villosum* and *Solanum nigrum* accessions and can be used to improve one another.

The significant variation seen among African nightshade accessions indicated that there exists diversity within cultivars belonging to the same species as well as among accession across different species and the variation observed could either be genetic or environmental.

These two traits leaf shape and leaf pubescence are important traits that can be exploited in developing African nightshade cultivars that are resistant to drought and pest and also disease tolerant.

The assessment of African nightshade accessions using SSR primers showed that there was genetic diversity recorded because accessions clustered into three different groups (I, II and III). The clusters and sub-clusters observed in the dendrogram indicated that there was rich genetic diversity within the African nightshade accessions collected from the three counties in Western Kenya.

It is concluded from the present assessment that African nightshade cultivars showed variations in their resistance response to *R. solanacearum*. *Solanum scabrum* improved genotype was found resistant, and two genotype *Solanum villosum* and *Solanum nigrum* as susceptible.

5.1.1Recommendation

This study recommends that further morphological characterization be carried out with both qualitative and quantitative traits and be narrowed down to studying accession belonging to the same species in Western Kenya after which superior cultivars within particular African nightshade species could be identified and documented to be accessed by scientists.

Solanum scabrum improved is recommended for cultivation under integrated production systems and in developing new resistant African nightshade cultivars.

This recommends that the genotypes should be evaluated at its local conditions against particular isolates of the pathogen of that area.

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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

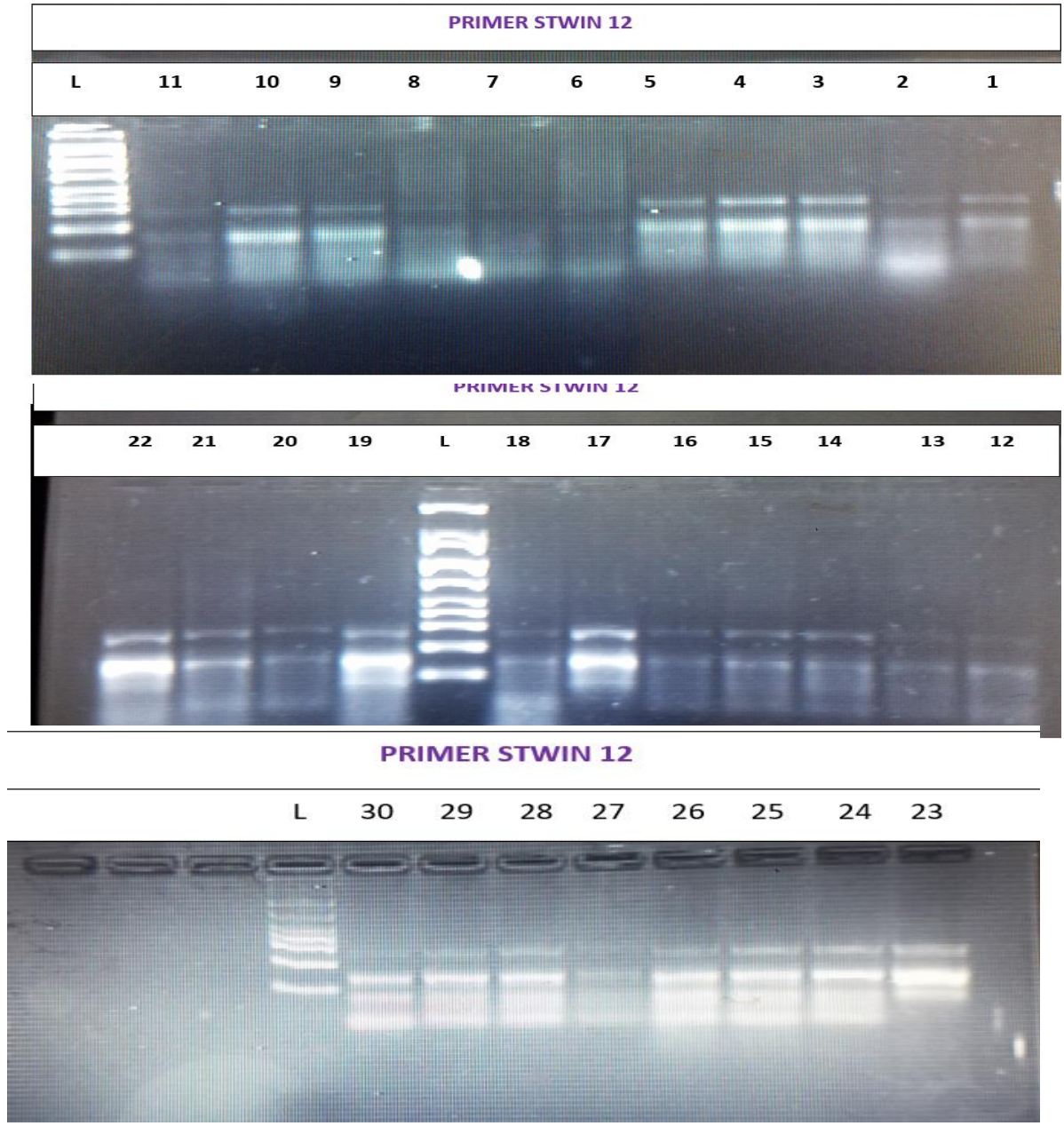
Trans Nzoia 2(*Solanum villosum)*	1	2	1	1	1	1	1
Trans Nzoia 3(*Solanum nigrum)*	2	1	2	2	1	1	1
Bungoma 7(*Solanum scabrum* improved)	3	2	1	1	2	2	2
Bungoma 8(*Solanum nigrum)*	2	2	2	2	1	1	1
Trans Nzoia 4(*Solanum scabrum* improved)	3	2	1	1	2	2	2
Bungoma 9(*Solanum villosum)*	1	2	1	1	1	1	1
Kakamega 7(*Solanum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

