MORPHOLOGICAL AND GENOTYPIC CHARACTERIZATION OF AFRICAN NIGHTSHADE CULTIVARS

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DEDICATION

I dedicate this work to my children Precious and Kylen, my husband and my parents for the assistance they gave me throughout the entire study period.

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ABBREVIATIONS AND ACRONYMS

ANS	African nightshade
STDs	Sexually transmitted diseases
UTIs	Urinary tract infections
ALVs	African Leafy vegetables
IPGRI	International Plant Genetic Resources Institute
AVRDC-TZ	World vegetable Centre Tanzania
KALRO	Kenya Agricultural and Livestock Research Institute
SSR	Simple sequence repeats
DNA	Deoxyribonucleic acid
ANOVA	Analysis of variance
AMOVA	Analysis of molecular variance
PVP	Participatory varietal selection
DUS	Distinct, uniform and stable
DARwin	Dissimilarity and Representation for windows
LSD	Least Significance Difference
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
GBK	Gene Bank of Kenya
PIC	Polymorphic Information Content

ABSTRACT

Characterization of African nightshade cultivars is important in identifying the genetic diversity that exists among different cultivars. Genetic diversity can be utilized in breeding programs to develop improved African nightshade cultivars that are high yielding (both leaf and fruit yields) and resistant to biotic and abiotic stresses. The aim of this study was to assess the existence of genetic diversity in different African nightshade cultivars through morphological and genotypic characterization. Germplasm were obtained from the Gene bank of Kenya, AVRDC-TZ and from Kenyan farmers. For morphological characterization the African nightshade cultivars were planted at KALRO Muguga and at KALRO Kisii and scored for several agro morphological characters based on International Plant Genetic Resources Institute (IPGRI) descriptors. Data was collected on both quantitative (plant height, internode length, leaf width leaf length, leaf length: leaf width ratio and number of berries per panicle) and qualitative (growth habit, stem color, leaf shape, leaf color, flower color, berry color and berry size) traits. Morphological data was then subjected to analysis of variance (ANOVA) using GenStat Version 15 while cluster analysis was done using DARwin 6 statistical software. Results showed that there was phenotypic variation among cultivars since they grouped into three clusters (AB and C). There were significant ($P \leq 0.05$) differences between cultivars in plant height, internode length, number of primary branches, leaf size and number of berries per panicle. Correlation studies showed significant strong correlation between leaf width and leaf length (r=0.949**) as well as between leaf size and leaf width (r=-0.772**). Plant height showed significant positive correlation with internode length (r=0.651**), number of branches (r=0.407**) while there was a negative significant correlation between plant height and leaf size (-0.409**). Number of berries and internode length were also significantly correlated (r=0.538**). The significant variations

seen among African nightshade cultivars showed that there exists diversity amongst them. Molecular characterization was done using SSR markers on twenty seven African nightshade cultivars. 5SSR primers yielded reproducible bands with 2 of them (CA158 and STI 001) were polymorphic while the rest (SSR 111, SSR 63 and TSR2) were monomorphic. The dendrogram revealed that the cultivars grouped into three clusters. These findings show that there is rich diversity both within and among African nightshade species which can be exploited for further breeding work.

Key words: Germplasm, characterization, cultivar, ANOVA, SSR.

CHAPTER ONE: INTRODUTION

1.1 BACKGROUND INFORMATION

There's a considerable decrease in varieties of crops grown in Africa today. 30,000 edible plant species are available for consumption but only 30 species are consumed world-wide. The widely consumed crops include maize, wheat and rice. Loss of diversity has been experienced through selection of best performing genotypes leading to loss of genotypes that might have been low yielding but a source of genes responsible for resistance or tolerance to biotic and abiotic stresses. Plant breeding has also partly led to loss of diversity through the development of genotypes adapted to specific environmental conditions and by the development of genetically uniform cultivars and promotion of few widely adapted varieties (Haussman et al., 2004).

Agriculture sector generates about 40% of gross domestic products (GDP) in sub- Saharan Africa and provides 60-80% employment. In Kenya, subsistence farming plays a vital role in the Agricultural economy and contributes 75% of total agricultural output with poverty affecting 56% of Kenyan population. Agriculture in Kenya contributes 53% Gross Domestic Production (GDP) through crop production and services associated which agriculture such as manufacturing and distribution of end products. Agriculture also offers 70% employment to Kenyans and forms 59% of the total export market (Owuor et al., 2016). Food insecurity, malnutrition and poverty is a major problem in Kenya with 60% of the people living in the rural areas living below poverty line with malnutrition resulting to above 50% of children either having stunted growth, being underweight or becoming anemic. Nevertheless, these malnutrition problem can be solved by consumption of African indigenous vegetables which are rich in both macro and micro nutrients (Abukutsa, 2009).

Indigenous vegetables such as African nightshade, moringa, pumpkin, amaranthus, cowpea, okra, jute mallow and Bambara groundnuts have been underutilized even though they are rich in nutrient, aid in cubing food insecurity and are a source of income. This is because of introduction of exotic vegetables which are more preferred, social economic values, cultural practices and lifestyle (Abukutsa, 2009). A survey done in Nairobi revealed that there was a strong association between the type of Indigenous vegetable species consumed by specific communities staying in the city and the indigenous food species found in their rural area (Abukutsa, 2009). This shows that people still hold on to their cultural practices even in urban areas and this is likely to affect the level of consumption of different indigenous vegetables (Maundu et al., 2009)

Plants belonging to *Solanaceae* family are abundant and found worldwide, they include economically important fruits and vegetables like eggplant, tomatoes and potatoes. *Solanum nigrum* is the most diverse plant species within the genus *Solanum (*Matasyoh et al., 2015). *S.nigrum* Linn. (Black Nightshade/African nightshade) is a dicotyledonous weed in the *Solanaceae* family characterized by green leaves which are either ovate or lanceolate in shape with entire to serrated leaf margins.

Solanum nigrum complex (African nightshade) comprises of several species which include *S. Schenopodioides, S. douglasii, S. furcatum*, *S. nigrum* L. subsp. *Nigrum, S. nigrum* L. subsp. *Schultesii, S. retroflexum*, *S. sarrachoides, S. scabrum*, *S. villosum, S. americanum, S. physalifolium, S. nigrescens, S. opacum. S. interius, S. pseudogracile, S. ptycanthum, S. excisirhombeum, S. fiebrigii, S. fragile, S. glandulosipilosum, S. insulae-paschalis, S. sinuatiexcisum and S. tweedianum* (Edmond and Chweya, 1997). Even though African nightshade species have been studied broadly, their correct taxonomic identification is yet to be established. This is because continued inter and intraspecific hybridization do occur naturally

among African nightshade species as well as due to inconsistent genetic variation. The susceptibility of morphological traits to phenotypic plasticity and the existence of many ploidy series have also caused problems to their taxonomic identification. 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).

The most commonly grown African nightshade (ANS) species in Kenya include *S. villosum, S. sarrachoides, S. scabrum, S.americanum* and *S. physalifolium* (Ojiewo et al., 2013b; Matasyoh and Bosire, 2016). Studies have shown that *S. scabrum, S.nigrum, S. villosum* were the species found in western, rift valley, and Nyanza regions in Kenya with *S. scabrum* being preferred in most regions except in Kisii and Nyamira where *S. nigrum* was preferred. *S. Villosum* was reported to have been preferred in most of the regions except in Kakamega and Kisumu while *S.nigrum* was preferred in all the regions except in Eldoret (Table 1) (Matasyoh and Mwaura, 2014; Matasyoh and Bosire, 2016).

Table 1: Geographical distribution of African nightshade species in Western, Nyanza and Rift valley regions of Kenya

	Kakamega	Busia	Bungoma	Kisii	Nyamira	Kisumu	Eldoret	Nakuru
S. scabrum	+	+	+	-	-	+	+	+
S. villosum	-	+	+	+	+	-	+	+
S. nigrum mil	+	+	+	+	+	+	-	+

Key: (+) – present, (-) – absent

Source: Matasyoh and Mwaura, 2014; Matasyoh and Bosire, 2016

Little studies have been done and reported on the size of land used for African nightshade production in Kenya. In 2013, African nightshade was gown on a land size of 2,687 hectare out of which 36% was produced in Kisii and Nyamira counties and this generated total income of

609 million Kenyan shillings per annum (Owuor et al., 2016). African nightshade has been reported to be grown on land size of about 0.25 h or less in Kisii, Nakuru and Kakamega counties by majority (80%) of growers with the farmers recording a mean income of between 11,762 and 4,701 shillings per month (Table 2) (Onyango et al.,2016)

Table 2. The average amount of money (KES) obtained by farmers from African nightshade sales per month

Amount of Money from ANS sales	Average amount of money from ANS sales per month		
	Nakuru	Kisii	Kakamega
Mean	11,762	9,589	4,701
Range	89,500	49,970	25,500
Minimum	500	30	500
Maximum	90,000	50,000	26,000

Source: Onyango et al., 2016

African nightshade has tiny flowers comprising of five petals that are white or purple in colour with berries that turn black on maturity and grows wild in uncultivated lands, rubbish pits and can also be grown in kitchen gardens. African nightshade mainly requires warm soils that are rich in nitrogen for its growth (Atanu et al., 2011; Jagatheeswari et al., 2013).

African nightshade is a widely consumed traditional leafy vegetables which is of high commercial importance because it generate income when sold and offer daily nutrition for subsistence farmers. African nightshade leaves have 87.2%water, 1.4g fiber, 20mg ascorbic acid, 442 mg calcium, 75mg phosphorus, 3660µg of beta carotene, 0.59mg riboflavin per 100g fresh weight (Ojiewo et al.,2013a: Klocke et al., 2016). African nightshade can provide the daily nutrients allowance required by an adult for calcium, iron, b-carotene, and ascorbic acid and 40% of protein if 100g of the fresh vegetable is consumed (Abukutsa et al., 2005). African nightshade

leaves contain substantial amounts of protein and amino acids such as methionine, minerals like calcium, iron and phosphorus, vitamins A and C, fat and fiber (Zebish et al., 2016). African nightshade berries have sap which contain high mounts of iron, calcium and vitamin B and significant amounts of vitamin C and Carotene while the seeds also have vitamin C and carotene. Nutrient composition of African nightshade however varies according to soil fertility of the site where it is grows, the age of the plant and the plant type (Jagatheeswari et al., 2013)

African nightshade is used worldwide for the treatment of various ailments such as throat and eye inflammation, shingles, ringworm, running ulcers, earache, testicular swelling, gout and ear pains, as a therapy for convulsions, pain reliever, an anti-helminthes, an antiseptic, anti-cancer and as an anti-dysentery. Juice made from the leaves can be combined with vinegar and used as a mouthwash and gargle. ANS is also used in the treatment of anthrax pustules, blood pressure, heart diseases, fevers, diarrhea, ulcers, eye infections, STDs, UTIs and tonsillitis (Jagatheeswari et al., 2013; Matasyoh and Mwaura, 2014).

The rise in consumer awareness on the nutritional, economical and medicinal value of African nightshade has simultaneously led to increased utilization which has in turn increased its market demand and value (Ojiewo et al., 2013). Efforts are being made to improve on African nightshade production through increased cultivation for commercial purposes so as to try and meet the demand in the market. Arise in demand and low supply of African nightshade has resulted into an increase in their market prices.

Short shelf life and high perishability of African nightshade are the main disadvantage limiting their transportation over long distances and storage prior to selling. However, farmers are making efforts to increase the supply by farming on contract in urban and semi urban areas (Ojiewo et al., 2013).

Major drawbacks faced during the production of African nightshade include, poor product image, lack of consumer awareness, poor and undeveloped seed systems, lack of quality seeds in the market, poor value chains (Mwangi and Kimathi, 2006) lack of improved high yielding genotypes and high cost of production which intern interferes with the trade (Ojiewo et al., 2013).

Germplasm characterization and evaluation provides an estimate of genetic diversity which is necessary for effective conservation of germplasm. Phenotypic characterization was the native way used to measure genetic diversity by plant breeders more so for crops thought to have little significance. However, diversity assessment that relies on phenotypic characterization alone might be biased since the morphological traits are always susceptible to environmental interferences. Genetic characterization should therefore be used alongside morphological characterization to ensure that true genetic diversity is measured (Mondini et al., 2009). Genetic characterization is carried out by the use of molecular marker technique which is a process by which genetic diversity is identified though existence of variation at specific gene loci.

Molecular markers are DNA fragments associated with a particular gene loci. The generation and use of molecular markers in diversity studies was a major development in molecular genetics. Molecular markers used for diversity studies include Inter-simple sequence repeat (ISSR) Random Amplified Polymorphic DNA (RAPD), DNA amplification fingerprinting (DAF), Arbitrarily primed polymerase chain reaction (APPCR), Amplified Fragment Length Polymorphism (AFLP), Sequence characterized amplified regions (SCARs), Restriction Fragment Length Polymorphism (RFLP), Simple sequence repeats (SSRs), Single Nucleotide Polymorphism (SNPs), Cleaved amplified polymorphic sequence (CAPS), Expressed sequence tags (ESTs), and sequence tagged sites (STSs) (Idrees and Irshad, 2014) . Molecular markers

are recommended for diversity studies since they have many advantages over morphological characterization because they are stable, found in all tissues and are not affected by the environment. An ideal molecular marker should be polymorphic, abundant, reliable, simple, quick, provide enough resolution of genetic variation and should use only amount of DNA for amplification (Mondini et al., 2009)

Diversity studies provide useful information for breeders to know the genetic relationships and distances between crops.

1.2 PROBLEM STATEMENT

Characterization and evaluation of African nightshade germplasm available has not been carried out thereby hindering the formation of African nightshade cultivars improvement breeding programs. Kenyans are increasingly becoming aware of the nutritional importance of African nightshade and this has led to the demand being more that the supply in urban and semi urban areas. 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 improved high yielding African nightshade varieties (Ojiewo et al., 2013). Studies have mainly been done on the nutritional importance of African nightshade so as to enhance its utilization and production however genetic diversity studies have not been exploited.

Few studies have been done on morphological characterization of African nightshade, however the information 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 authors tend to treat different African nightshade species as belonging to one species *S.nigrum L*. (Nandhini and Paramaguru, 2013). It is therefore important to understand morphological traits and genetic diversity of existing African nightshade cultivars for parental selection as well as for the development of an effective breeding program for African nightshade cultivar improvement.

1.3 JUSTIFICATION

Cultivar characterization and evaluation of genetic materials has an important role in the measurement of genetic diversity hence necessary in effective conservation of biodiversity and in the African nightshade improvement program (Ojiewo et al., 2013).

Determination of genetic diversity of any given crop species is a suitable precursor for crop improvement because it is a starting point and a guide to selection of parental lines and on which breeding design is to be developed (Bhat and Kudesia, 2011).

Laws for plant variety protection also requires morphological description of a crop and this could be generated through morphological characterization since varietal testing for distinctness, uniformity and stability (DUS) is an important requirement before the release of a new African nightshade cultivar (Nandhini et al., 2014).

Due to the difficulty in separation of environmental effect from genetic effects using morphological characterization alone as a method of assessing genetic diversity, it is advisable to combine both morphological and molecular characterization method for genetic diversity studies (Dhasmana et al., 2007).

Morphological and molecular characterization of African nightshade will help in the differentiation of one species from the other hence assists in the taxonomic identification of different cultivars that have not been characterized before.

1.4 OBJECTIVES

1.4.1 OVERALL OBJECTIVE

The main objectives of this study was to contribute to African nightshade improvement through documenting the existing variations and genetic diversity by morphological and molecular characterization.

1.4.2 SPECIFIC OBJECTIVES

- To assess variation among different African nightshade cultivars using morphological traits.
- To identify genetic diversity among African nightshade cultivars using SSR markers

1.5 RESEARCH QUESTIONS

- Does morphological traits variation occur among African nightshade cultivars?
- Does genetic diversity exist among African nightshade cultivars?

1.6 HYPOTHESIS

- There is no phenotypic variations among African nightshade cultivars under study.
- There is no genetic diversity among African nightshade cultivars under study.

CHAPTER 2: LITERATURE REVIEW

2.1 African nightshade genetics and cytogenetics.

The genus *Solanum* is the biggest in the *Solanaceae* family containing about 1700 species that are distributed worldwide. *Solanum* consists of a number of species including *Solanum nigrum* complex or African nightshades also known as Black nightshade. African nightshade has an average plant height of 75cm. The leaves are ovate or lanceolate shaped with serrated or entire margin and are green with leaves surface being either glabrous or pubescent. African nightshade flowers have yellow anthers which are conspicuous and are surrounded by white petals. The berries are either dull black, purple-black, red or orange in colour (Matasyoh et al., 2015).

African nightshade is a large group of plants that herbaceous and are made up of about 30 species that vary substantially in morphological features hence forming a polyploidy series, with chromosome number of x=12, diploid ($2n = 2x = 24$), tetraploid ($2n = 4x = 48$) and hexaploid ($2n = 6x = 72$) species. African nightshade complex is self-pollinated but to a less extent of cross pollination do happen (Jacoby and Labuschagne, 2006). Variations among diploid species arise due to differences in the DNA nucleotide sequences (Obute et al., 2015**)**. Polyploidy and hybridization are an important source of diversity and therefore members of the group are morphologically diverse. Natural inter- and intraspecific hybrids have been defined and there are plants with higher level of ploidy among diploid and hexaploid group (Poczai and Hyvonen, 2010). Hybridization across species give rise to sterile plants because their F_1 or F_2 generation experience genetic breakdown however backcrossing the sterile hybrids to the parental species can restore their fertility. Nevertheless, cross-ploidy hybridization is restricted by cross incompatibility inflicted by pollination and fertilization barriers which weakens the

chromosomes. Increase in heterozygosity nevertheless leads to hybrid vigor (Ojiewo et al., 2013a).