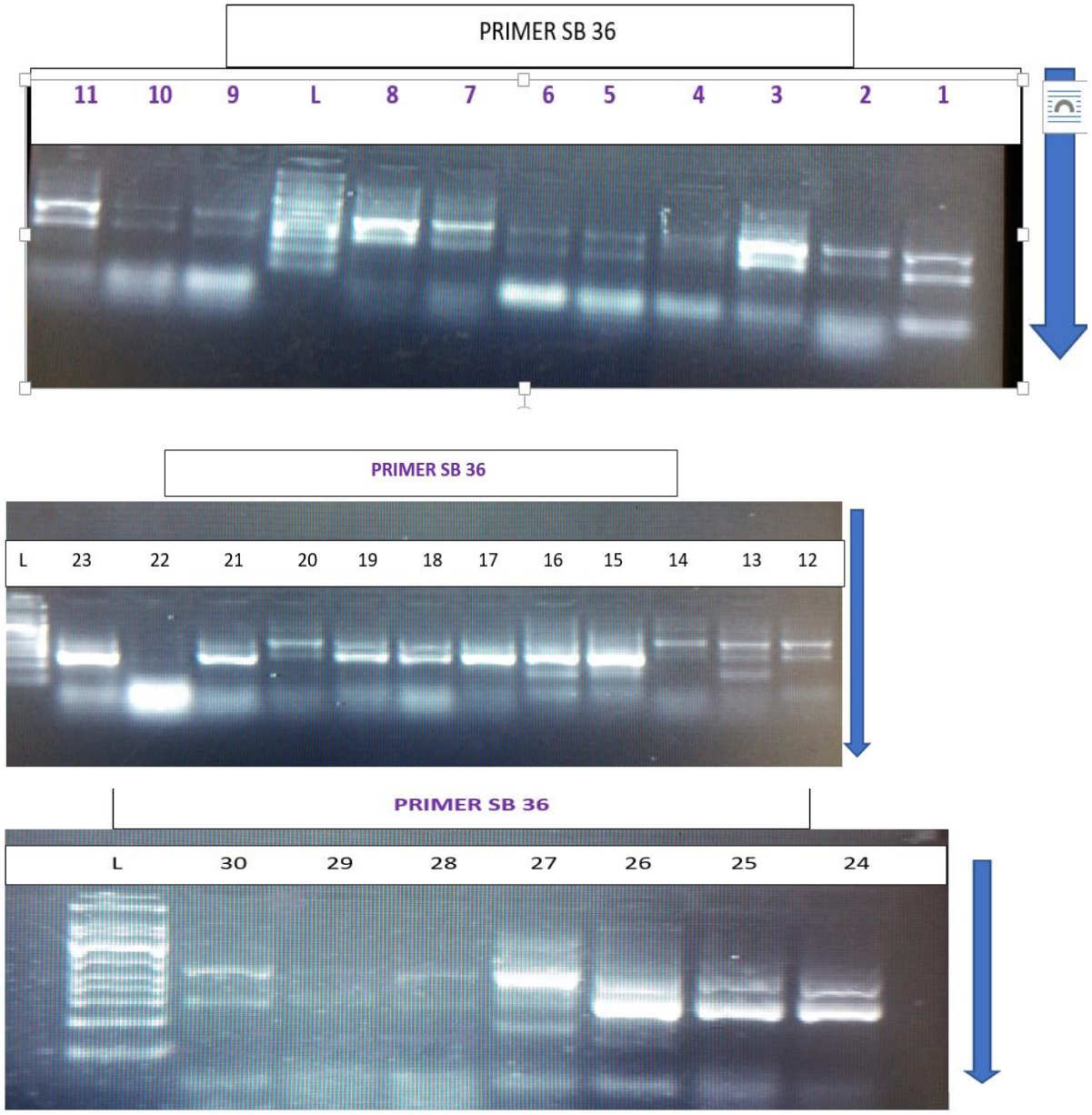
Plant type: 1=Semi erect2=Erect; Leaf surface: 1=Glaborous (sparsely), 2=pubescent (Densely). Leaf margin: 1 =Sinuate –dentate, 2 =Entire, 3 = Lobed; Leaf shape: 1 = Lanceolate, 2 = Ovate, 3 = Rhomboid; Stem ridge: 1= Smooth ridges, 2= Dented; Fruit colour: 1= Orange, 2= Dark purple, 3=Black; Inflorescence type:1=Simple,2=Forked. Source: Singh 2003.

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



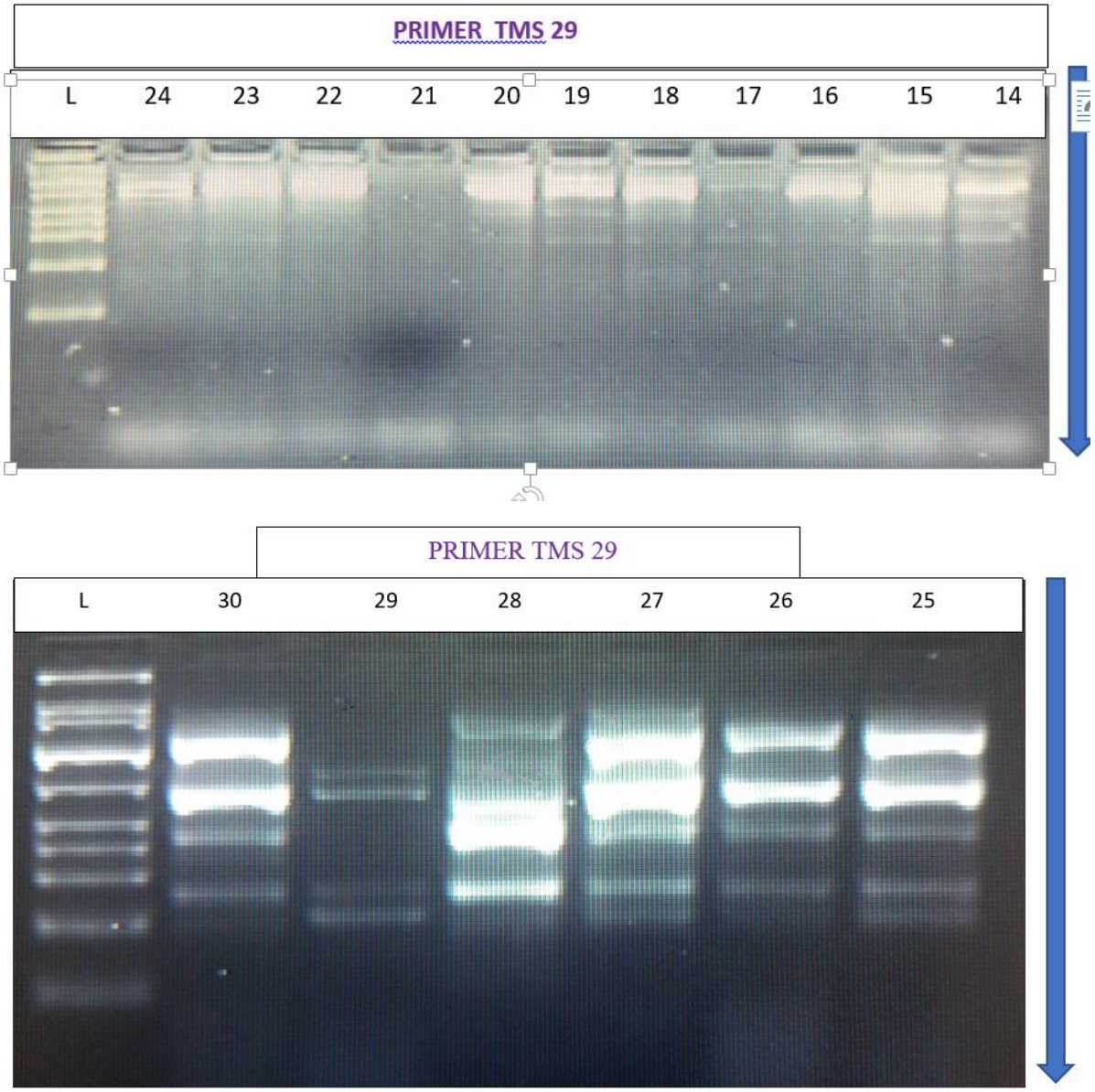
1= Bungoma 1 (S.N), 2= Kakamega 1(S.V), 3= Trans Nzoia 1 (SN), 4= Bungoma 2(SN), 5= Kakamega 2(SS), 6= Bungoma 3 (SS), 7=Kakamega 3 (SS),8= Kakamega 4(SV), 9= Kakamega 5(SV), 10= Bungoma 4 (SN), 11= Bungoma 5(SN), 12= Kakamega 6 (SN), 13= Bungoma 6(SN), 14= Trans Nzoia 2(SV), 15= Trans Nzoia 3(SN), 16= 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