2.2 African nightshade species, ecotypes ecology, origin and distribution

African nightshade complex comprises of different species which include *S. nigrum, S. villosum, S. scabrum, S.americanum, S. burkankii, S. sarrachoides and Schenopodioides* among others. Genetic variations occur both within cultivars in a given species and between species and it is this variations that brings about genetic diversity. Some of these species might be phenotypically similar giving rise to taxonomical problem. Though morphologically similar, they differ genetically and that is why African nightshade has been reported to be rich in genetic diversity (Ojiewo et al., 2013 a). Phenotypic variation among different species can be observed in terms of plant growth habits, stem colour and ridging, leaf shape and pubescence among others. During their senescence, their flower and berry sizes reduces considerably becoming fewer and smaller respectively than usual. African nightshade can grow under different environmental conditions and this accounts for their distribution throughout the world. However, the best growing condition for them is under cool high moisture conditions in medium to high altitude with temperature ranges of 15-30ºC for germination and 20-30ºC for growth and low light intensity for germination. They require an annual rainfall of between 500 to 1200mm. Broad leaved African nightshade genotypes are more susceptible to water stress as compared to narrow leaved ones (Ojiewo et al., 2013b). Different African nightshade species are thought to originate from different parts of the world. The center of origin of diploid species such as *S. americanum* and *S. sarrachoides* is South America, the tetraploid species (*S. villosum* and *S. retroflexum*) and hexaploid species *S.nigrum* and *S. scabrum* are thought to have originated from Africa, Europe and Asia. African nightshade hexaploid species is thought to have been derived from a cross

between the tetraploid *Solanum villosum* Mill. And the diploid *Solanum americanum* Mill. (Edmond and Chewea, 1997).

2.3 Production, utilization and markets of African nightshade in Kenya.

Kenya faces major food insecurity with 56% of Kenyans living below poverty line. 50% of Kenyan population lacks adequate food because of prolonged drought, extreme poverty and high population growth rate. This has led to over dependence on nutritionally poor diets leading to malnourishment and child mortality in the rural and semi urban areas. Food security and proper nutrition can be achieved in developing countries through increased awareness, utilization and consumption of traditional leafy vegetables which are rich in nutrients as compared to the exotic vegetables (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 traditional leafy vegetables that continue to be cultivated by farmers from many Kenyan communities (Ondieki et al., 2011). African nightshade is among the most highly supplied and widely consumed traditional leafy vegetables in the country with Kenyan farmers producing yields of 1.5-3.0 tones/ha (Ojiewo et al., 2013). Early flowering and extreme fruiting hinders leaf expansion resulting to lower yields. Increased utilization of traditional vegetables over exotic ones have been documented in Eastern Africa since traditional vegetables require less efforts to produce and are cost effective for rural households with low sources of income (Ojiewo et al., 2013).

Species of vegetable African nightshade vary in their growth habits, leaf yield and nutrition. The most common species grown in Kenya are *S. villosum, S. scabrum and S. sarrachoides. S. villosum* species include *Solanum villosum* subsp. *Villosum* (finely lobed dentate leaf margins and mature berries are orange dull in colour) and *Solanum villosum* Miller subsp. *Miniatum*

(entire, sinuate, sinuate-dented or dentate leaf margins and mature berries are orange dull in colour). *Solanum scabrum* Miller is characterized by entire to sinuate leaf margins with dark purplish black mature berries whereas *Solanum sarrachoides* Sendtner 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 the most common and promising of all African nightshade species in Kenya and can be differentiated from others by its vigorously growing broad leaves and large purple berries and it shows variation in leaf size and plant height but its leaf production remains higher than those of the narrow leafed species like *Solanum villosum* and *Solanum eldoretianum*. *Solanum scabrum* is one of the most distributed and consumed African nightshade species in the country (Abukutsa et al., 2005). In Kenya, African nightshade is usually grown in home gardens intercropped 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 increase 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). Consumption of African indigenous vegetables in Nairobi in 2003 was estimated to be 31 tons 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 were considered old fashioned and a poor man's food 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. Lack of awareness on the nutritional importance of indigenous vegetables and inadequate of agronomic information on African nightshade production has led to the vegetable being underutilized. Poor quality seed has resulted into limited production hence the increasing demand can't be met. African nightshade is also high perishability and if it doesn't reach the market on time traders face huge loses (Mwangi and Kimathi, 2006). This constrains have resulted into low vegetable production of between 1-3 tons per hectare and this is bellow optimum production levels required of 20-40 tons per hectare(Abukutsa et al., 2005; Mwangi and Kimathi, 2006)

2.4 African nightshade diversity, collection and conservation in Kenya

Germplasm can be gathered from the wild, conserved and domesticated either by collecting seeds or vegetative parts used for propagation depending on the breeding system of the species (Rao, 2004). The gene bank of Kenya has gathered approximately around 43 accessions of African nightshade complex. The vegetables were obtained at an altitude of between 650m to 2200 m above sea level and from different environments. Of the forty three accessions samples, nine were uninhabited, thirteen weedy, twelve indigenous and nine accessions were without any data. The Kenyan collections are currently being multiplied and evaluated. Seeds are being preserved at temperatures of 12-15°C and at a relative humidity of about 40 %.(Matasyoh et al., 2015).

African nightshade has been regarded as an insignificant crop by the relevant institution hence no finances have been allocated for its research and there's also lack of enough personnel assigned the duty of evaluation and preserving of African nightshade germplasm.

Conservation of African nightshade genetic resource should start with characterization of existing cultivars (Manoko et al., 2007).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 examples Manoko et al., (2007) found out that cultivars within *S. scabrum* and *S.nigrum* exhibited genetic variation.

2.5 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 morphologically and genetically though characterization (Matta et al., 2015). The higher the genetic diversity, the wider the genetic distance between parental lines the higher the hybrid vigor 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 maybe different from those obtained from principle component analysis and therefore principle component analysis maybe 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 the use of descriptors and the results are normally presented inform of a dendrogram which further shows the genetic interaction within the clusters. Genetic variations can be assessed using pedigree, morphological, biochemical and molecular data (Osawaru et al., 2015). Morphological characterization is cost effective because it does not require expensive and complex technology however, big amount of land is required for laying out the field experiments and this makes it more laborious as compared to molecular characterization. Phenotypic traits are normally prone to environmental interferences hence affect 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).

Molecular analysis is the assessment of genetic diversity by the use of various DNA molecular markers which corrects the mistakes incurred during phenotypic characterization (Mondini et al., 2009). Molecular markers may or may not concur 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 regardless of 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. Morphological

characterization allows for an elaborate 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 provides an elaborate measure of genetic diversity since it is not affected by environmental interferences. Molecular markers also provide a large 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).

2.5 a) Morphological measurements

Traditionally, diversity was assessed by measuring variation in phenotypic traits such as flower colour, growth habit or quantitative agronomic traits like yield potential, stress tolerance, etc., which are of direct interest to users. The main disadvantage experienced during morphological characterization is that the genetic information provided by morphological characters is often restricted and expression of quantitative traits is subjected to environmental interferences (Rao, 2004).

Morphological characterization is necessary in identifying genetic variations that exist within and between species and it is these variations that are responsible for the genetic diversity observed. Genetically diverse parents can be used in crop improvement program to develop high yielding and stable genotypes. Morphological descriptions of genotypes are also required for plant protection under plant variety protection (PVP) legislation, because varietal testing for distinctness, uniformity and stability (DUS) is the basis for grant of protection for new varieties.

Knowledge of genetic variability available within species will greatly assist in exploitation of variability for different breeding programs (Nandhini et al., 2014). Knowledge of phenotypic variation and relationships among genotypes will assist breeders to develop appropriate breeding strategies and to create the most adaptive and productive genotypes. Assessment of morphological and agronomic traits would be useful in the development of new varieties which are high yielding with better adaptation to biotic and abiotic stresses (Stoilova and Pereira, 2013).

Morphological measurements for African nightshade used for assessing diversity include phenotypic features such as plant stand, plant height, growth habit of the plant, internode length, number of nodes, number of primary branches, angle of branching, length and width of the leaf, leaf shape, flower colour, fruit colour, number of fruits per panicle and days to 50% flowering (Ondieki et al., 2011).

2.5 b) Genotypic measurements

Introduction of molecular genetic markers made it easy to differentiate wanted from unwanted agronomic genes in segregating populations. If relationships are established between a heritable agronomic trait and a genetic marker, markers can be used to identify the genes loci. Such relationships allow direct selection for the trait using marker assisted selection in a backcrossing program. Molecular markers which densely cover an entire crop genome can be applied to develop a molecular map for a crop, which could be used to determine linkage between a specific molecular marker and a strongly heritable trait. This holds great promise for breeding programs as many traits are difficult to choose directly from breeding populations (Rao 2004).

Genetic marker assessment involves the measurement of genetic diversity as shown by variation of genes at specific loci and provides information about the level of diversity available within and between populations and how the diversity is distributed (Parmar et al., 2013).

Microsatellites (SSR) are used in genetic diversity studies because they are co-dominant, abundance, diverse, reproducible and highly polymorphic (Mondini et al., 2009). SSR are abundant and this makes them suitable for use in the construction of high-density genetic maps (Demir et al., 2010). Diversity among SSR markers is measured by the number of nucleotide base pair repeat sequences. This variation in DNA sequence can be used to locate a nearby gene. SSR markers are considered highly polymorphic as the number of repeats can vary greatly among plants. The nature of SSR gives them a number of advantages over other molecular markers. SSRs are evenly distributed all over the genome, very small quantities of DNA are required for screening and analysis may be semi-automated (Parmar et al., 2013). SSRs have been demonstrated to be the most suitable markers for genetic diversity evaluation in germplasm collections in different plant species.

2.5 c) Hierarchical cluster analysis

Hierarchical cluster analysis is a method used in genetic diversity studies when classifying cultivars into groups based on their level of similarities and differences and this is done through hierarchical method that interconnects cultivars and then generates a dendrogram in which similar cultivars group together using UPGMA and Ward's method algorithms (Khodadadi et al., 2011; Hashash 2016). Hierarchical cluster analysis aims at classifying sample into homogeneous groups based on the evolutionary distance between individuals. It uses dissimilarity function such as squared Euclidian distance, Manhattan distance or Pearson correlation (Kimes et al., 2014).

Euclidian distance is the most commonly used for cluster analysis and calculates distance based on squared Euclidian distance equation.

$$\sum_{j}^{k} = (a_j - b_j)^2$$

In the above equation, a and b refer to the two cases being compared on the j variable, where k is the total number of variables included in the cluster analysis. This algorithm makes it possible for the distance between two cultivars to be calculated across all variables and reflected in a single distance value in a proximity matrix (Yim and Ramdeen, 2015). Cluster analysis is also used for selection of cultivars for improvement.

2.5 d) Principal component analysis

Principal component analysis is a method that identifies and clarifies on the contribution of the most significant traits to the total genetic variability. Principal component analysis is used mainly for genetic divergence studies to identify and select best performing cultivars for crop improvement as well as in evaluation of the contribution of individual traits in the overall variation recorded (Hashash, 2016). Principle component analysis is carried out by first of all extraction of all the variables, identification of significant components that remain in the model, rotation of the matrix based on factor loading to get solutions, interpretation of solutions, generation of scores for each factor and finally production of results in a tabular form (Petrisor et al., 2012) . The main disadvantage of Principle component analysis is that it may not be the most appropriate measure of association since it's mainly based on correlation or covariance coefficient.

2.5 **e) Principle coordinate analysis (Metric multidimensional scaling)**

Principal coordinate analysis is a method of factorial analysis that is based on Eigen value equation and uses any measure of association. Principle coordinate analysis calculates distance matrix and generates a two or three dimension graph **(**Santo, 2012). When the space defined by distance between units is of high dimension, Principle coordinate Analysis looks for a subspace of low dimension that can be read and at the same time makes sure the distances between the units is as close as possible to the original distances (Perrier et al., 2003).

2.5 f) Unweighted Pair Group Method with Arithmetic mean (UPGMA)

UPGMA is a method of measuring linkage in a group based on their average distance. The distance between two clusters is described as the mean of the distance between all samples generated from different groups. UPGMA constructs a phylogenetic tree through clustering where two clusters are merged generating a new node on the tree hence the dendrogram is built from the leaves towards the roots producing a tree (Kimes et al., 2014). Cultivars are grouped according to their levels of similarities and dissimilarities with members of a given cluster having more similarity as opposed to those of a different cluster ((Perrier et al., 2003).

2.5 g) Neighbour-joining

Neighbour joining is a method used for evolutionary tree construction based on genetic distance. During construction of the tree, the nearest nodes are selected and referred to as neighbours and this process is repeated until all the nodes are paired. The neighbours become the leaves of the tree and are the operational taxonomic unit (OTU) (Mailund and Pedersen, 2004). Neighbour joining method is used for diversity studies and generates dendrogram with clusters which are separated based on the bootstrap values (Targonska et al., 2016).

2.5 h) Bootstrap values

Bootstrap value is a percentage value on a dendrogram in which a branch is formed. Dendrogram are usually presented with bootstrap values associated at their nodes which are used to measure of support on a dendrogram (Soltis and Soltis, 2003).Bootstrapping generates a support value for each node putting into consideration all the samples that support that node. The highest bootstrap value is 100% with values above 70% considered high and values between 70% and 50% considered weak while values below 50% considered insignificant hence ignored (Soltis and Soltis, 2003; Landis, 2012). Bootstrap values of 50% though considered weak have been reported to be statistically significant (Chemutai et al., 2016). Bootstraps values are important in diversity studies of cultivars because they assist in the identification of diverse clusters from which diverse parental lines could be picked for further breeding work.

2.5 1) Nei genetic distance.

Genetic distance is the level of genetic variation between different species or populations that is numerically measured in terms of the number of nucleotide difference per gene (Nei, 2001).

Nei genetic distance measures the number of nucleotide substitution per locus that arises after separation of any two population. Nei genetic distance measures the total allele differences per loci and can be estimated from data of distantly related individuals based on the amino acid sequence in their protein. When enough data is available, the genetic distance between any two cultivars can be measured without taking into account their ploidy series or mating scheme (Nei, 1972).

2.6) Molecular markers used in diversity studies in Indigenous vegetables

Molecular makers have been used to assess genetic diversity in various Indigenous vegetable hence providing vegetable breeders with important information on the performance, agronomic

qualities and adaptation of the cultivars (Singh et al., 2011). Tomatoes, potatoes, eggplant and African nightshade are vegetable belonging to the genus *Solanum* of the *Solanaceae* family. Genetic diversity studies have been carried out on tomatoes using different molecular markers such as Random Amplified Polymorphic DNA (RAPD) (Sharifova et al., 2013), restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified length polymorphic (AFLP) (Claudio et al., 2004) and inter-simple sequence repeat (ISSR) (Hassan et al., 2013**).** In eggplant, genetic studies have been done using RAPD, AFLP, SSR and ISSR molecular markers (Demir et al., 2010) while in potatoes diversity studies, RAPD, AFLP (Teixeira *et al*.,2013), RAPD and SSR (Rocha et al., 2010: Favoretto et al., 2011) markers have been used. Genetic diversity studies in African nightshade have been carried out using molecular markers such as AFLP (Mwai, 2007) SCoT, ISSR (Poczai and Hyvonen, 2010) and RAPD (Dhasmana et al., 2007).

Simple sequence repeat (microsatellites, SSR) molecular marker has been used for genetic diversity studies of vegetables belonging to the genus *Solanum* such as potato (*Solanum tuberosum* L.), eggplant (*S. melongena* L.) and tomato (*S. lycopersicum* L.) (Zhu et al., 2012) but have not for African nightshade. SSR makers have major advantage over other molecular markers such as RFLP and RAPD due to their high polymorphic rates. SSR primers developed for one species can be used to identify polymorphism at homologous site in related species and this would avail SSR primer for use in a number of related species at a minimum cost. Advanced genomic homology might translate into more conservation of SSR- flanking regions resulting into primer transferability across different related species (Rossetto, 2017).

CHAPTER 3

MORPHOLOGICAL CHARACTERIZATION OF AFRICAN NIGHTSHADE CULTIVAS

3.1 ABSTRACT

This study was conducted to morphologically characterize nineteen African nightshade cultivars in order to establish the level of genetic variation amongst them. The cultivars were characterized for several agro morphological characters based on IPGRI descriptors and most of them had lanceolate leaves (68%), leaves with entire margins (74%), leaves with glabrous surfaces (79%), stems with ridges (89%) and flower with white petals (95%). Genetic diversity was estimated using DARwin 6 statistical software in which the cultivars clustered into 3 groups based on their qualitative traits. Analysis of variance (ANOVA) was done using GenStat 15th

edition for quantitative traits and the mean separated by LSD. The results indicated that there was significant difference at $p \leq 0.05$ in plant height, internode length, number of primary branches, leaf length, leaf width, leaf size and number of berries per panicle within cultivars planted both in Kisii and Muguga. Combined ANOVA for Kisii and Muguga indicated that there was significance difference at $p \leq 0.05$ in cultivar x location in plant height, leaf length and in the number of primary branches with cultivars recording higher plant height in Muguga (74.66cm) than in Kisii (69.96cm), longer leaves in Muguga (13.47cm) than in Kisii (12.10) and producing more primary branches in Muguga (17) than in Kisii (14). There was significant correlation at $p \leq 0.05$ reported between quantitative traits both within and among African nightshade species both in Kisii and Muguga with high significant correlation at $p \leq 0.05$ being recorded between leaf length and leaf width in Kisii (r= 0.9509**) and in Muguga (r= 0.9276). The significant differences seen within African nightshade cultivars in both qualitative and quantitative traits indicated that there exists diversity within them that can be used for improvement and or development of new varieties in African nightshade breeding programs.

Key words: African nightshade, cultivar, characterization, diversity, variation

3.2 INTRODUCTION

Variability among germplasm is a prerequisite of any breeding program. Genetic variation would assist in the selection of parental lines to be in cooperated into a breeding program and may enhance improved food production by improving already existing varieties or developing new high yielding varieties. The existing variations within and among cultivars can be measured through diversity studies. The higher the variation and diversity between parents the more the hybrid vigor in the F_1 generation (Khodadi et al., 2011).

Quantitative traits of African nightshade could be evaluated under different environmental conditions to assess their stability in different environments. A cultivar may respond differently to different environmental conditions leading to morphological expressions and responses as a result of genetic x environmental (G*E) interaction. The knowledge of G*E interaction is crucial for breeding work since it enables breeders to identify superior and stable cultivars for different environments (El-Soda et al., 2014). This is due to the fact that environmental stresses may

trigger varied plant responses, by either altering the gene expression or cellular metabolism thereby modifying plant growth and productivity (Anjum et al., 2011).

Correlation is a measure of the association between pair of variables. Even though a lot of genetic diversity and interrelationship studied have been reported on major crops, such information is very scanty for minor crops such as African nightshade. Vegetative yield for African nightshade can be improved by the study and knowledge of interrelationship between different quantitative traits and their influence on yield either directly or indirectly (Shukla et al., 2010). Correlation studies also allow for the simultaneous selection of more than one trait at a time during plant breeding (Meitei et al., 2014) with traits that show significant positive correlations being improved simultaneously while those traits that show significant negative correlations being improved independently (Nyadanu et al.,2014).

3.3 MATERIALS AND METHODS

3.3.1 Germplasm assembly and collection.

The study consisted of 19 African nightshade cultivars comprising of local landraces and wild types. The germplasm was assembled from AVRDC TZ (World vegetable center), Gene bank of Kenya and from Kenyan farmers landraces (Table 3).

Table 3: African nightshade cultivars used in the study, their source and species

Cultivar code/name	Collected from	Species
GBK 050572	Gene bank of Kenya	*S. nigrum*
GBK 050287	Gene bank of Kenya	*S.americanum*
GBK 050292	Gene bank of Kenya	*S.americanum*
Kisii 1	Kenyan farmer at Kisii, Kenya	*S. sarrachoides*
Kisii 2	Kenyan farmer at Kisii, Kenya	*S. sarrachoides*
OARA 1	Kenyan farmer at Kisii, Kenya	*S. americanum*
OARA 2	Kenyan farmer at Kisii, Kenya	*S. nigrum*
Improved type	Agrovet in Kenya	*S. scabrum*
Managu Nakuru county	Farmers at Nakuru, Kenya	*S. scabrum*
Kakamega1	Farmers at Kakamega, Kenya	*S. villosum*
Kakamega 2	Farmers at Kakamega, Kenya	*S. villosum*
Kakamega 3	Farmers at Kakamega, Kenya	*S. villosum*
BG 14	AVRDC –TZ	*S. scabrum*

BG 22	AVRDC-TZ	*S. scabrum*
BG 23	AVRDC-TZ	*S. scabrum*
SS 49	AVRDC-TZ	*S. villosum*
MW 17	AVRDC-TZ	*S.americanum*
MW 13	AVRDC-TZ	*S. sarrachoides*
RC 11	AVRDC-TZ	*S. americanum*

3.3.2 Study site

Evaluation of 19 African nightshade cultivars (Table 3) was conducted at the Kenya Agricultural and Livestock Research Organization (KALRO) stations in Kisii and Muguga during the 2015 long rains (March, April and May) and short rains (September, October and November) seasons. KALRO Kisii is located at a latitude of 0°39'S, longitudes 34°38'E and an altitude of 1792m above sea level with a gentle sloppy landscape. The soils type is mainly loam soils classified as phaeozems (FAO/UNESCO, 1974). The area receives annual convectional type of rainfall of between 1200 mm to 2000 mm occasioned by its proximity to Lake Victoria and its hilly topography with an average daily temperature range between18-22°C.

KALRO-Muguga is located latitude 1°13' S, longitude 36º38' E, and altitude of 2096 m above sea level, 27 km North West of Nairobi, Kenya. This area receives bimodal mean rainfall of between 900 mm to 1000 mm annually with long rains of 550 mm falling in mid-March to June and the short rains of 400 mm falling in mid-October to December. The area has minimum temperature of 7°C and maximum of 20ºC.It is classified as sub humid with a well-drained, very deep, dark reddish brown to dark red, friable clay soil classified as humic nitisols

3.2.3 Field operations and experimental design

Land was ploughed and harrowed until fine tilth was achieved. Twenty genotypes of African nightshade were then planted using Alpha Lattice design in 3 replications at KALRO Muguga and KALRO Kisii. Seeds were drilled at a depth of 1cm on single row plots of 200 cm long with 15 cm spacing between plants, 60cm between rows and 75 cm between blocks during the short and long rainy seasons. No artificial fertilizer was used but farm yard manure was applied at the rate 0.5 kg per single row plot and thoroughly mixed with the 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 DATA COLLECTION

Ten African nightshade plants per plot were then tagged and morphological traits data among the cultivars captured using the International Plant Genetic Resource Institute (IPGRI) descriptors (Edmonds 1972) as follows:

i. Plant height (cm): Was determined by measuring the plants length from ground level to the tip of the main stem of the plant at 50% flowering while internodes length (cm) was measured by marking the fourth internodes and the lengths determined between the adjacent nodes.

ii. Number of primary branches: This was determined by physically counting the total number of primary branches directly attached to the main stem.

iii. Leaf margin: The margins of the leaves were observed and recorded using the IPGRI descriptors.

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

v. Leaf size was determined by measuring the leaf length (cm) from its tip to the petiole and the leaf width (cm) by measuring the leaf diameter at the middle of the leaf from one end to the next using a 30cm ruler then calculating the ratio of leaf length to leaf width. The smaller the ratio the bigger the leaf and the bigger the ratio the smaller the leaf. Leafs which were measured were randomly picked factoring both the biggest, the smallest and medium sized leaves per plant.

vi. Leaf colour: The colour of the leaves was determined by visually observing and recording the observed colours and the leaves were either pale green, intermediate green or dark green.

vii. Stem colour: The colours were determined by visually observing and recording the observed colour at 50% flowering.

viii. Stem pubescence: Pubescence was determined by feeling the texture of the entire stem of the plant using hand and scoring for presence or absence of hairs and ridges.

ix. Fruit colour: The colour was determined by visually observing and recording the colour of mature and ripe fruits. The colours of the mature fruits were either green, purple or orange.

Table 4: Qualitative traits and their scores according to the IPGRI descriptors

Cultivar	Plant type	Leaf colour	Leaf shape	Leaf margin	Leaf hairiness	Flower colour	Branching habit	Stem ridging	Stem colour	Stem pubescence	Berry colour
GBK genotype	1	7	1	1	1	1	3	2	3	1	1
GBK 050572	2	5	2	1	1	1	3	1	2	2	3
GBK050287	3	5	2	1	2	1	5	2	5	1	3
GBK050292	3	7	2	1	2	1	5	2	4	1	3
Nakuru county	1	7	1	1	1	1	3	1	3	2	1
Kisii 1	3	7	2	1	2	1	5	2	4	1	3
Kisii 2	3	7	2	1	2	1	5	2	4	1	3
OARA type 1	2	7	3	2	3	1	5	2	5	1	1
Improved type	1	3	1	1	2	1	3	2	1	1	1
Kakamega 1	2	5	2	2	2	1	3	2	2	1	2
Kakamega 2	2	5	2	2	1	1	3	2	1	1	2
Kakamega 3	2	7	2	3	2	1	3	2	1	1	2
AVRDC BG14	3	5	2	1	2	1	3	2	3	1	3
AVRDC BG22	3	3	2	1	2	1	5	2	2	1	3
AVRDC BG23	3	7	2	1	2	1	5	2	5	1	3
AVRDC W13	3	5	2	1	2	1	5	2	4	1	3
AVRDC SS49	1	7	1	1	2	2	3	2	2	1	1

AVRDC RC11	3	5	2	1	2	1	5	2	4	1	3
OARA TYPE 2	2	3	3	2	3	1	3	2	4	1	1

IPGRI Descriptors
Plant type: 1=Erect, 2=Semi erect 3=Creeping/prostrate; Branching habits: 3=Upright, 5=Spreading dropping; Leaf colour: 3= Light green, 5= green, 7=dark gre0en; Leaf hairiness: 1=Glabrous, 2=pubescent, 3= pubescent with very dense and long trachomes. Leaf margin: 1 =Entire, 2 =Sinuate -dentate, 3 = Lobed; Leaf shape: 1 = Ovate, 2 = Lanceolate, 3 = Rhomboid; Stem colour: 1=Purple, 2=Orange, 3=Green; Stem colour:1 =Green, 2 =Green with purple shades, 3 =Purple with green shades, 4 Purple, 5 =Dark purple; Stem pubescence: 1= present, 2= absent; Flower colour: 1 = White, 2 = Purple; Fruit colour : 1= Purple, 2= Orange, 3= Green.
Source: Edmonds 1972.

3.5 DATA ANALYSIS

Qualitative data was analyzed using excel and frequency distribution pie charts drawn to establish the percentage frequency of African nightshade cultivars having each trait. Qualitative traits were further analyzed using DARwin (Dissimilarity and Representation for windows) 6 software (Perrier et al., 2006) and an unweighted neighbor joining dendrogram drawn based on dissimilarity index. The dendrogram was drawn using Neighbour joining unweighted tree which is a method of reconstructing phylogenetic trees and computing the lengths of its branches where the two nearest nodes of the tree referred to as neighbours are the leaves of the tree and are the operational taxonomic units (OUT's). The process is repeated until all the nodes are paired together (Perrier et al., 2003). Dendrogram has bootstrap values that varies from 1% to 100% of all the total replications. Bootstraps calculate a support value for each node (Where the leaves or branches of the tree meet) based on the fraction of the samples that meet at the node with the highest value being 100% (Holmes, 2003).

Quantitative data were subjected to analysis of variance (ANOVA) using GENSTAT statistical software (GENSTAT 15th) edition and where the traits were significant their means compared using LSD. Correlation analysis of traits was also performed using GENSTAT 15th edition to

assess the interrelationship within African nightshade morphological traits and a probability level of $p \leq 0.05$ was considered statistically significant while probability of ≤ 0.01 was considered to be highly significant.

3.6 RESULTS

3.6.1 Qualitative traits

When the 19 African nightshade cultivars were planted, 9 cultivars had creeping plant type, 6 were semi erect while 4 were erect (Figure 1) which accounted for 47%, 32% and 21% respectively.

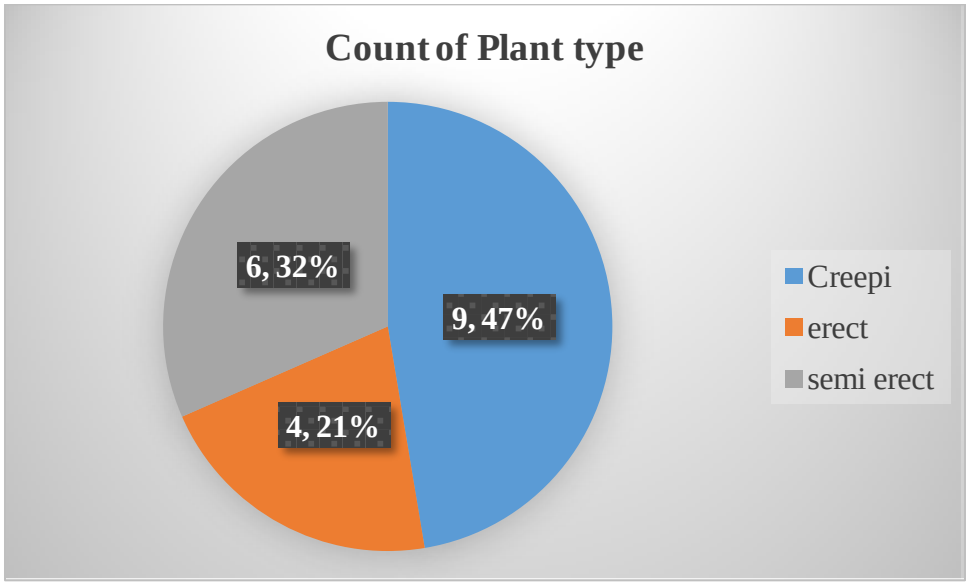


Figure 1: Distribution of 19 African nightshade cultivars according to plant type.

13 out of the 19 African nightshade cultivars hade lanceolate leaf shapes, 4 had ovate while only 2 cultivars which were OARA type 1 and 2 had rhomboid leaf shapes (Figure 2) and this accounted for 68% , 21% and 11%, respectively.

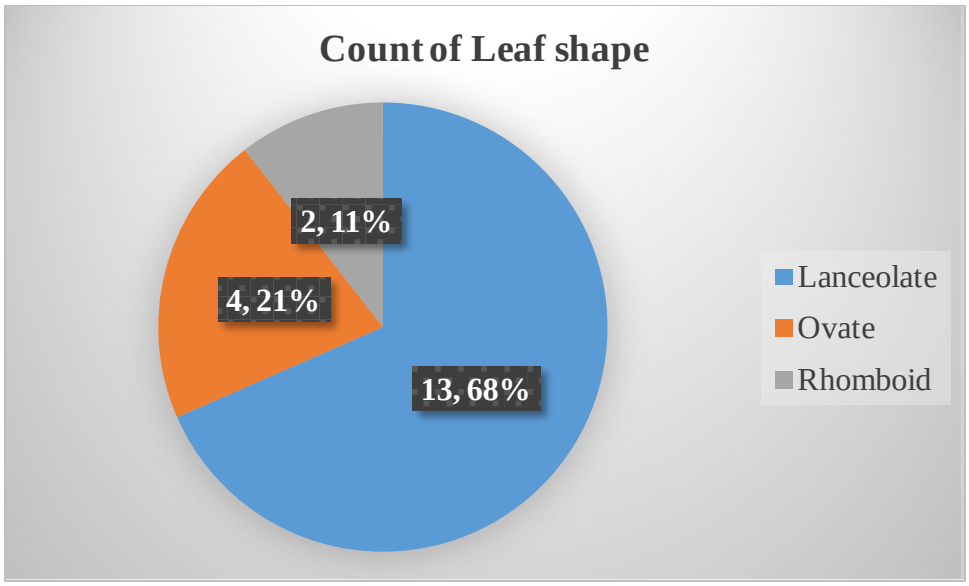


Figure 2: Distribution of 19 African nightshade cultivars according to leaf shape.

Most (74%) African nightshade cultivars had entire leaf margins followed by 21% having sinuate to dentate while only Kakamega type 3 (5%) had lobed leaf margins (Figure 3).

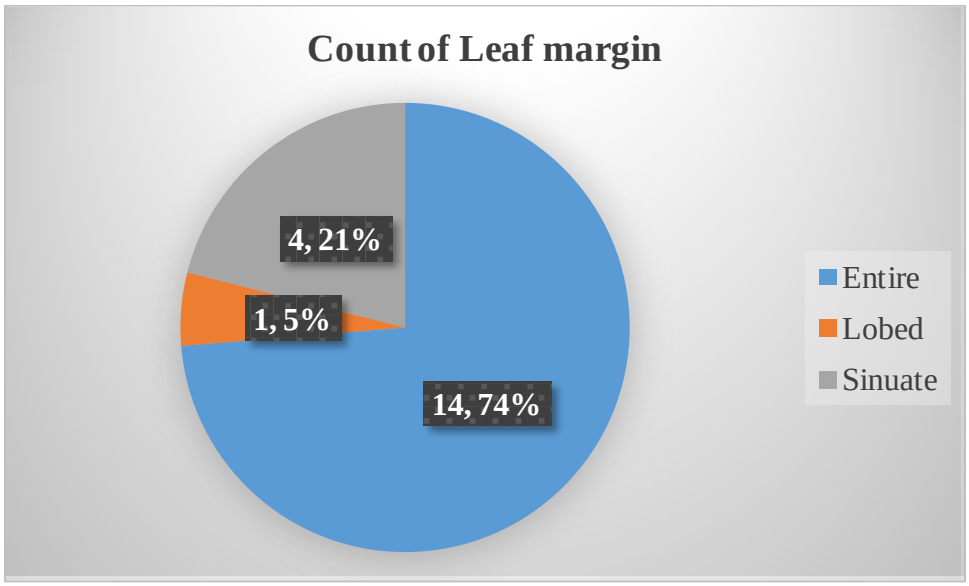


Figure 3: Distribution of 19 African nightshade cultivars according to leaf shape.

Only 4 African nightshade cultivars (21%) had glabrous leaf surfaces (GBK genotype, GBK 050572), Nakuru county and Kakamega type 2) while the rest (15 cultivars) had pubescent leaf

surfaces representing 79% of the total population (Figure 4). The degree of hairiness varied from one cultivar to the next with OARA type 1 and 2 having the longest trachomes.

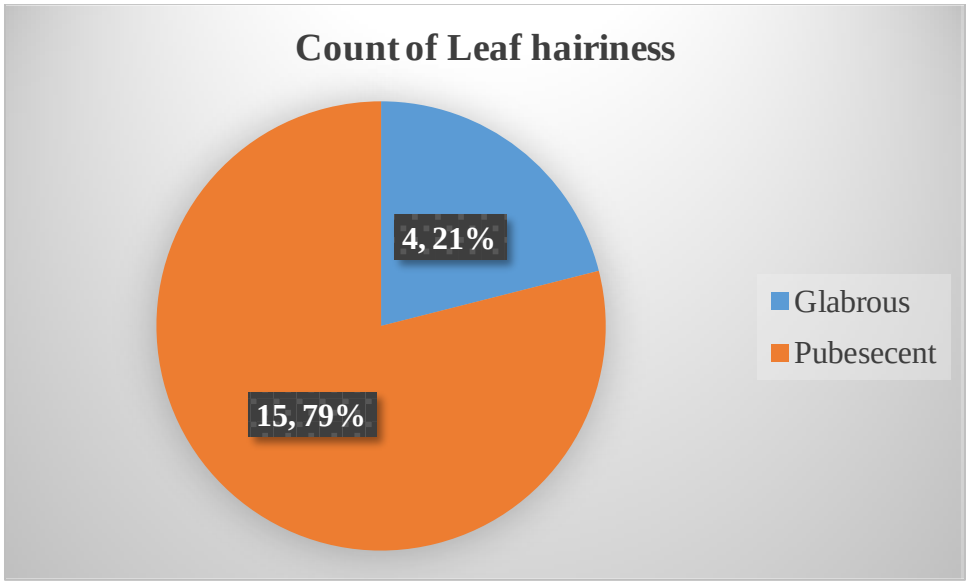


Figure 4: Distribution of 19 African nightshade cultivars according to leaf hairiness**.**

7 African nightshade cultivars had purple stems, 4 (GBK genotype, Kakamega type1, AVDC BG 22 and AVRDC SS49) had green stems with purple shades, 3 (Kakamega 1, Kakamega 2 and improved type) had entire green stems and 3 cultivars (GBK 050287, OARA 1 and AVRDC BG 23) had dark purple stems while the remaining 2 (Nakuru county and AVRDC BG 14) cultivars had purple stems with green shades (Figure 5).