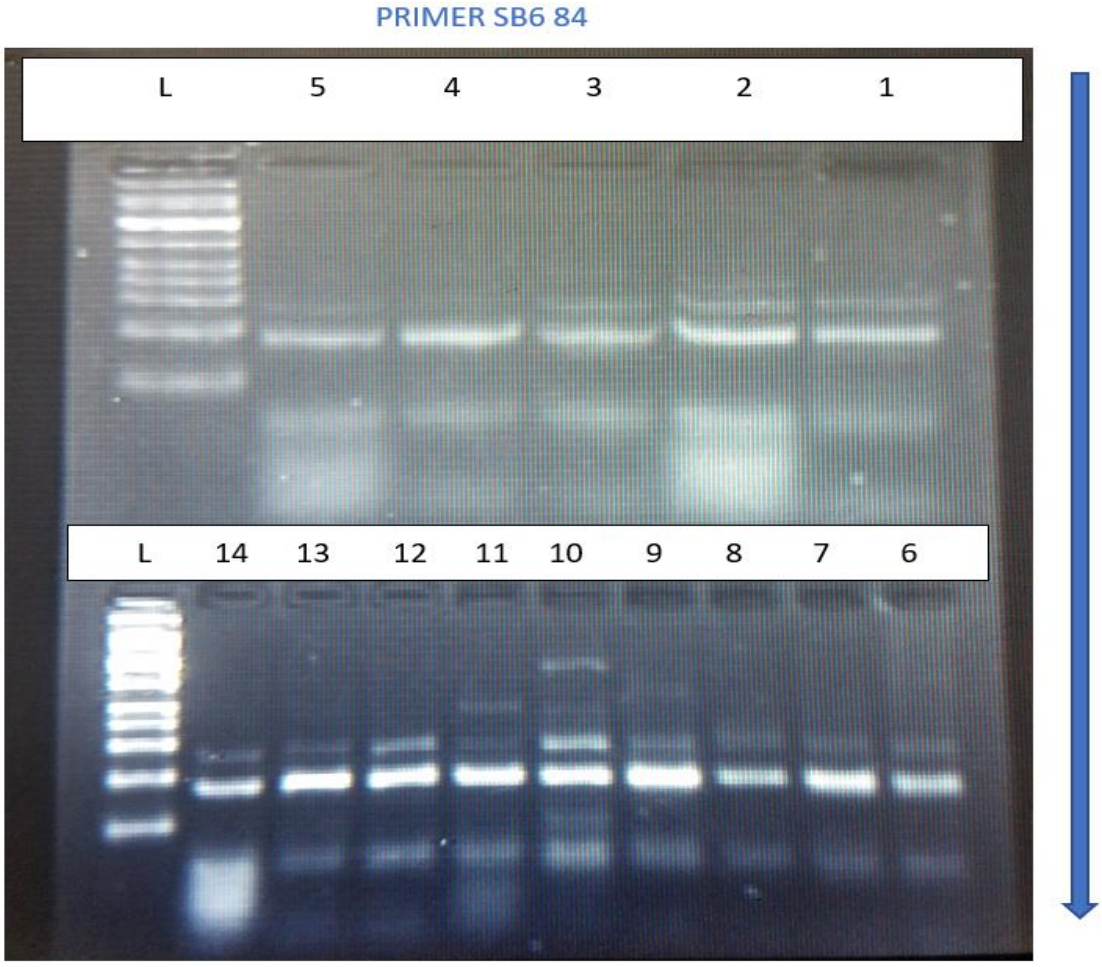
1= Bungoma 1 (S.N), 2= Kakamega 1(S.V), 3= Trans Nzoia 1 (SN), 4= Bungoma 2(SN), 5= Kakamega 2(SS), 6= Bungoma 3 (SS), 7=Kakamega 3 (SS),8= Kakamega 4(SV), 9= Kakamega 5(SV), 10= Bungoma 4 (SN), 11= Bungoma 5(SN), 12= Kakamega 6 (SN), 13= Bungoma 6(SN), 14= Trans Nzoia 2(SV), 15= Trans Nzoia 3(SN), 16= 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*)