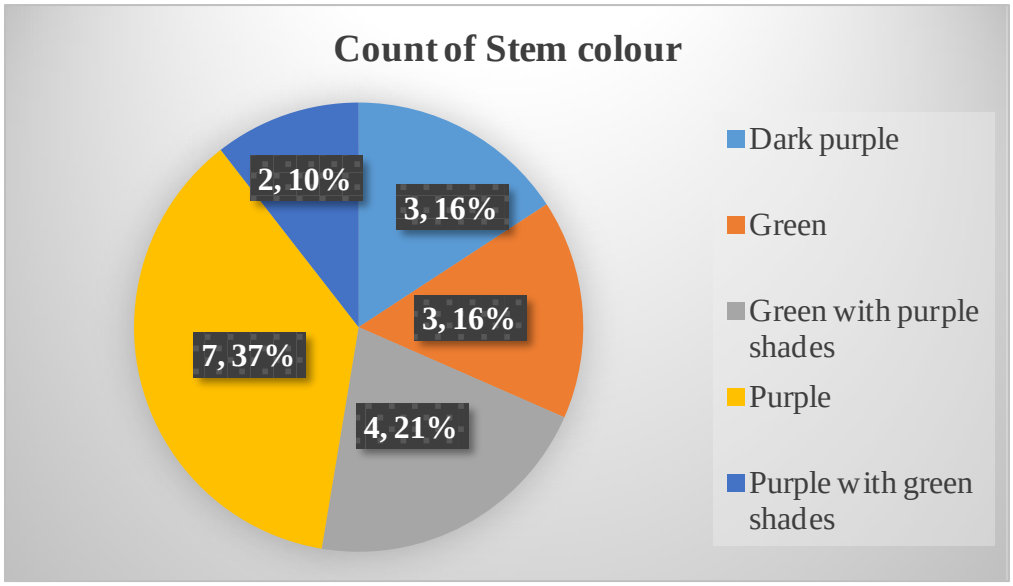


Figure 5: Distribution of 19 African nightshade cultivars based on stem colour.

Most on the African nightshade cultivars (17) had stem ridges while only 2 (GBK 050572) and Nakuru county) cultivars lacked the stem ridges and that formed 89% and 11% respectively (Figure 6).

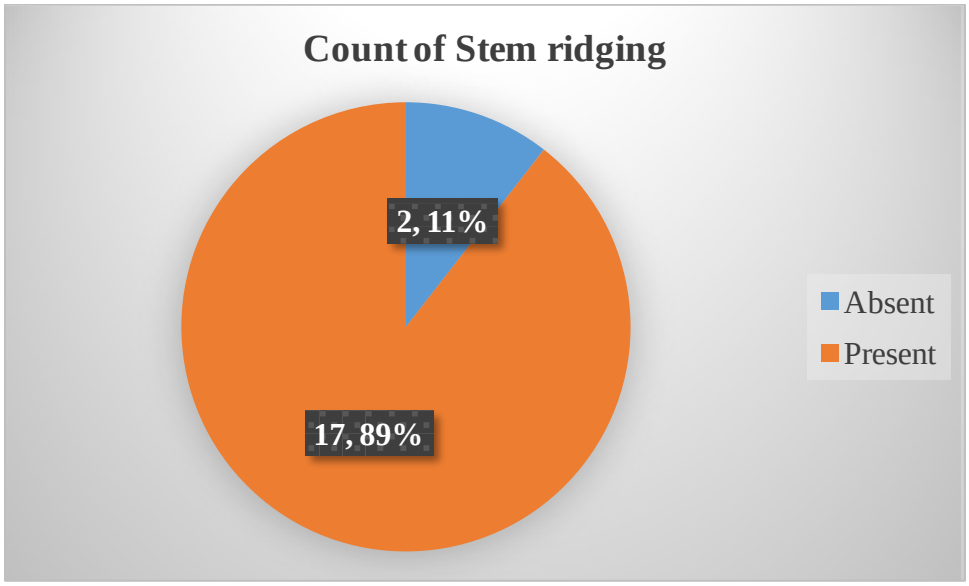


Figure 6: Distribution of 19 African nightshade cultivars based on stem ridging.

53% of African nightshade cultivars grown had spreading drooping branching habits while the remaining 47% had upright branching habits (Figure 7)

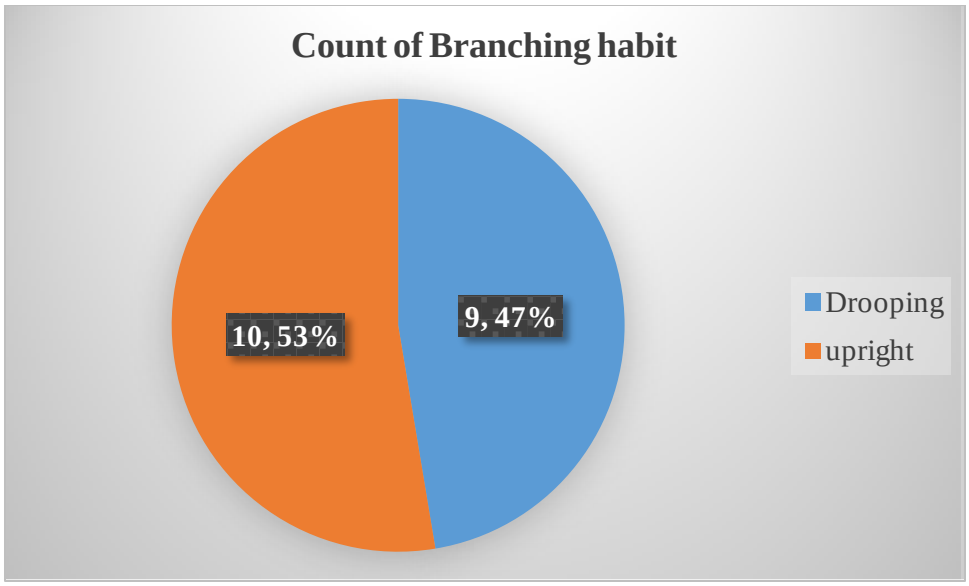


Figure 7: Distribution of 19 African nightshade cultivars based on branching habits.

All the 18 African nightshade cultivars had flowers with white petals the only exceptional being AVRDC SS49 which had flowers with purple petals (Figure 8).

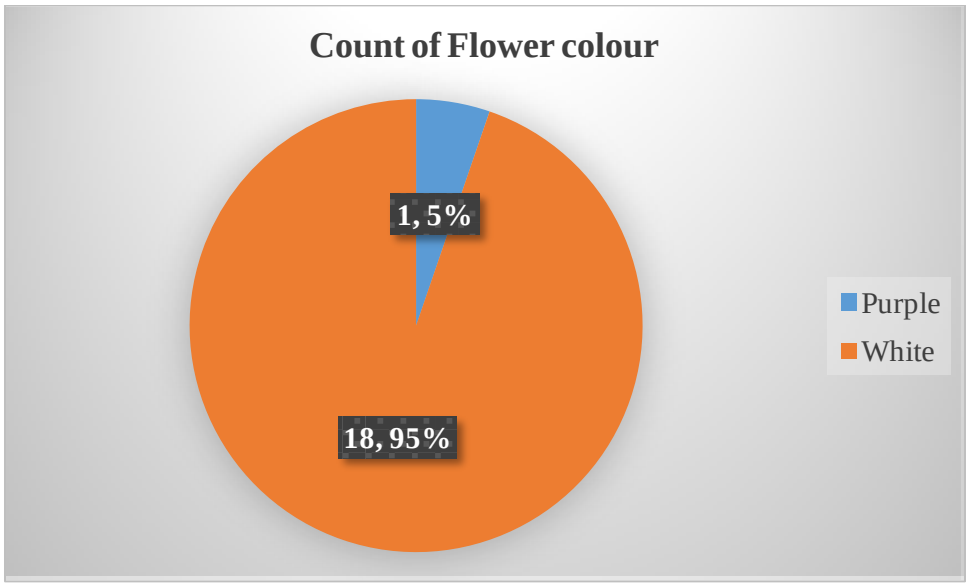


Figure 8: Distribution of 19 African nightshade cultivars based on flower colour.

19 African nightshade grown produced berries of different colours with the Kakamega types (type 1, 2 and 3) producing orange berries, GBK genotype, improved type, Nakuru county,

AVRDC SS49, OARA type 1 and OARA type 2 producing purple berries while the rest produced berries that remained green at maturity (Table 9).

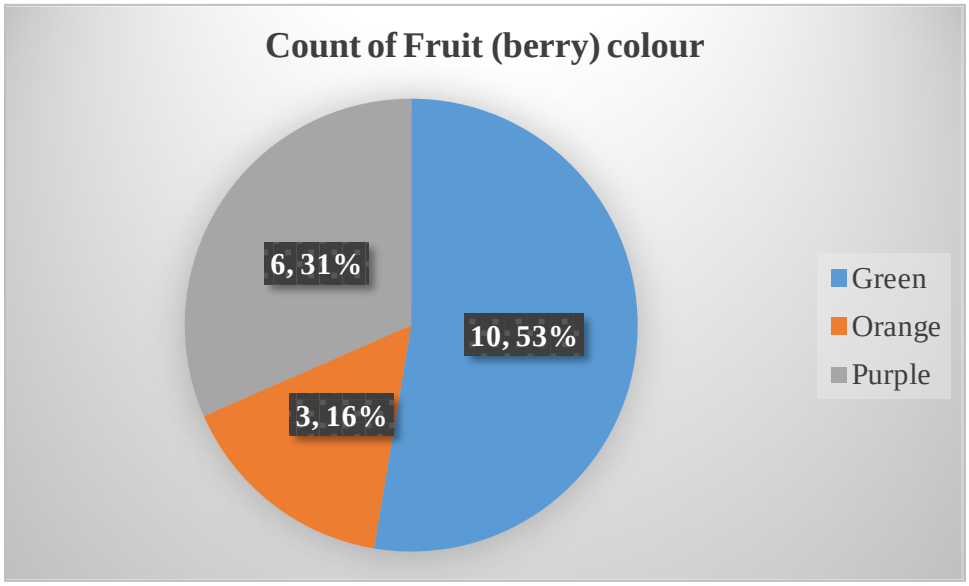


Figure 9: Distribution of 19 African nightshade cultivars based on the colour of the berries

The above qualitative traits were used to generate a dendrogram in which African nightshade cultivars grouped into different clusters based on their morphological traits similarities and difference. The cultivars clustered into three distinct groups namely group A, B, and C based on plant type, branching habit, leaf shape, leaf margin, leaf pubescence, leaf and stem colour, stem ridging, stem pubescence and fruit colour (Figure 10). Group A consisted of two sub clusters representing two African nightshade species *S. americanum* (OARA1, AVRDC RC11, GBK 050287, and GBK 050292)which had rhomboid shaped leaves with sinuate to dentate and entire margins, white flower petals and small purple berries when mature (Figure 11; d. e, i, l) and a semi erect plant type (figure 12 ; b, c) and *S. sarrachoides* (Kisii 1, Kisii 2 and AVRDC MW 13) characterized by lanceolate shaped leaves with entire margins, white flower petals and its berries remain green when mature and having prostrate/creeping plant type (Figure 11; b, i, m).

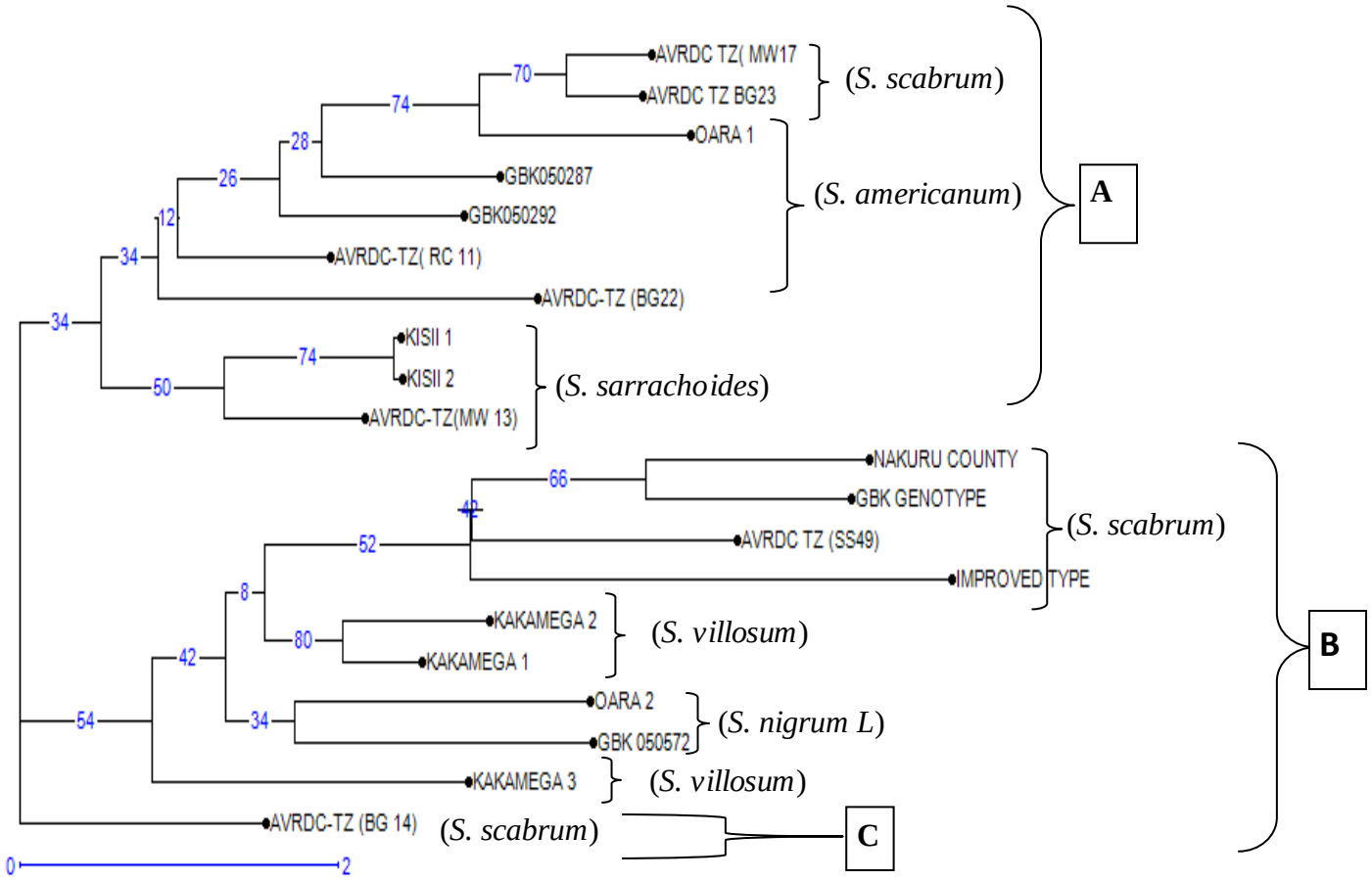


Figure 10: Genetic distance dendrogram of African nightshade cultivars

Group B consisted of species *S scabrum* (Managu from Nakuru County, AVRDC SS49, improved type and AVRDC GB14) whose cultivars were characterized by having ovate leaves with entire margins, either purple or white flower petals that produced large purple berries when mature and had erect plant type (Figure 11; a, h, i, k and Figure 12 a), *S. villosum* (Kakameg1, Kakamega 2, Kakamega 3) whose cultivars had lanceolate leaves with lobed and sinuate to dentate leaf margins, white flower petals that produced green berries which turned to orange when mature and had semi erect plant type (Figure 11; c, i. j and Figure 12 b) and species *S. nigrum* L (OARA type 2) characterized by rhomboid shaped leaves with sinuate to dentate and entire margins, white flower petals that produced small purple berries when mature and had semi

erect plant type (Figure 11; d. e, i, l). Group C consisted of one cultivar which was identified as *S scabrum*.

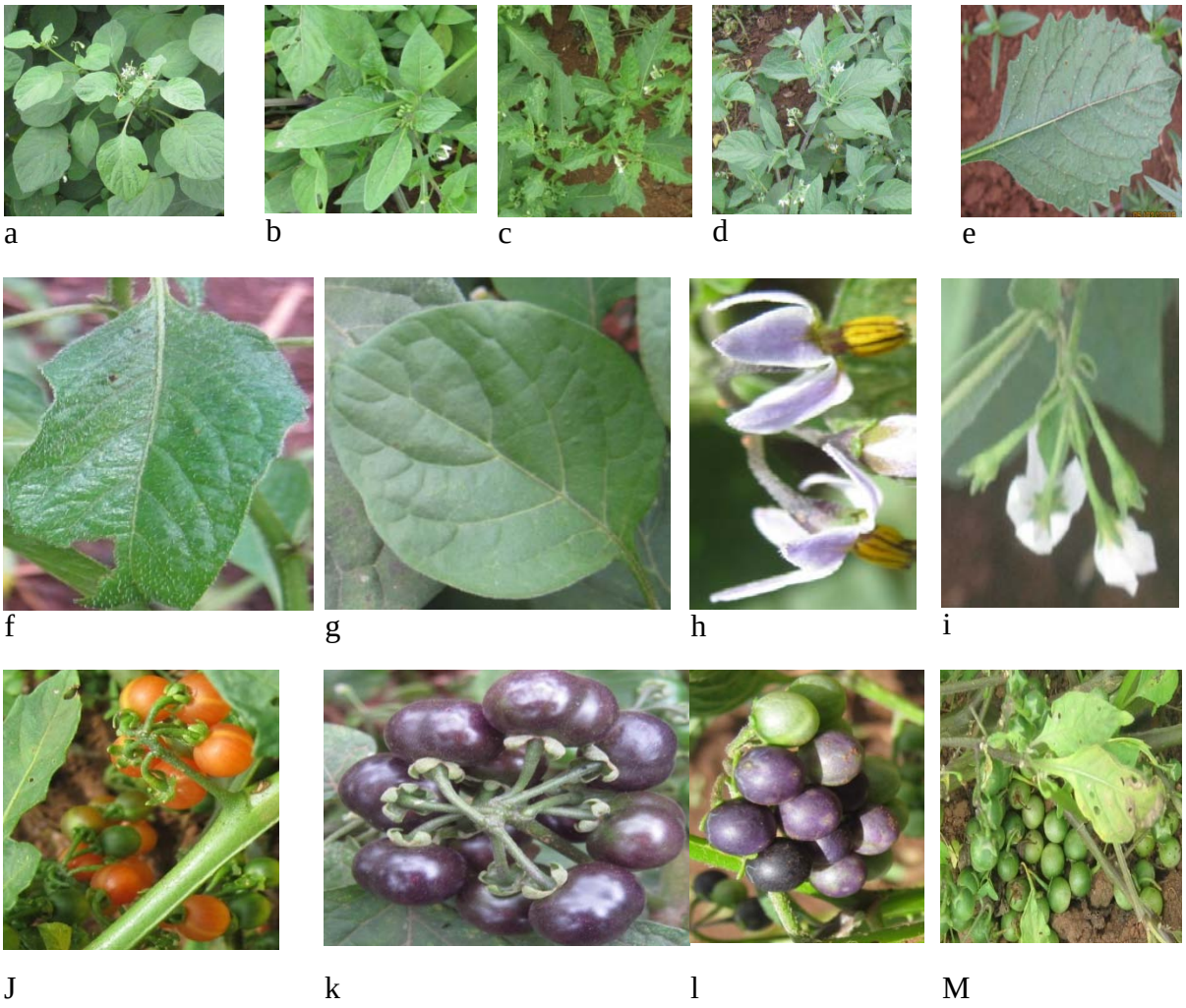


Figure 11: African nightshade cultivars exhibiting diversity in leaf shape, leaf margin, leaf pubescent, flower colour and fruit colour; a-Ovate leaves with entire margins, b- Lanceolate with entire margins, c- Lanceolate leaves with sinuate to dentate margins, d- Rhomboid leaves with entire margins, e- Rhomboid leaf with sinuate-dentate margins, f –Pubescent leaf. g- Glabrous leaf. h- Purple flower petals. i- White flower petals. j- Orange berries. k- Purple berries. l- Purple berries. m- Green berries

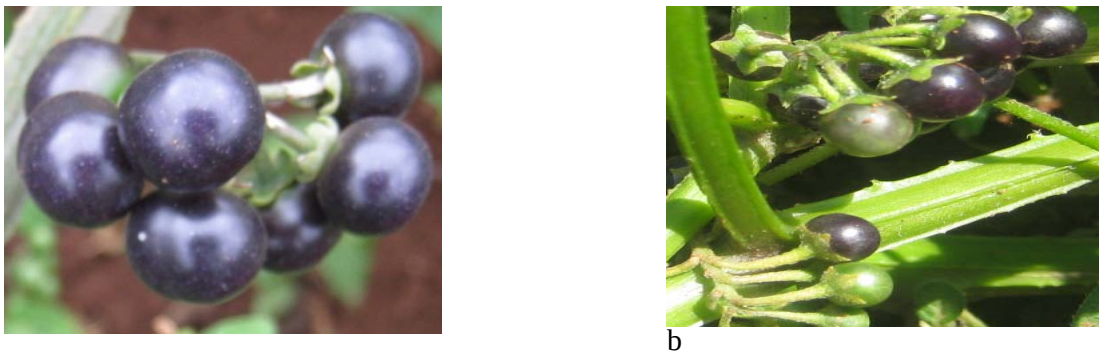
Bootstrap values of between 0-100 percent were shown on branches with the scale giving level of dissimilarities. Cultivars in cluster group A had 34% bootstrap value while those in group B which had 54 % bootstrap value. The highest bootstrap value was 80% representing cluster

group consisting of Kakamega 1 and Kakamega 2 followed by 74% bootstrap value representing cluster group of Kisii type 1 and Kisii type 2 (Figure 10).

a b C

Figure 12: African nightshade cultivars exhibiting diversity in plant type; a- Erect. b- Semi erect .c- Prostate/ Creeping.

Variation in stem colour of cultivars belonging to African nightshade species was also observed ranging from green, mixture of green and purple to dark purple stems with *S. scabrum* (improved type), *S. villosum* (Kakamega 2 and Kakamega 3) having green stems, *S. scabrum*(AVRDC-TZ SS49, BG 14, BG 22 and Nakuru County), *S. villosum* (Kakamega 1), having a mixture of green and purple while the remaining cultivars from African nightshade species *S. sarrachoides*, *S. americanum* and *S. nigrum* L, had purple stems (Figure 13; c, d and e).



A b

c d e

Figure 13: African nightshade cultivars exhibiting diversity in berry size and stem pigmentation; a- Big size purple berries. b- Small size purple berries. c- Green stems. d- Purple stem. e- Mixture of purple and green stems.

3.6.2 Quantitative traits

3.6.2.1 Analysis of variance

Analysis of variance carried out for quantitative traits of different African nightshade cultivars grown in Kisii during the long rains season showed that there was high significant difference at $p \leq 0.05$ between the cultivars in plant height, internode length, leaf length, leaf width, leaf size, number of primary branches and number of berries per panicle (Table 5). The highest coefficient of variation (CV %) was found for plant height (21.4%) followed by leaf length (20.8%) and leaf length (19.6%) while the lowest was 5.3% for number of berries per panicle. This means that there were less experimental errors in the counting of number of berries per panicle compared to the measurement of plant height, leaf length and leaf width (Table 5).

Table 5: Analysis of variance for Kisii in 2015 long rains season for quantitative traits of 19 African nightshade cultivars.

Traits	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
Residual	190.5	0.7992	5.250	1.057	0.1649	5.575	0.1453
Mean square	954.9	16.4651	69.492	28.028	0.6434	50.569	4.3807
V.R	5.01	20.60	13.24	26.51	3.90	9.07	30.16
Fpr.	<.001**	<.001**	<.001**	<.001**	<.001**	<.001**	<.001**
CV (%)	21.4	15.2	19.6	20.8	15.8	14.6	5.3

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) =Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level.
V.R. = variance ratio; Fpr. =F probability. CV=Coefficient of variation.

African nightshade cultivar AVRDC BG23 recorded the highest plant height of 92cm while AVRDC MW 13 recorded the lowest plant height of 23cm with most cultivars belonging to *S. scabrum* species having plant height of between 76.4 cm to 92cm (Table 5).There was significance difference in internode length at $p \leq 0.05$ with improved type recording the longest internode of 10.87cm while AVRDC BG14 had the shortest internode of 2.71cm (Table 6).

Table 6: Means for Kisii in 2015 long rains season for quantitative traits of 19 African nightshade cultivars

	Cultivars	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
S. scabrum	GBK genotype	76.4	5.26	17.11	8.41	2.1	13	7
S. scabrum	AVRDC BG14	27.7	2.71	9.31	3.00	3.1	16	6
S. sarrachoides	AVRDC MW13	23.0	1.81	7.42	2.55	2.5	16	5
S. americanum	GBK050282	63.3	8.11	8.45	2.48	3.2	22	7
S.americanum	OARA 1	68.5	4.67	5.55	2.15	2.6	23	6
S.americanum	GBK050292	47.1	3.28	8.75	3.03	3.0	12	6
S. scabrum	Improved type	80.2	10.87	19.29	12.13	1.6	11	9
S. americanum	AVRDC RC11	52.0	5.33	8.53	3.10	2.6	15	8
S. scabrum	AVRDC SS49	56.9	3.33	23.65	11.59	2.2	9	7
S. nigrum	GBK 050572	68.4	6.31	15.09	5.43	4.3	10	10
S. scabrum	AVRDC BG23	92.3	8.27	5.43	2.50	2.1	23	7
S. villosum	Kakamega 1	66.7	6.49	10.16	3.92	2.3	15	8
S. villosum	Kakamega2	79.0	6.64	12.97	5.33	2.5	14	9
S. villosum	Kakamega 3	64.5	5.09	11.93	5.44	2.3	18	8
S. scabrum	Nakuru county	85.2	7.81	16.88	8.61	2.0	20	8
S. scabrum	AVRDC BG22	72.0	5.87	11.40	4.15	2.6	14	6
S. sarrachoides	Kisii 1	59.3	3.56	8.92	2.87	3.0	17	7
S. sarrachoides	Kisii 2	63.4	8.23	9.16	3.13	3.0	18	7
S. nigrum	OARA 2	79.2	8.08	11.75	4.17	2.6	20	7
	Grand mean	64.5	5.88	11.67	4.95	2.6	16	7.2
	Lsd	22.86	1.48	3.79	1.70	0.67	3.9	0.63
	CV (%)	21.4	15.2	19.6	20.8	15.8	14.6	5.3

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle. Lsd= Least significant difference of means (5% level); CV=Coefficient of variation

The smaller the ratio of leaf length to leaf width, the bigger the leaf size while the bigger the ratio the smaller the leaf size. Improved type cultivar had the biggest leaves (1.6) followed by GBK genotype (2.1) while GBK 050572 had the smallest leaves (4.3). Cultivar AVRDC SS 49

produced the longest leaves of 23.65cm while OARA type 1 produces the shortest leaves of 5.55cm and improved type produced the widest leaves of 12.3cm whereas OARA type 1 produced the narrowest leaves of 2.15cm.There was no significant difference in leaf size at $p \leq 0.05$ between cultivars GBK genotype, AVRDC MW 13, AVRDC RC 11, AVRDC BG22, AVRDC BG23, OARA (1and 2) and Kakamega (I, 2 and 3) (Table 6).

GBK050282 produced the highest number of primary branches (22) followed by OARA type 2 (20) while AVRDC SS 49 had the least number of primary branches (9).There was no significance difference in the number of primary branches at $p \leq 0.05$ between GBK genotype, AVRDC BG14,GBK 050292, improved type, Kakamega (type1 and 2) and AVRDC BG 22. GBK 050572 produced the highest number of berries per panicle (10) while AVRDC MW 13 produced the least number of berries per panicle (5) (Table 6).

Analysis of variance carried out for quantitative traits of different African nightshade cultivars planted in Kisii in 2015 during the short rains season showed that there was high significant difference at $p \leq 0.05$ between the cultivars in plant height, internode length, leaf length, leaf width, leaf size, number of primary branches and number of berries per panicle (Table 7).

Table 7: Analysis of variance for Kisii in 2015 short rains season for quantitative traits of 19 African nightshade cultivars

Traits	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
Residual	89.98	0.4251	3.619	0.8309	0.006382	1.659	0.06578
Mean square	1336.03	14.5886	71.143	27.3358	0.699516	60.667	4.66096
V.R	14.85	34.32	19.66	32.90	109.61	36.57	70.85
Fpr.	<.001**	<.001**	<.001**	<.001**	<.001**	<.001**	<.001**
CV (%)	13.9	11.0	15.9	18.1	3.1	9.6	3.5

Key: PH (cm) =Plant height, IL=Internode length (cm), LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level.
V.R. = variance ratio; Fpr. =F probability. CV=Coefficient of variation

The highest coefficient of variation (CV %) was recorded for leaf width (18.1%) followed by leaf length (15.1%) while the lowest coefficient of variation was for leaf size (3.1%) indicating

that there was less experimental errors in the calculation of leaf size when compared to leaf width and leaf length (Table 7).

African nightshade cultivar AVRDC BG23 recorded the highest plant height of 120cm while AVRDC MW13 recorded the lowest plant height of 21cm (Table 8).

Table 8: Means for Kisii in 2015 short rains season for quantitative traits of 19 African nightshade cultivars

	Cultivars	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
S. scabrum	GBK genotype	78.1	5.44	17.32	8.66	2.04	10	7
S. scabrum	AVRDC BG14	26.6	2.82	9.46	3.00	3.19	10	6
S. sarrachoides	AVRDC MW13	21.0	1.71	7.12	2.55	2.80	10	5
S. americanum	GBK050282	64.9	8.16	8.52	2.48	3.45	19	7
S.americanum	OARA 1	68.0	4.50	5.70	2.15	2.58	22	6
S.americanum	GBK050292	80	5.88	10.62	3.56	2.98	20	7
S. scabrum	Improved type	85.4	10.80	18.94	11.70	1.62	8	9
S. americanum	AVRDC RC11	51.1	5.13	8.20	3.00	2.78	12	7
S. scabrum	AVRDC SS49	59.9	3.33	24.25	11.74	2.08	6	7
S. nigrum	GBK 050572	70.1	6.96	15.64	5.64	2.78	8	11
S. scabrum	AVRDC BG23	120	8.00	5.40	2.60	2.28	20	7
S. villosum	Kakamega 1	69.1	6.77	10.16	3.91	2.61	13	8
S. villosum	Kakamega 2	79.0	6.54	12.97	5.33	2.45	13	9
S. villosum	Kakamega 3	66.9	4.99	12.35	5.58	2.22	16	8
S. scabrum	Nakuru county	83.6	7.55	16.81	8.59	1.96	17	8
S. scabrum	AVRDC BG22	72.0	5.80	11.40	4.15	2.75	12	6
S. sarrachoides	Kisii 1	61.1	3.34	9.25	2.87	3.26	14	7
S. sarrachoides	Kisii 2	73.3	7.16	9.22	3.05	3.04	11	7
S. nigrum	OARA 2	63.7	7.82	13.45	5.02	2.72	12	7
	Grand mean	68.1	5.93	11.94	5.03	2.61	13	7
	Lsd	15.71	1.08	3.15	1.51	0.13	2.1	0.42
	CV (%)	13.9	11	15.9	18.1	3.1	9.6	3.5

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle.
Lsd= Least significant difference of means (5% level); CV=Coefficient of variation

Improved type cultivar had the longest internode of 10.80cm while AVRDC BG 14 had the shortest internode of 2.8 cm. African nightshade cultivar AVRDC SS 49 produced the longest leaves (24.11 cm) and widest leaves (11.7cm) while Kisii 2 produced the shortest leaves (2.22cm) followed by Kisii 1 (2.25 cm). Improved type cultivar had the biggest leaves of 1.62

followed by Nakuru County (1.96) while GBK 050282 had the smallest leaves (3.45). There was significant difference at $p \leq 0.05$ in leaf size between Kakamega 1 and 3 but there was no significance difference at $p \leq 0.05$ in leaf size between Kakamega 1 and 2 and between Kakamega 2 and 3. There was significance difference at $p \leq 0.05$ between Kisii 1 and Kisii 2 in leaf size (Table 8).

African nightshade cultivar OARA 1 recorded the highest number of primary branches (22) followed by GBK 050292 (20) and AVRDC BG 23(20) while AVRDC SS 49 had only 6 primary branches hence recording the lowest number of primary branches among the cultivars. GBK 050572 had the highest number of berries in a panicle (11) while OARA 2 had the lowest number of berries per panicle (6). There was no significant difference in number of berries per panicle among cultivars GBK 050292, AVRDC SS49, Kisii 1 and Kisii 2 (Table 8).

Combined analysis of variance for African nightshade cultivars grown in Kisii both in the first (2015 long rains) and the second (2015 short rains) season showed that there was high significant difference at $p \leq 0.05$ among cultivars in both the long and short rains seasons in plant height, internode length, leaf length, leaf width, leaf size, number of primary branches and number of berries per panicle. There was no significant difference at $p \leq 0.05$ between the long and short rains seasons in plant height, internode length, leaf length, leaf width, leaf size and number of berries per panicle with the only significant difference at $p \leq 0.05$ being recorded in the number of primary branches between long rains season (16) and in the short rains season (13) (Table 9; Table 10). This showed that cultivars performed better in the long rains season than in the short rains season in terms of number of primary branches produced. There was significant ($p \leq 0.05$) correlation in cultivar x season interaction in number of primary branches whereas there was no significant difference in cultivar x season interaction at $p \leq 0.05$ in plant height, internode length,

leaf length, leaf width, leaf size and number of berries per panicle (Table 9). This shows that the significant difference seen at ≤ 0.05 in the number of primary branches produced by the cultivars grown in Kisii in the long and short rains seasons was as a result of the different environmental conditions experienced within the two season with performance in season one being better than that of season two.

Table 9: Analysis of variance for combined data of the long and short rains season in Kisii for quantitative traits of 19 African nightshade cultivars

Traits	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
Residual	139.0	0.6014	4.433	0.9474	0.009184	3.695	0.1042
Mean square cultivar	2123.5	30.3249	140.100	55.2613	1.360915	95.186	8.8663
Mean square season	372.9	0.0789	2.002	0.2018	0.020011	221.680	0.0763
Mean square cultivar x season	167.5	0.7288	0.534	0.1020	0.006817	16.051	0.1753
V.R cultivar	15.28	50.43	31.60	58.33	148.18	25.76	85.13
V.R season	2.68	0.13	0.45	0.21	2.18	59.99	0.73
V.R cultivar x season	1.21	1.21	0.12	0.11	0.74	4.34	1.68
Fpr. Cultivar	<.001**	<.001**	<.001**	<.001**	<.001**	<.001**	<.001**
Fpr. Season	0.106	0.718	0.504	0.646	0.144	<.001**	0.395
Fpr. Cultivar x season	0.279	0.274	1.000	1.000	0.757	<.001**	0.062
CV (%)	17.8	13.1	17.8	19.5	3.7	13	4.5

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level.
V.R. = variance ratio; Fpr. =F probability. CV=Coefficient of variation

The highest coefficient of variation (CV %) was for leaf length (19.5%) followed by plant height (17.8%) and leaf length (17.8%) while the lowest was for leaf size 3.7% which means that experimental errors incurred in the calculation of leaf size were less than those incurred in the measurement of all the other traits (leaf width, leaf length, plant height, internode length, number of primary branches and number of berries per panicle) (Table 9).

Table 10: Means for combined data for short and long rains seasons in Kisii for quantitative traits of 19 African nightshade cultivars

	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
Season 1	64.5	5.9	11.7	4.9	2.6	16.2	7.2
Season 2	68.1	5.9	11.9	5.0	2.6	13.4	7.2

Lsd	4.40	0.29	0.79	0.36	0.04	0.72	0.12
CV (%)	17.8	13.1	17.8	19.5	3.7	13	4.5

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle.
Lsd= Least significant difference of means (5% level); CV=Coefficient of variation

In Kisii, AVRDC BG 23 recorded the highest plant height in both 2015 long and short rains (92cm and 120cm respectively) (Table 11).