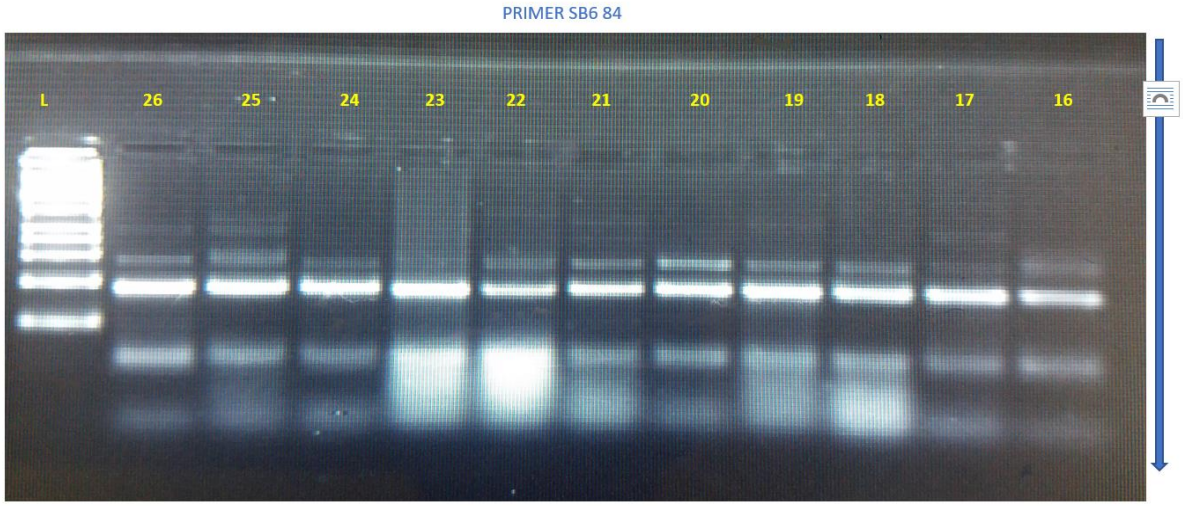
Appendix E PCR products amplified with primer TMS 29 and visualized under UV light.