Table 11: Means for combined data for 2015 long and short rains seasons in Kisii for quantitative traits of 19 African nightshade cultivars

Cultivars	PH(cm)		IL(cm)		LL(cm)		LW(cm)		LS		No PB		No BP	
Seasons	1	2	1	2	1	2	1	2	1	2	1	2	1	2
GBK genotype	76.4	78.1	5.3	5.4	17.3	17.3	8.4	8.7	2.0	2.0	12.7	9.6	6.9	6.9
AVRDC BG14	27.7	26.6	2.7	2.8	9.3	9.5	3.0	3.0	3.1	3.2	15.7	10.4	6.0	6.0
AVRDC MW13	23.0	21.0	1.8	1.7	7.4	7.1	2.5	2.5	2.9	2.8	16.3	10.4	5.0	5.0
GBK050282	63.3	64.9	8.1	8.2	8.4	8.5	2.5	2.5	3.4	3.5	22.3	19.4	6.8	6.8
OARA 1	68.5	68.0	4.7	4.5	5.6	5.7	2.1	2.1	2.6	2.6	21.7	21.5	6.0	6.0
GBK050292	47.1	60.0	3.3	5.9	8.8	10.6	3.0	3.6	2.9	3.0	12.3	20.0	6.1	7.0
Improved type	80.2	85.4	10.9	10.8	19.3	18.9	12.1	12.7	1.6	1.6	11.3	8.3	8.6	8.6
AVRDC RC11	52.0	51.1	5.3	5.1	8.5	8.2	3.1	3.0	2.8	2.8	15.3	12.3	8.1	7.2
AVRDC SS49	56.9	59.9	3.3	3.3	23.7	14.2	11.6	11.7	2.0	2.1	9.0	6.2	6.9	7.0
GBK 050572	68.4	70.1	6.3	7.0	15.1	15.6	5.4	5.6	2.8	2.8	10.0	7.6	10.2	10.8
AVRDC BG23	92.3	120.0	8.3	8.0	5.4	5.4	2.5	2.6	2.2	2.3	23.3	20.0	7.0	7.0
Kakamega 1	66.7	69.1	6.5	6.8	10.2	10.2	3.9	3.9	2.6	2.6	15.3	13.4	7.6	7.6
Kakamega2 2	79.0	79.0	6.6	6.5	13.0	13.0	5.3	5.3	2.4	2.4	14.3	13.2	8.8	8.8
Kakamega 3	64.5	66.9	5.1	5.0	11.9	12.3	5.4	5.6	2.2	2.2	18.0	15.5	8.0	8.0
Nakuru county	85.1	83.6	7.8	7.5	16.9	16.8	8.6	8.6	2.0	2.0	19.7	17.1	7.8	7.8
AVRDC BG22	72.0	72.0	5.9	5.8	11.4	11.4	4.2	4.2	2.7	2.7	14.3	12.5	6.0	6.0
Kisii1	59.3	61.1	3.6	3.3	8.9	9.3	2.9	2.9	3.1	3.3	17.0	14.3	7.0	7.0
Kisii2	63.4	73.3	8.2	7.2	9.2	9.2	3.1	3.1	2.9	3.0	18.3	10.5	6.9	7.0
OARA2	79.2	63.7	8.1	7.8	11.8	13.5	4.4	5.0	2.8	2.7	20.0	11.5	6.9	7.2
Grand mean	66.3		5.9		11.8		5.0		2.6		14.8		7.2	
Lsd	19.18		1.26		3.43		1.58		0.16		3.13		0.53	
CV	17.8		13.1		17.8		19.5		3.7		13		4.5	

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle. Lsd= Least significant difference of means (5% level); CV=Coefficient of variation

Cultivar AVRDC MW 13 recorded the lowest plant height of 23cm and 21 cm in long and short rains season respectively (Table 11). Improved type cultivar had the biggest internode length (10.9cm and 10.8 cm respectively) and the biggest leaves (1.6) in both long and short rains

season (Table 11). In 2015 long and short rains season, African nightshade cultivar GBK 050282 and OARA 1 produced the highest number of primary branches (22) while in the short rains season only OARA 1 produced the highest number of primary branches (22). In both the short and long rain season (2015) GBK 05572 produced the highest number of berries per panicle (10 and 11 respectively) with VRDC MW13 producing the least berries (5) (Table 11).

Analysis of variance for Muguga in 2015 long rains season showed that there was high significant difference at $p \leq 0.05$ in plant height, internode length, leaf length, leaf size, number of primary branches and number of berries per panicle (Table 12). The highest coefficient of variation (CV%) was found for internode length (54.2%) followed by that of leaf length (40.8%) while the lowest coefficient of variation was recorded for number of berries per panicle (7%) an indication that few experimental errors were done during the counting of number of berries per panicle as compared to in the measurement of the internode length and leaf length (Table 12).

Table 12: Analysis of variance for Muguga 2015 long rains season for quantitative traits of 19 African nightshade cultivars

Traits	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
Residual	164.7	16.25	6.256	5.472	0.2267	20.14	0.2609
Mean square	613.5	24.32	48.516	17.888	0.5266	55.09	2.8237
V.R	3.73	1.50	7.76	3.27	2.32	2.73	10.82
Fpr.	0.004**	0.200	<.001**	0.008	0.041*	0.020*	<.001**
CV (%)	20.3	54.2	20.2	40.8	19.8	28.2	7.0

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level.
V.R. = variance ratio; Fpr. =F probability. CV=Coefficient of variation

AVRDC BG 23 had the highest plant height of 100cm long while AVRDC MW 13 had the lowest plant height of 23cm.There was no significant difference in plant height at $p \leq 0.05$ among African nightshade cultivars Kakamega (1, 2 and 3), Kisii (1 and2), OARA (1 and 2), Nakuru county, GBK 050282, AVRDC BG 22, GBK genotype, GBK 050572 and GBK 050292 African nightshade cultivar Kakamega type 3 had the longest internode of 15.36 cm long followed by

GBK 050292 (12.44cm), improved type (11.47 cm) then Kakamega type 1 (11.17cm) whereas AVRDC MW had the shortest internode of 1.86 cm (Table 13).

Table 13: Means for Muguga 2015 long rains season for quantitative traits of 19 African nightshade cultivars

Species	Cultivars	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
S. scabrum	GBK genotype	56.35	7.97	12.22	7.96	1.78	17	7
S. scabrum	AVRDC BG14	28.31	3.11	10.23	3.10	3.31	11	6
S. sarrachoides	AVRDC MW13	23.01	1.86	7.31	2.77	2.65	11	6
S. americanum	GBK050282	67.45	8.58	8.76	2.74	3.23	20	7
S.americanum	OARA 1	71.00	5.25	6.65	2.57	2.51	30	7
S.americanum	GBK050292	75.08	12.44	10.92	7.74	1.97	22	7
S. scabrum	Improved type	65.64	11.47	16.68	10.20	1.65	13	8
S. americanum	AVRDC RC11	53.00	5.55	7.40	3.20	2.35	14	8
S. scabrum	AVRDC SS49	54.49	3.92	25.43	12.87	1.98	13	6
S. nigrum	GBK 050572	69.15	6.98	15.82	5.22	3.05	7	11
S. scabrum	AVRDC BG23	100	8.50	5.60	2.80	2.11	19	7
S. villosum	Kakamega 1	70.64	11.17	10.37	7.34	1.86	19	7
S. villosum	Kakamega 2	54.15	15.36	13.74	5.93	2.38	18	9
S. villosum	Kakamega 3	79.79	8.07	15.55	5.83	2.00	13	8
S. scabrum	Nakuru county	79.85	7.62	18.05	9.34	1.97	20	8
S. scabrum	AVRDC BG22	69.50	5.90	11.50	3.82	3.03	13	6
S. sarrachoides	Kisii 1	68.80	4.33	14.76	7.57	2.39	18	8
S. sarrachoides	Kisii 2	56.87	3.73	8.07	2.81	2.89	13	7
S. nigrum	OARA 2	54.40	8.55	16.09	5.97	2.64	10	8
Grand mean		63.18	7.93	12.38	5.78	2.41	15.94	7
Lsd		26.96	8.45	5.56	4.91	1.00	9.43	1.07
CV (%)		20.3	54.2	20.2	40.8	19.8	28.2	7.0

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle.
Lsd= Least significant difference of means (5% level); CV=Coefficient of variation

AVRDC MW cultivar had the widest leaf size of 12.87cm while OARA 1 had the narrowest leaves of 2.57 cm and AVRDC SS49 had the longest leaves of 25.43cm whereas AVRDC BG 23 had the shortest leaves of 5.6cm. African nightshade cultivar improved type had the biggest leaves (1.65) while AVRDC BG 14 had the smallest leaves (3.31) (Table 13).

Cultivar OARA 1 had the highest number of primary branches (30) whereas GBK 050572 had the lowest number of primary branches (7). There was no significant difference at $p \leq 0.05$ in

number of primary branches among cultivars AVRDC BG14, improved type, AVRDC RC11, AVRDC SS49, AVRDC MW13, GBK Genotype, GBK050282, AVRDC BG23, Kakamega (1,2 and 3), Nakuru county, AVRDC BG22 and Kisii(1 and 2). Only cultivar GBK 050572 produced 11 berries per panicle while the rest produced between 9 and 6 berries per panicle (Table 13).

Analysis of variance for African nightshade cultivars grown in Muguga in 2015 during short rains season showed that there was high significant difference at $p \leq 0.05$ among cultivars in plant height, internode length, leaf length, leaf width, leaf size, number of primary branches and in the number of berries per panicle (Table 14).

Table 14: Analysis of variance for Muguga 2015 short rains season for quantitative traits of 19 African nightshade cultivars

Traits	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
Residual	4.884	1.527	1.298	1.313	0.1649	3.189	0.2374
Mean square	1576.688	21.941	111.193	34.714	0.6434	114.656	4.7877
V.R	322.84	14.37	85.68	26.43	3.90	35.95	20.16
Fpr.	<.001**	<.001**	<.001**	<.001**	<.001**	<.001**	<.001**
CV (%)	0.3	17.5	8.5	20.2	15.8	10.6	6.4

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level.
V.R. = variance ratio; Fpr. =F probability. CV=Coefficient of variation.

The highest coefficient of variation (CV%) was found for leaf width (20.2%) followed by internode length (17.5%) and the lowest coefficient of variation was for plant height (0.3%) which means that less experimental errors were encountered in the measurement of plant height compared to the measurement of leaf width and internode length (Table 14).

African nightshade cultivar AVRDC BG23 had the highest plant height of 122.7 cm followed by improved type which had a plant height of 106.4 cm while AVRDC MW 13 had the lowest plant height of 26.5 cm (Table 15).

Table 15: Means for Muguga 2015 short rains season for quantitative traits of 19 African nightshade cultivars

Species	Cultivars	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
S. scabrum	GBK genotype	65.4	6.0	19.1	9.0	2.1	16	7
S. scabrum	AVRDC BG10	29.1	2.6	10.4	3.4	3.1	11	6
S. sarrachoides	AVRDC MW13	26.5	2.4	7.8	3.1	2.5	12	5
S. americanum	GBK050282	68.4	8.7	9.1	2.8	3.2	22	7
S.americanum	OARA 1	72.6	5.2	6.9	2.7	2.6	36	6
S.americanum	GBK050292	83.2	7.4	11.8	3.6	3.0	23	7
S. scabrum	Improved type	106.4	12.5	20.2	12.7	1.6	10	9
S. americanum	AVRDC RC11	54.6	5.6	8.7	3.4	2.6	14	8
S. scabrum	AVRDC SS49	69.2	4.7	31.7	14.5	2.2	20	7
S. nigrum	GBK 050572	74.9	9.7	16.4	5.0	3.4	8	11
S. scabrum	AVRDC BG23	122.7	9.4	5.8	2.8	2.1	21	7
S. villosum	Kakamega 1	71.8	7.7	10.6	4.2	2.3	17	8
S. villosum	Kakamega 2	82.7	8.2	14.1	5.9	2.5	15	9
S. villosum	Kakamega 3	72.3	5.5	14.1	6.0	2.3	13	9
S. scabrum	Nakuru county	87.9	7.3	18.8	9.3	2.0	21	8
S. scabrum	AVRDC BG22	93.2	6.2	11.7	4.4	2.6	14	7
S. sarrachoides	Kisii 1	65.5	4.6	12.4	5.5	3.0	16	8
S. sarrachoides	Kisii 2	76.7	11.1	10.3	3.4	3.0	18	7
S. nigrum	OARA 2	95.4	9.5	16.2	6.3	2.6	13	8
Grand mean		74.7	7.1	13.5	5.7	2.6	16.8	7.6
Lsd		3.66	2.05	1.89	1.90	0.67	2.96	0.81
CV (%)		0.3	17.5	8.5	20.2	15.8	10.6	6.4

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle.
Lsd= Least significant difference of means (5% level); CV=Coefficient of variation

There was no significant difference at $p \leq 0.05$ between Kakamega 1 and 3 in terms of plant height whereas there was significant difference at $p \leq 0.05$ between Kakamega 1 and 2 and between Kakamega 2 and 3 in plant height. There was also significant difference at $p \leq 0.05$ between Kisii 1 and Kisii 2. Improved type had the longest internode (12.5 cm) followed by Kisii 2 (11.1cm) whereas AVRDC BG 10 had the shortest internode of 2.6cm (Table 15).

There was no significant difference at $p \leq 0.05$ in internode length among cultivars Kakamega (1 and 2), GBK 050282, GBK 050572, AVRDC BG 23 and OARA 2(Table 11). Cultivar AVRDC

SS49 had the longest leaves of 31.7cm while AVRDC GB 23 had the shortest leaves of 5.8cm. There was significant difference at $p \leq 0.05$ between Kakamega 2 and Kakamega (Table 15).

African nightshade cultivar AVRDC SS 49 had the widest leaves 14.5 cm followed by improved type which had leaf width of 12.7cm and OARA 1 had the narrowest leaves of 2.7 cm. There was no significance difference at $p \leq 0.05$ in leaf width among cultivars AVRDC BG 10, AVRDC MW 13, GBK 050282, OARA 1, GBK 050292, AVRDC RC11, AVRDC BG 23 and Kisii 2. Cultivar improved type had the biggest leaf size of 1.6 while GBK 050572 had the smallest leaf size of 3.4 and there was no significance difference at $p \leq 0.05$ among cultivars GBK genotype, AVRDC MW 13, OARA (1 and 2), Kakamega (1, 2 and 3), AVRDC RC11, AVRDC SS49, AVRDC GB23, Nakuru county and AVRDC BG 22 (Table 13). Cultivar OARA 1 had produced the highest number of primary branches (36) whereas GBK 050572 produced the lowest number of primary branches. There was no significance difference in number of primary branches between AVRDC BG 10, AVRDC MW 13, Kakamega 3 and OARA. GBK 05072 cultivar produced the highest number of berries per panicle (11) however, there was significant difference at $p \leq 0.05$ in number of berries per panicle produced among cultivar GBK genotype, GBK 050282, GBK 050292, AVRDC SS 49, AVRDC BG 23, AVRDC BG22 and Kisii 2. There was also significant difference at $p \leq 0.05$ in number of berries per panicle produced among cultivar AVRDC RC11, Kakamega 1, Nakuru County, Kisii 1 and OARA 2 (Table 15).

Combined analysis of variance for African nightshade cultivars grown in Muguga both in the long and short rains seasons showed that there was high significant difference at $p \leq 0.05$ among cultivars in both seasons in plant height, internode length, leaf length, leaf width, leaf size, number of primary branches and number of berries per panicle. There was significant difference at $p \leq 0.05$ between the long and short rains season in plant height, leaf length and number of

berries per panicle which means that the difference seen between the two seasons was purely as a result of the different environmental conditions experience between the seasons. There was no significant difference at $p \leq 0.05$ between the long and short rains seasons in internode length, leaf width, leaf size and in the number of primary branches (Table 16). African nightshade cultivars produced taller plants in the long rains season (74.5cm) as compared to the short rains season, the plants also had longer leaves in the long rains season (13.4cm) than in the short rains season (12.4cm). They produced more number of berries per panicle in the long rains season (8) than in short rains season (7) (Table 17). There was also significant difference at $p \leq 0.05$ in cultivar x season interaction in plant height which means that the different in plant height observed between the long and short rains season in Muguga was as a result of the contribution of both the genotype and the environmental condition experience between the two seasons. However, there was no significance at $p \leq 0.05$ difference in cultivar x season interaction in internode length, leaf length, leaf width, leaf size, number of primary branches and in the number of berries per panicle.

The highest coefficient of variation (CV %) found was for leaf width (29.9%) followed by number of primary branches (23.3%) while the lowest coefficient of variation was for number of primary berries per panicle (6.9%). There were less experimental errors in the counting of number of berries per panicle than in the measurement of leaf length and in the counting of number of primary branches (Table 16).

African nightshade cultivar AVRDC BG23 had the highest plant height of 100cm and 123.5 cm in both the long and short rains season respectively in Muguga while AVRDC MW 13 had the lowest plant height of 23cm and 27.2cm in long and short rains season respectively with all cultivars performing better (in long rains season all cultivars recorded higher plant height

compared to short rains season) in long rains season than in short rains season in terms of plant height (Table 17).

Table 16: Analysis of variance for combined data of the long and short rains seasons (Muguga) for quantitative traits of 19 African nightshade cultivar

Traits	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
Residual	84.97	9.178	3.413	2.899	0.1724	14.36	0.2662
Mean square cultivar	1457.26	30.802	115.616	38.974	0.8643	124.11	5.8353
Mean square season	2430.33	4.133	18.266	0.582	0.4819	9.96	1.2884
Mean square cultivar x season	193.43	10.041	5.913	3.426	0.1672	7.32	0.1518
V.R cultivar	17.15	3.36	33.88	13.44	5.01	8.64	21.92
V.R season	28.60	0.45	5.35	0.20	2.80	0.69	4.84
V.R cultivar x season	2.28	1.09	1.73	1.18	0.97	0.51	0.57
Fpr. Cultivar	<.001**	<.001	<.001**	<.001**	<.001**	<.001**	<.001**
Fpr. Season	<.001**	0.506	0.026*	0.657	0.103	0.410	0.034*
Fpr. Cultivar x season	0.017*	0.395	0.078	0.324	0.511	0.936	0.898
CV (%)	13.4	42.3	14.4	29.9	16.3	23.3	6.9

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL= (cm) Leaf length, LW= Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level.
V.R. = variance ratio; Fpr. =F probability. CV=Coefficient of variation

Table 17: Means for combined data of the long and short rains seasons (Muguga) for quantitative traits of 19 African nightshade cultivars

	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
Long rains season	74.5	6.9	13.4	5.6	2.6	16.7	7.6
Short rains season 2	63.2	7.4	12.4	5.8	2.4	15.9	7.3
Lsd	4.28	1.41	0.86	0.78	0.19	1.76	0.24
CV (%)	13.4	14.3	14.4	29.9	16.3	23.3	6.9

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL=Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle.
Lsd= Least significant difference of means (5% level); CV=Coefficient of variation

In the long rains season Kakamega 2 recorded the highest internode length (15.4cm) while improved type recorded the highest internode length (12.7cm) in season two, cultivars from Kakamega (Kakamega 1, 2 and 3) had longer internodes in season one than in season two while cultivars from Kisii (Kisii 1 and 2) had longer internode in season two than in short rains season (Table 18).

Table 18: Means for combined data for the long and short rains seasons (Muguga) for quantitative traits of 19 African nightshade cultivars

Cultivars	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP

Seasons	1	2	1	2	1	2	1	2	1	2	1	2	1	2
GBK genotype	59.3	63.0	8.0	3.5	12.2	19.0	8.0	9.1	1.8	2.1	17	13	7	7
AVRDC BG10	28.3	28.7	3.1	2.4	10.2	10.6	3.1	3.4	3.3	3.1	11	12	6	6
AVRDC MW13	23.0	27.2	1.9	2.6	7.3	7.9	2.8	3.2	2.6	2.5	11	12	6	5
GBK050282	67.4	69.1	8.6	8.8	8.8	9.0	2.7	2.9	3.2	3.1	12	22	7	7
OARA 1	71.0	73.6	5.2	5.2	6.6	6.8	2.6	2.7	2.5	2.5	30	36	7	6
GBK050292	75.1	82.7	12.4	7.3	10.9	11.7	7.7	3.6	2.0	2.8	22	23	7	7
Improved type	65.6	104.1	11.5	12.7	16.9	20.5	10.2	12.6	1.6	1.6	13	10	8	9
AVRDC RC11	53.0	54.5	5.6	5.8	7.4	8.6	3.2	3.3	2.4	2.6	14	14	8	8
AVRDC SS49	54.5	69.3	3.6	4.1	25.4	31.7	12.9	14.6	2.0	2.1	13	19	6	7
GBK 050572	69.1	76.0	7.0	9.5	15.8	16.2	5.2	5.4	3.1	3.0	7	8	11	11
AVRDC BG23	100.0	123.5	8.5	9.5	5.6	5.8	2.8	2.7	2.1	2.1	19	21	7	7
Kakamega 1	70.6	71.5	11.2	7.6	10.4	10.7	7.3	4.2	1.9	2.4	19	17	7	8
Kakamega2	54.1	82.8	15.4	8.0	13.7	14.3	5.9	6.3	2.4	2.3	18	16	9	9
Kakamega 3	79.8	72.2	8.1	5.5	15.6	14.2	5.8	5.9	2.0	2.4	13	14	8	9
Nakuru county	79.8	87.1	7.6	7.1	18.0	19.2	9.3	9.6	2.0	2.0	20	21	8	8
AVRDC BG22	69.5	92.3	5.9	6.2	11.5	11.5	3.8	4.4	3.0	2.6	14	14	6	7
Kisii1	68.8	65.7	4.3	4.7	14.8	10.0	7.6	2.7	2.4	3.8	18	16	8	8
Kisii2	56.9	76.7	3.7	11.2	8.1	10.3	2.8	3.4	2.9	3.0	13	18	7	7
OARA2	54.4	95.1	8.6	9.7	16.1	16.2	6.0	6.2	2.6	2.6	10	13	7	8
Grand mean	68.8		7.2		12.9		5.7		2.5		16.3		7.5	
Lsd	18.68		6.14		3.74		3.45		0.84		7.68		1.05	
CV	13.4		14.3		14.4		29.9		16.3		23.5		6.9	

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle.
Lsd= Least significant difference of means (5% level); CV=Coefficient of variation

In both the short and long rains season, AVRDC SS49 recorded the highest leaf length of 25.4 cm and 31.7 cm respectively while AVRDC BG 23 recorded the lowest leaf length of 5.6cm and 5.8cm respectively. All African nightshade cultivars had longer leaves the short rains season when compared to long rains season. AVRDC SS49 had the widest leaves of 12.9cm and 14.6cm in both season long and short rains seasons respectively while improved type had the biggest leaf size (1.6) in both long and short rains seasons. Cultivar OARA type 1 produced the highest number of primary branches (30 and 36) in both season one and two respectively (Table 18).

Combined analysis of variance for African nightshade cultivars grown in both locations (Kisii and Muguga) showed that there was high significant difference at ≤ 0.05 among cultivars in both locations in plant height, internode length, leaf length, leaf width, leaf size, number of

primary branches and number of berries per panicle. There was significance difference at $p \leq 0.05$ between the two locations (Kisii and Muguga) in plant height, internode length, leaf length, leaf width, number of primary branches and number of berries per panicle. There was no significant difference at $p \leq 0.05$ between locations in leaf size (Table 19).

Table 19: Analysis of variance for combined data of the 2 locations (Kisii and Muguga) for quantitative traits of 19 African nightshade cultivars

Traits	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
Residual	9.079	0.8320	0.9304	0.7191	0.08860	1.955	0.1324
Mean square cultivar	2945.749	37.0466	195.0729	65.2753	1.31429	151.472	9.3578
Mean square location	629.591	29.1809	53.8134	8.8533	0.02004	226.419	3.0854
Mean square cultivar x location	42.941	0.7999	1.6580	0.5844	0.06285	14.819	0.1403
V.R cultivar	324.46	44.53	209.67	90.77	14.83	77.48	70.70
V.R Location	69.35	35.07	57.84	12.31	0.23	115.82	23.31
V.R cultivar x season	4.73	0.96	1.78	0.81	0.71	7.58	1.06
Fpr. Cultivar	<.001**	<.001**	<.001**	<.001**	<.001**	<.001**	<.001**
Fpr. Location	<.001**	<.001**	<.001**	<.001**	0.636	<.001**	<.001**
Fpr. Cultivar x location	<.001**	0.512	0.044*	0.680	0.791	<.001**	0.408
CV (%)	4.2	13.9	7.5	15.7	11.5	9.1	4.9

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level.
V.R. = variance ratio; Fpr. =F probability. CV=Coefficient of variation

There was significant difference at $p \leq 0.05$ in interaction between cultivar and location in plant height, leaf length and in number of primary branches. There was no significant difference at $p \leq 0.05$ in cultivar x location interaction in internode length, leaf width, leaf size and in number of berries per panicle (Table 19).The highest coefficient of variation (CV%) was recorded in leaf width (15.7%) followed by leaf size (11.5%) while the lowest coefficient of variation was for number of berries per panicle (4.9%) which means that less experimental errors were encountered in the counting of number of berries per panicle compared to in the measurement of leaf length and in the calculation of leaf size (Table 19).

All African nightshade cultivars recorded more plant height in Muguga (74.66cm) that in Kisii (69.96cm) and this maybe be an indication that the significant difference at $p \leq 0.05$ in the

environmental conditions experienced between Kisii and Muguga were responsible for the difference in plant height observed among the African nightshade cultivars grown in the two location (Table 20).

Table 20: Means for combined data of the 2 location (Kisii and Muguga) for quantitative traits of 19 African nightshade cultivars

Location	PH(cm)	IL(cm)	LL(cm)	LW(cm)	LS	No PB	No BP
Kisii	69.96	6.05	12.10	5.12	2.59	14.02	7.28
Muguga	74.66	7.07	13.47	5.68	2.57	16.84	7.61
Lsd	1.13	0.34	0.36	0.32	0.11	0.52	0.14
CV (%)	42.	13.9	7.5	15.7	11.5	9.1	4.9

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle. Lsd= Least significant difference of means (5% level); CV=Coefficient of variation

African nightshade cultivar AVRDC SS 49 produced the longest and widest leaves in both Kisii and Muguga whereas OARA 1 produced the most number of primary branches in both Kisii and Muguga with most branches being produced from Muguga (35) than from Kisii (22) African nightshade cultivar AVRDC BG 23 had the highest plant height in both Kisii and Muguga but it was longer in Muguga (122.7cm) than in Kisii (117.7 cm) while improved type was the second highest in plant height and just like AVRDC BG 23, it performed better in Muguga (106cm) as compared to Kisii (93cm) (Table 21).

Table 21: Means for combined data of the 2 locations (Kisii and Muguga) for quantitative traits of 19 African nightshade cultivars

Cultivars	PH(cm)		IL(cm)		No PB		LS		LL(cm)		LW(cm)		No BP	
Location	Kis	Mu	Kis	Mu	Kis	Mu	Kis	Mu	Kis	Mu	Kis	Mu	Kis	Mu
GBK Genotype	78.1	65.4	5.4	6.0	11	16	2.0	2.1	17.3	19.1	8.7	9.0	7	7
AVRDC BG 14	26.3	29.1	2.8	2.6	10	11	3.2	3.1	9.5	10.4	3.0	3.4	6	6
AVRDC MW 3	21.3	26.5	1.7	2.4	11	11	2.8	2.5	7.1	7.8	2.6	3.1	5	5
GBK 050287	64.9	68.4	8.2	8.7	19	22	3.4	3.2	8.5	9.1	2.5	2.2	7	7
OARA 1	68.0	72.6	4.5	5.2	22	35	2.7	2.6	5.7	6.9	2.1	2.7	6	6
GBK 050292	80.1	83.2	5.9	7.4	21	23	3.1	3.0	10.6	11.8	3.4	3.6	7	7
Improved type	93.6	106.4	10.7	12.5	8	10	1.6	1.6	18.9	20.2	11.7	12.7	9	9
AVRDC RC 11	51.1	54.6	5.1	5.6	12	14	2.7	2.6	8.2	8.7	3.0	3.4	7	8
AVRDC SS49	66.9	69.2	3.3	4.7	15	19	2.1	2.2	27.3	31.7	13.2	14.5	7	7
GBK 050572	69.1	74.9	7.3	9.7	8	8	2.8	3.4	15.6	16.4	5.6	5.0	11	11

AVRDC BG23	117.7	122.7	8.0	9.4	20	21	2.2	2.1	5.3	5.8	2.5	2.8	7	7
Kakamega 1	69.1	71.8	6.8	7.7	13	17	2.6	2.3	10.2	10.6	3.9	4.2	8	8
Kakamega 2	79.0	82.7	6.5	8.2	13	15	2.4	2.5	13	14.1	5.3	5.9	9	9
Kakamega 3	66.9	72.3	5.0	5.5	16	13	2.2	2.4	12.4	14.1	5.6	6.0	8	9
Nakuru county	82.2	87.9	7.6	7.3	17	21	2	2.0	16.8	18.8	8.6	9.3	8	8
AVRDC BC22	80.0	93.2	5.8	6.2	13	14	2.8	2.6	11.4	11.7	4.1	4.4	6	7
Kisii 1	61.1	65.5	3.3	4.6	14	16	3.2	3.0	9.3	12.4	2.9	5.5	7	8
Kisii 2	65.0	76.7	8.9	11.1	14	17	3.0	3.0	9.2	10.3	3.1	3.4	14	14
OARA 2	88.8	95.4	8.2	9.5	10	12	2.4	2.6	13.6	16.2	5.7	6.3	8	8
Grand mean	72.31		6.56		15		2.58		12.79		5.40		7.00	
Lsd	4.90		1.48		2.28		0.48		1.57		1.38		0.59	
CV%	4.20		13.90		9.10		11.50		7.50		15.70		4.90	

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle.
Kis=Kisii, Mu=Muguga
Lsd= Least significant difference of means (5% level); CV=Coefficient of variation

African nightshade cultivars grown in Muguga also produced plants with longer internodes (7.07cm) than those grown in Kisii (6.05cm) (Table 20). African nightshade grown in Muguga had longer and wider leaves than those grown in Kisii). The leaf size of all African nightshade cultivars did not change significantly across the two locations (Kisii and Muguga). There was significant variation observed in the number of berries per panicle produced in Kisii (7) and those produced in Muguga (8) (Table 20).

3.6.2.2 Correlations among quantitative traits of 19 African nightshade.

When quantitative traits of African nightshade cultivars grown in Kisii during the long rains season were correlated, there was a high positive significant ($p \leq 0.05$) correlation between plant height and internode length (r = 0.7026**) as well as between plant height and number of berries per panicle (r = 0.4145**). Number of berries per panicle and leaf length, leaf width and number of berries per panicle(r = 04116** and r = 03852** respectively) also had high significant ($p \leq 0.05$) positive correlation. There was a significant ($p \leq 0.05$) positive correlation between leaf width and plant height and between leaf width and internode length (r= 0.3011*) and (r=

0.2368*) respectively. There was highly negative significant ($p \leq 0.05$) correlation between number of berries per panicle and internode length (r -0.5211**) and between plant height and leaf size (r = -0.4303**) (Table 22). There was also a high negative significant correlation between leaf size and leaf length (r = -0.6180**) as well as between leaf size and leaf width (r = -0.7873**). Number of primary branches had a significant ($p \leq 0.05$) negative correlation with leaf length (r= -0.5532**) and leaf width (– 0.4913**) (Table 22).

Table 22: Phenotypic correlations between various traits scored in 19 African nightshade cultivars planted in Kisii during the 2015 long rains season

	PH(cm)	IL(cm)	No PB	LL(cm)	LW(cm)	LS	No BP
PH(cm)	1.0000						
IL(cm)	0.7026**	1.0000					
No PB	0.1617	0.2368	1.0000				
LL(cm)	0.2543	0.1778	-0.5532**	1.0000			
LW(cm)	0.3011*	0.2687*	-0.4913**	0.9509**	1.0000		
LS	-0.4303**	-0.3213*	0.2490	-0.6180**	-0.7873**	1.0000	
No BP	0.4145**	0.5211**	-0.2509	0.4116**	0.3852**	-0.3205*	1.0000

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level

When quantitative traits of African nightshade cultivars grown in Kisii during the short rains season (2015) were correlated, there was a high positive significant ($p \leq 0.05$) correlation between plant height and internode length (r= 02819**) as well as between plant height and number of primary branches (r = 0.2609**). There was also negative significant ($p \leq 0.05$) correlation between internode length and number of primary branches (r=-0.2707**) and between leaf width and internode length (r=-0.3659**). Negative significant ($p \leq 0.05$) correlation was also recorded between number of berries per panicle and internode length (r= 0.3844**), leaf width and leaf length (r= 0.8812**), leaf length and number of berries per panicle (r= 0.4383) and between leaf width and number of berries per panicle (r= .0.3421) (Table 23). Similarly, there was high

significant ($p \leq 0.05$) negative correlation between leaf size and plant height (r= -0.3041**), internode length and plant leaf size (r= -0.2551**) as well as between number of primary branches and leaf length (r = -0.3755**). Leaf length and leaf size (r= -0.3894**), leaf width and leaf size (r= -.6529**) had negative significant ($p \leq 0.05$) correlation (Table 23).

Table 23: Phenotypic correlations between various traits scored in 19 African nightshade cultivars planted in Kisii during the 2015 short rains season

	PH(cm)	IL(cm)	No PB	LL(cm)	LW(cm)	LS	No BP
PH(cm)	1.0000						
IL(cm)	0.2819**	1.0000					
No PB	0.2609**	0.2707**	1.0000				
LL(cm)	0.1587	0.1962*	-0.3755**	1.0000			
LW(cm)	0.2153*	0.3659**	-0.1600	0.8812**	1.0000		
LS	-0.3041**	-0.2551**	-0.0545	-0.3894**	-0.6529**	1.0000	
No BP	0.3209**	0.3844**	-0.2094*	0.4383**	0.3421**	-0.2179*	1.0000

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level

Combined correlation studies carried out for quantitative traits of African nightshade cultivars grown in Kisii during both the long and short rains seasons in 2015 showed that there was high significant ($p \leq 0.05$) positive correlation between plant height and internode length (r= 0.6740**) as well as between plant height and number of primary branches (r=-0.2493**). There was also a significant ($p \leq 0.05$) positive correlation between leaf width and plant height (0.2537**) and between number of berries per panicle and plant height (r = 0.3796**). Internode length had a positive significant ($p \leq 0.05$) correlation with leaf width (r= 0.2710**) while leaf length had a positive significant correlation with leaf width (r= 0.9499**). There was a high significant ($p \leq 0.05$) negative correlation between number of primary branches and leaf length (r= -0.55516**), leaf width and number of primary branches (r= -0.4845**) as well as between number of primary branches and number of berries per panicle (r= -0.2508**) (Table 24).

Table 24: Phenotypic correlations between various traits scored in 19 African nightshade cultivars planted in Kisii during the 2015 long and short rains seasons combined

	PH(cm)	IL(cm)	No PB	LL(cm)	LW(cm)	LS	No BP
PH(cm)	1.0000						
IL(cm)	0.6740**	1.0000					
No PB	0.2493**	0.1708	1.0000				
LL(cm)	0.1811	0.1821	-0.5516**	1.0000			
LW(cm)	0.2537**	0.2710**	-0.4845**	0.9499**	1.0000		
LS	-0.0012	-0.1140	0.1412	-0.0342	-0.0872	1.0000	
No BP	0.3796**	0.5285**	-0.2508**	0.4256**	0.3881**	-0.0804	1.0000

Key: PH=Plant height, IL=Internode length, LL=Leaf length, LW= Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level.

Correlation results for quantitative traits of African nightshade cultivars grown in Muguga during the long rains season in 2015 showed that there was a positive high significant ($p \leq 0.05$) correlation between leaf length and leaf width (0.8004**) as well as between internode length and leaf width (r= 0.3722*). There was also a significant ($p \leq 0.05$) positive correlation between leaf length and number of berries per panicle (r=0.3451*). There was a high significant ($p \leq 0.05$) negative correlation between internode length and leaf size (r= -0.4932**) and between leaf width and leaf size (r= -0.7414**). Number of primary branches had a significant ($p \leq 0.05$) negative correlation with leaf size (r= - 0.3270*) whereas leaf width had a significant negative correlation with leaf size (r= -0.3218*) (Table 25).

Table 25: Phenotypic correlations between various traits scored in 19 African nightshade cultivars planted in Muguga during the 2015 long rains season

	PH(cm)	IL(cm)	No PB	LL(cm)	LW(cm)	LS	No BP
PH(cm)	1.0000						
IL(cm)	0.1807	1.0000					
No PB	0.2648	0.3144	1.0000				
LL(cm)	0.0725	0.1206	-0.2529	1.0000			
LW(cm)	0.1226	0.3722*	0.0726	0.8004**	1.0000		

LS	-0.2547	-0.4932**	-0.3270*	-0.3218*	-0.7414**	1.0000	
No BP	0.2767	0.2796	-0.1822	0.3451*	0.2304	-0.0899	1.0000

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level

Correlation studies of quantitative traits of African nightshade cultivars grown in Muguga during the short rains season in 2015 showed a high positive significant ($p \leq 0.05$) correlation between plant height and internode length (r= - 0.7172**) as well as between number of berries per panicle and plant height (r= -0.4309**). Internode length and number of berries per panicle also recorded a high significant ($p \leq 0.05$) positive correlation (r= 0.5833**) as well as leaf length and leaf width (r= 0.9276**). There was a high significant ($p \leq 0.05$) negative correlation between number of primary branches and number of berries per panicle (r=-0.3557**) and between leaf length and leaf size (r= -0.3421**). There was also a high significant negative correlation between leaf width and leaf size (r= -0.6111**). There was significant ($p \leq 0.05$) negative correlation plant height and leaf size (r= -0.3136*) (Table 26).