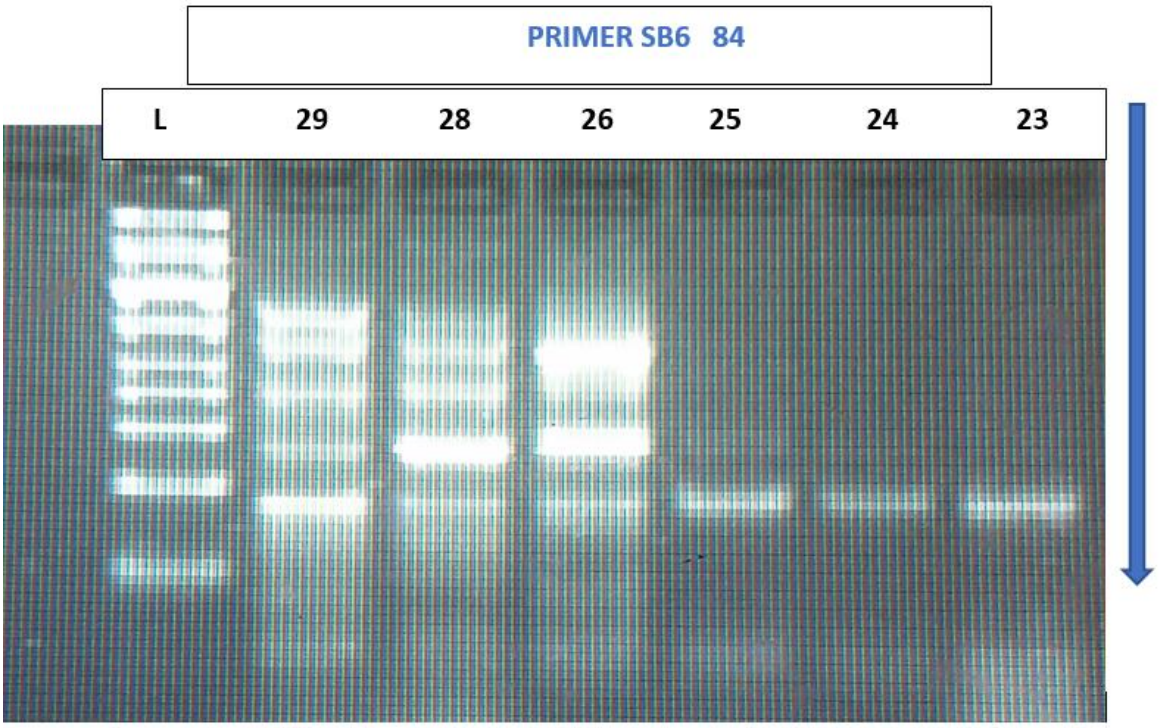


1= Bungoma 1 (S.N), 2= Kakamega 1(S.V), 3= Trans Nzoia 1 (SN), 4= Bungoma 2(SN), 5= Kakamega 2(SS), 6= Bungoma 3 (SS), 7=Kakamega 3 (SS),8= Kakamega 4(SV), 9= Kakamega 5(SV), 10= Bungoma 4 (SN), 11= Bungoma 5(SN), 12= Kakamega 6 (SN), 13= Bungoma 6(SN), 14= Trans Nzoia 2(SV), 15= Trans Nzoia 3(SN), 16= 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

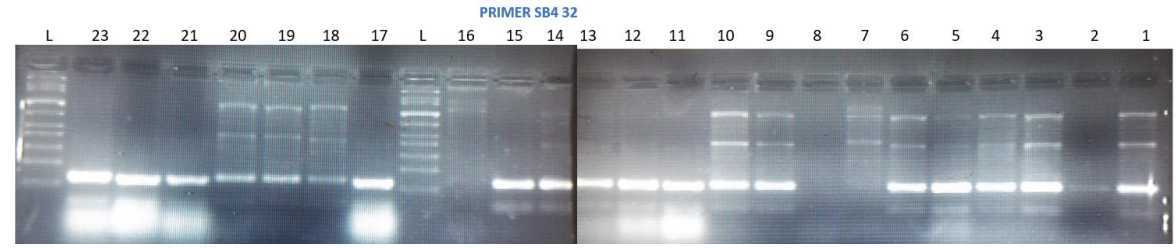


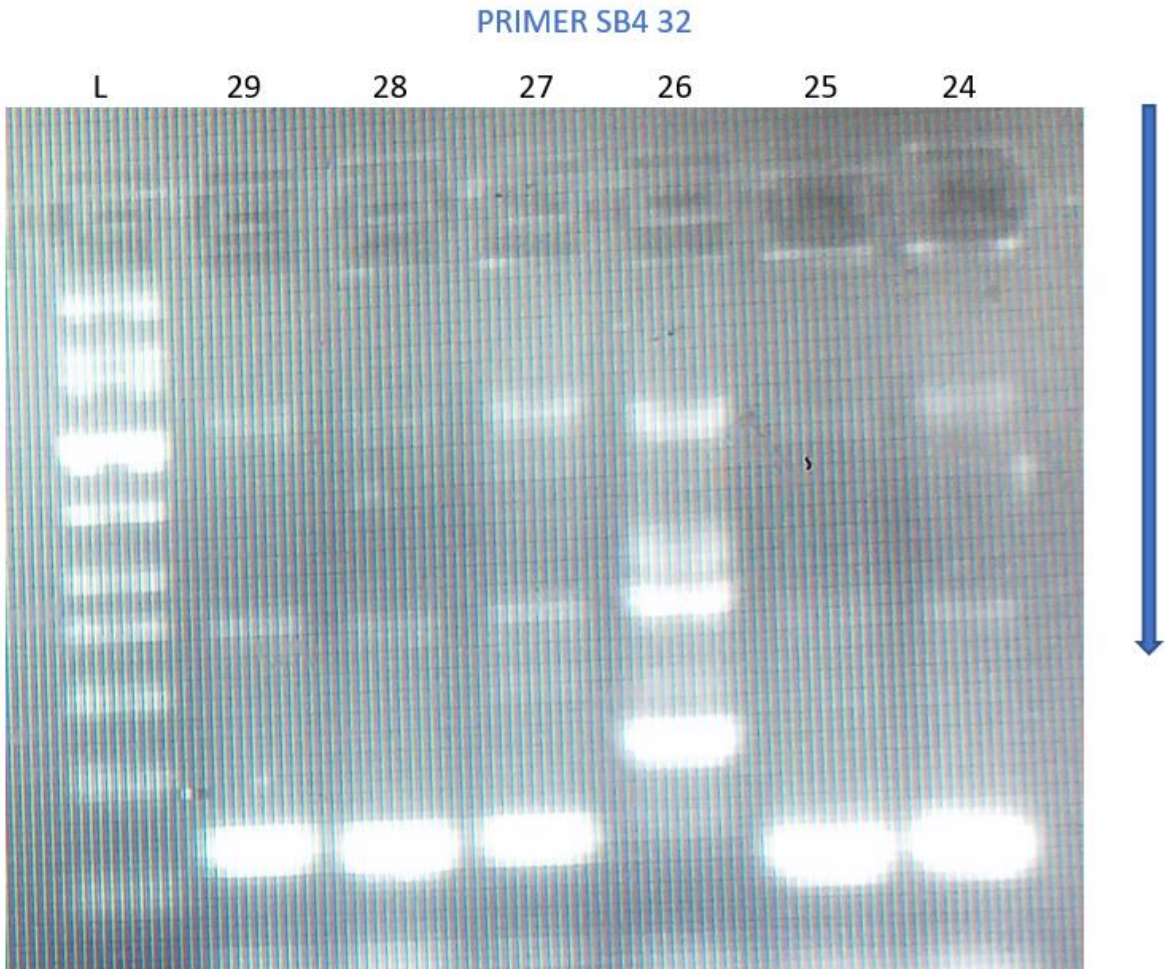




1= Bungoma 1 (S.N), 2= Kakamega 1(S.V), 3= Trans Nzoia 1 (SN), 4= Bungoma 2(SN), 5= Kakamega 2(SS), 6= Bungoma 3 (SS), 7=Kakamega 3 (SS),8= Kakamega 4(SV), 9= Kakamega 5(SV), 10= Bungoma 4 (SN), 11= Bungoma 5(SN), 12= Kakamega 6 (SN), 13= Bungoma 6(SN), 14= Trans Nzoia 2(SV), 15= Trans Nzoia 3(SN), 16= 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*)

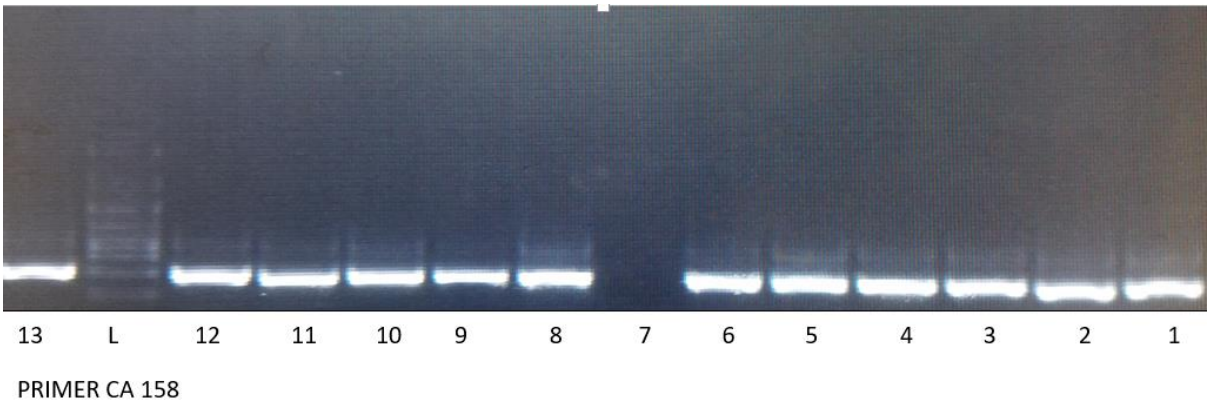
Appendix G: PCR products amplified with primer SB4 32 and visualized under UV light.

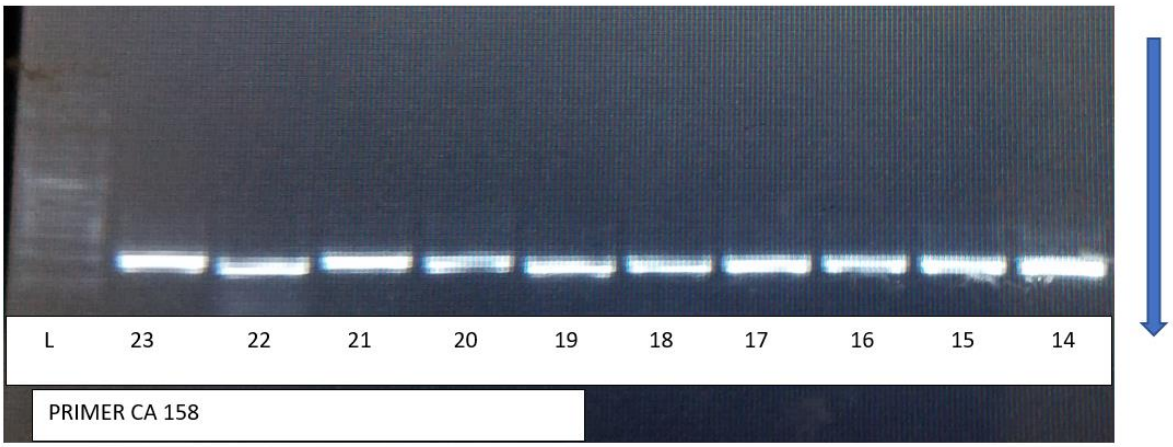




1= Bungoma 1 (S.N), 2= Kakamega 1(S.V), 3= Trans Nzoia 1 (SN), 4= Bungoma 2(SN), 5= Kakamega 2(SS), 6= Bungoma 3 (SS), 7=Kakamega 3 (SS),8= Kakamega 4(SV), 9= Kakamega 5(SV), 10= Bungoma 4 (SN), 11= Bungoma 5(SN), 12= Kakamega 6 (SN), 13= Bungoma 6(SN), 14= Trans Nzoia 2(SV), 15= Trans Nzoia 3(SN), 16= 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*)