Table 26: Phenotypic correlations between various traits scored in 19 African nightshade cultivars planted in Muguga in 2015 short rains season

	PH(cm)	IL(cm)	No PB	LL(cm)	LW(cm)	LS	No BP
PH(cm)	1.0000						
IL(cm)	0.7172**	1.0000					
No PB	0.1750	-0.0225	1.0000				
LL(cm)	0.1347	0.0918	-0.2095	1.0000			
LW(cm)	0.2197	0.1263	-0.1853	0.9276**	1.0000		
LS	-0.3136*	-0.0821	-0.0115	-0.3421**	-0.6111**	1.0000	
No BP	0.4309**	0.5833**	-0.3557**	0.2417	0.1746	0.0647	1.0000

Key: PH=Plant height, IL=Internode length, LL=Leaf length, LW= Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level

Combined correlation study carried between different quantitative traits of African nightshade cultivars grown in Muguga during both the long and short rains seasons in 2015 indicated that there was high significant ($p \leq 0.05$) positive correlation between plant height and internode length (r= 0.3652**) as well as between plant height and number of berries per panicle (r= 0.3754**). Internode length and number of berries per panicle had a high significant positive correlation (r= 0.3839**) and also plant height and number of primary branches (r= 0.2385*). There was a significant ($p \leq 0.05$) positive correlation between internode length and leaf width (r= 0.2590*) as well as between leaf length and number of berries per panicle (0.2812*). There was a high significant negative correlation between internode length and leaf size (r= -0.3768) and between leaf length and leaf size (r= -0.3383**). There was a significant ($p \leq 0.05$) negative correlation between plant height and leaf size (r = -0.2513*), number of primary branches and leaf length (r= -0.2443*) and also between number of berries per panicle and number of primary branches (r= -0.2548*) (Table 27).

Table 27: Phenotypic correlations between various traits scored in 19 African nightshade cultivars planted in Muguga during both the 2015 long and short rains seasons

	PH(cm)	IL(cm)	No PB	LL(cm)	LW(cm)	LS	No BP
PH(cm)	1.0000						
IL(cm)	0.3652**	1.0000					
No PB	0.2385*	0.1812	1.0000				
LL(cm)	0.1250	0.0721	-0.2443*	1.0000			
LW(cm)	0.1596	0.2590*	-0.0667	0.8711**	1.0000		
LS	-0.2513*	-0.3768**	-0.1808	-0.3383**	-0.6766*	1.0000	
No BP	0.3754**	0.3839**	-0.2548*	0.2812*	0.1975	-0.0305	1.0000

Key: PH (cm) =Plant height, IL (cm) =Internode length, LL (cm) =Leaf length, LW (cm) = Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level

Combined correlation for different quantitative traits of African nightshade cultivars grown in both Kisii and Muguga showed that there was high significant ($p \leq 0.05$) positive correlation between plant height and internode length (r= 0.7108**) as well as between plant height and leaf width (r= 0.2581**). Plant height had a high significant ($p \leq 0.05$) positive correlation with number of berries per panicle (r = 0.4203**) while internode length had a high significant positive correlation with number of berries per panicle (r= 0.5711**). There was also a high significant ($p \leq 0.05$) positive correlation between leaf length and number of berries per panicle (r= 03249**) and between leaf width and number of berries per panicle (r= 0.2719**). There was significant ($p \leq 0.05$) positive correlation between plant height and number of primary branches (r= 0.2279*) (Table 28).

Table 28: Phenotypic correlations between various traits scored in 19 African nightshade cultivars planted in Kisii and Muguga 2015 short and long rains combined

	PH(cm)	IL(cm)	No PB	LL(cm)	LW(cm)	LS	No BP
PH(cm)	1.0000						
IL(cm)	0.7108**	1.0000					
No PB	0.2279*	0.0307	1.0000				
LL(cm)	0.1710	0.1196	-0.2151*	1.0000			
LW(cm)	0.2581**	0.1695	-0.2024*	0.9387**	1.0000		
LS	-0.4043**	-0.1711	0.0534	-0.4409**	-0.6739**	1.0000	
No BP	0.4203**	0.5711**	-0.2855**	0.3249**	0.2719**	-0.1083	1.0000

Key: PH=Plant height, IL=Internode length, LL=Leaf length, LW= Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle, **= Significant at 1% level; * = Significant at 5% level

There was a high significant ($p \leq 0.05$) negative correlation plant height and leaf size (r= -0.4203), number of berries per panicle and number of primary branches (r= -0.2855**), leaf length and leaf size (= -0.4409**), leaf width and leaf size (r= -0.6739**). There was also a significant ($p \leq 0.05$) negative correlation between plant height and leaf size (r= -0.4043*), number of primary branches and leaf length (r= -0.2151*) and between number of primary branches and leaf width (r=-0.2024*) (Table 28).

Further correlation results were obtained for quantitative traits of African nightshade cultivars belonging to different species. When different quantitative traits of African nightshade cultivars belonging to *S. scabrum* species (improved type, Nakuru County, AVRDC SS 49, AVRDC BG 14, and AVRDC BG 23) were correlated the results showed that there was few significant ($p \leq 0.05$) correlation between various morphological traits (Table 29).

Table 29: Phenotypic correlation between quantitative traits of African nightshade cultivars belonging to *S. scabrum* species.

	PH(cm)	No PB	IL(cm)	LL(cm)	LS	LW(cm)	No BP
PH(cm)	1.0000						
No PB	0.6198	1.0000					
IL(cm)	0.8810*	0.7931	1.0000				
LL(cm)	-0.3114	-0.7391	-0.3062	1.0000			
LS	-0.6372	0.0267	-0.4996	-0.5069	1.0000		
LW(cm)	-0.0706	-0.6669	-0.1645	0.9531**	-0.7078	1.0000	

No BP	0.5473	-0.0719	0.3304	0.4004	-0.8917*	0.6486	1.0000

Key: PH=Plant height, IL=Internode length, LL=Leaf length, LW= Leaf width, LS=Leaf size, No PB= Number of primary branches, No BP =Number of berries per panicle.

**= Significant at 1% level; * = Significant at 5% level

There was a high significant positive correlation at between leaf length and leaf width (r= 0.9531**), there was also a significant positive correlation at $p \leq 0.05$ between plant height and internode length (r= 0.8810*) whereas there was a significant negative correlation at $p \leq 0.05$ between leaf size and number of berries per panicle (r= -0.8917*) (Table 29).

Phenotypic correlation of quantitative traits of African nightshade cultivars belonging to *S.americanum* species (OARA 1, AVRDC-TZ (RC11), AVRDC BG 22, GBK 050287 and 050292) showed that there was only high significant correlation at $p \leq 0.05$ between leaf length and leaf width (r= 0.9943**), all the remaining correlation between various traits were not significant (Table 30).

Table 30: Phenotypic correlation between quantitative traits of African nightshade cultivars belonging to *S. americanum* species.

	PH(cm)	IL(cm)	No PB	LL(cm)	LW(cm)	LS	No BP
PH (cm)	1.0000						
IL(cm)	0.8165	1.0000					
No PB	-0.2425	-0.5572	1.000				
LL(cm)	0.5708	0.1889	0.6561	1.0000			
LW(cm)	0.5057	0.1582	0.7037	0.9943**	1.0000		
LS	-0.2087	0.0242	-0.8425	-0.8925	-0.9349	1.0000	
No BP	0.6145	0.3754	-0.5772	-0.0491	-0.1546	0.4937	1.0000

Key: PH=Plant height, IL=Internode length, No PB= Number of primary branches, LL=Leaf length, LW= Leaf width, LS=Leaf size, No BP =Number of berries per panicle.

**= Significant at 1% level; * = Significant at 5% level

There was only one high significant negative correlation at $p \leq 0.05$ recoded between quantitative traits of African nightshade cultivars belonging to S. villosum species (Kakamega1, 2 and 3) which was between number of primary branches and leaf size (r= -1.000**). All the remaining quantitative traits recorded non-significant correlation results (Table31).

When various phenotypic traits of African nightshade cultivars belonging to S. sarrachoides species (AVRDC MW13, Kisii type 1 and Kisii type 2) were correlated, there was no significant correlation at $p \leq 0.05$ recorded between the traits (Table 32).

Table 31: Phenotypic correlation between quantitative traits of African nightshade cultivars belonging to *S. villosum* species.

	PH(cm)	IL(cm)	No PB	LL(cm)	LW(cm)	LS	No BP
PH(cm)	1.0000						
IL(cm)	0.7834	1.0000					
No PB	-0.7269	-0.9963	1.0000				
LL(cm)	0.3157	-0.3423	0.4221	1.0000			
LW(cm)	0.1315	-0.5131	0.5852	0.9821	1.0000		
LS	0.7269	0.9963	-1.0000**	-0.4221	-0.5852	1.0000	

No BP	0.9582	0.5729	-0.5000	0.5740	0.4196	0.5000	1.0000

Key: PH=Plant height, IL=Internode length, No PB= Number of primary branches, LL=Leaf length, LW= Leaf width, LS=Leaf size, No BP =Number of berries per panicle
**= Significant at 1% level; * = Significant at 5% level.

Table 32: Phenotypic correlation between quantitative traits of African nightshade cultivars belonging to *S. sarrachoides* species.

	PH	IL	No PB	LL	LW	LS	No PB
PH	1.0000						
IL	0.7886	1.0000					
No PB	0.8191	0.3286	1.000				
LL	0.4494	0.6717	-0.0807	1.000			
LW	0.1673	0.6503	-0.4260	0.8830	1.0000		
LS	0.4521	0.1172	0.3706	0.5874	0.1577	1.0000	
No BP	-0.5926	-0.1345	-0.9141	0.4480	0.6621	0.0000	1.0000

Key: PH=Plant height, IL=Internode length, No PB= Number of primary branches, LL=Leaf length, LW= Leaf width, LS=Leaf size, No BP =Number of berries per panicle
**= Significant at 1% level; * = Significant at 5% level

3.7 DISCUSSION

3.7.1 Qualitative traits and quantitative traits

Significant variation was observed for all the qualitative traits among the 19 African nightshade cultivars and this implies that qualitative traits can be used as a measure of diversity among African nightshade cultivars. This 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 variation 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 stem and fruit colours expressed by different African nightshade cultivars could be as a result of anthocyanin concentration in the plants and could be influenced by environmental factors. Stem colours observed could also be as a result of stress experienced by the plant or age of the plant since the level of anthocyanin in African nightshade plant increases with an increase in the age of the plant (Manoko, 2008).

The dendrogram showed that African nightshade cultivars in group B were more closely related (54%) as compared to those in group A (34%). This was because the higher the bootstrap value the more closely related or the more similar the individuals within that cluster are (Holmes, 2003) for example Kakamega 1 and 2 where cultivars with the highest similarities (80%) followed by Kisii 1 and 2 (74%). Bootstrap values of below 50% are however considered to be low and therefore statistically insignificant (Soltis and Soltis, 2003).

Kakamega 1 and 2 cultivars belonged to *S. villosum* subspecies *Miniatum* which has entire, sinuate, sinuate-dented or dentate leaf margins while Kakamega 3 cultivar belonged to *S. villosum* subsp. *Villosum* which has lobed leaf margin (Ashilenje et al., 2012). The observed long chaining with the dendrogram was suggestive of a rich diversity within the African nightshade cultivars assessed since the shorter the length 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.

African nightshade species *S. americanum* and *S. sarrachoides* have been shown to cluster to cluster together in the dendrogram and this could be because both of them belonging to the

diploid species. Similar results were obtained by Olet et al., (2011) working on *Solanum nigrum* species found in Uganda. *S. scabrum* species have been shown in the dendrogram to have appeared in both cluster B and C and this maybe because there are two different *S. scabrum* sub species namely *S. scabrum* subsp *scabrum* characterized by a many leaves with less fruits and its more than 1.5m tall and *S. scabrum* subsp. *Laevis* which is < 1 m tall and has fewer leaves but produces a large number of fruits. The latter is the wild form of *S. scabrum* and has been previously been mistaken to be *S. nigrum* L (Poczai et al., 2010).

African nightshade cultivars with lanceolate leaves such as cultivars belonging to *S.americanum*, *S. nigrum* L, *S. sarrachoides* and *S. villosum* species have been shown to tolerate water stress more than cultivars with ovate leaves (Ojiewo et al., 2013). This is because lanceolate leaves are narrow hence have a smaller surface area exposed to the atmosphere for transpiration and therefore they lose less water as compared to ovate leaves which are broad, have a large surface area exposed for transpiration and lose a lot of water loss when exposed to water stress conditions. Different African nightshade species differed in terms of plant growth habit and that could affect their photosynthetic activities and could intern lead to difference in fresh leaf yield among the species. For instance, plant orientation has been indicated to determine the exposure of leaves and other vegetative parts to sunlight for photosynthesis, and that might affect the yield obtained. The upright and erect African nightshade cultivars are also thought to be better yielding due to their plant orientation (Nandhini et al., 2014). In addition, Sekyere et al., (2011) working with okra reported that genotypes with erect plant type had high dry matter production leading to an increase in yield.

All cultivars belonging to African nightshade species *S. sarrachoides, S. nigrum L, S. villosum, S. americanum* and *S. scabrum* had leaves with pubescent surfaces (trachomes/hairs) the only

exception being cultivar from Nakuru County (*S. scabrum* species) that had glabrous leaf surface. The degree of pubescence also varied from one African nightshade species to the next with cultivars within *S. nigrum* L species having the longest and most dense trachomes. Cultivars with pubescence have been shown to be tolerant to pests and insects (War et al., 2012) since trachomes (hairs) hinder insects and pests from laying eggs, feeding and also interferes with their larval feeding (Steinite and Ievinsh, 2003). The trachomes 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).

Manoko (2007) also reported that the degree of leaf pubescence could be a as a result of environmental influence or an adaptation mechanism by the plant for resistance to pest and insects.

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 diversity 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.

Only AVRDC –TZ (SS 49) from the *S. scabrum* species produced flowers with purple petals, all the other cultivars produced flowers with white petals an indication that flower colour doesn't necessarily determine the colour of the berries observed since all the cultivars in the *S. scabrum* species produced purple fruits despite some of them having white flowers. There is no documentation showing the effect of stem, flower and berry colour on yield.

For qualitative traits, most variation observed were across species and not within species for instance all cultivars belonging to *S. villosum* had semi erect plant type, produced white flowers and produces mature orange berries while the colour of mature berries varied across species with all the cultivars of the *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 observes on the cultivars within a certain species at Kisii were the very same once observed at Muguga for example, the leaf shape of cultivars within *S. scabrum* species was ovate both at Kisii and Muguga.

Quantitative traits such as plant height, internode length and number of primary branches shows variabitity from one location to the next mainly and from one season to the next in one location mainly because they are mostly affected by the environmental factors (Madic et al., 2016) and this is supported by results from the study that showed that cultivars grown Muguga had longer plant height and internode length than their counterparts grown in Kisii while at the same time

producing more primary branches in Muguga than in Kisii. African nightshade cultivars also produced more number of berries per panicle in season one (16) as compared to season two (13). As much as the number of berries per panicle varied across African nightshade species, the timing of berry harvesting is also very important since seeds for *S.americanum, S. villosum* and *S.nigrum* L species abscise at maturity, some fall off from the panicle while some are eaten by birds and therefore there is need for timing their harvesting. Berries of *S. scabrum* are not eaten by birds and remain attached to the panicle at maturity until they are plucked off during harvesting. African nightshade seeds are very important to farmers since they are a means through which they are propagated and most farmers use seeds from their last harvest for the next season planting.

Significant difference in cultivar x location (environment) in plant height, leaf length and in the number of primary branches in the two sites (Kisii and Muguga) among the African nightshade cultivars might be an indication that the traits were not stable across the sites since they were influenced by environmental factors hence their variation.

3.7.2 Correlations among quantitative traits of 19 African nightshade

The positive correlation between plant height and number of primary branches recorded implies that taller plants tend to have many primary branches when compared to shorter plants. This positive correlation is important in as far as it translates to taller plants having more primary branches per plant hence increased leaf yield. A high significant correlation between plant height and number of branches was in *Capsicum annuum* L genotypes was recorded by Maga et al., (2012). Positive correlation between plant height and number of branches was also been reported by Ali et al., (2013). Nevertheless, an increase in plant height may result into decrease in leaf size as shown by the negative correlation between leaf size and plant height. These means that as

much as an increase in plant height may lead to an increase in number of primary branches and an increase in the number of leaves, the leaves may be very small in size hence weigh less and therefore will not be of economic importance. That is why farmers prefer African nightshade cultivars of average height which have bigger leaves and more weight. Plant height is a very important factor when breeding for genotypes tolerant to lodging and in such cases shorter plants are preferred more to taller plants because they are able to resist lodging and offer better response to fertilizer application (Medagam et al., 2015). However taller plants are also preferred in some instance because they are thought to be stronger and do not fall easily in production levels compared to shorter plants (Medagam et al., 2015).

High significant correlation between plant height and leaf length shows that taller plants tend to have longer leaves while high significant correlation between leaf length and leaf width implies that an increase in length may lead to an increase in leaf width. Sahai et al., (2013) also reported a high significant correlation between leaf length and plant height and also between leaf length and leaf width in *Vigna unguilata*. Similar findings were also made by Nyadanu et al., (2014).

Plants with wider leaves resulted into big sized leaves. The strong positive correlation between leaf width and leaf size implied that wider leaves have bigger leaf size and they may weigh more as compared to narrower leaves which are small in size. African nightshade vegetable leaf yield is measured in terms of weight in Kilograms in the market when being sold and it would only take few of big leaves to weigh one kg as compared to many small leaves required to weigh the same kg. The weight of African nightshade cultivars with smaller leaves can therefore be improved by increasing their number of leaves produces per plant.

Leaf size can be increased by increasing the width and length of the leaves as indicated by the results. Positive correlation between leaf size and number of branches indicates that an increase

in the number of branches per plant might lead to an increase in number of leaves and intern to an increase in leaf yield. This findings are in line with those of Nyadanu et al., (2014).

African nightshade *S. scabrum* species had the biggest leaves followed by *S. villosum* while *S. sarrachoides* had the smallest leaves hence for commercial purposes *S. scabrum* may be preferred. This is supported by the findings of Ojiewo et al., (2013b) who reported that when farmer participatory varietal selection was carried out for African nightshade species, most farmers were reported by to have preferred *S. scabrum* species as compared to other African nightshade species since they associated longer and broader leaves with high yield and used this trait as a selection criterion for African nightshade production.

Negative correlation between leaf size and number of berries per panicle recorded in the results was due to the fact that when African nightshade cultivars increase the number of berries produces, it uses the stored photosynthates that would have otherwise been used for vegetative production. This would in turn lead to leaf senescence by reducing the amount of assimilates present in the leaves thereby producing smaller leaves (Ojiewo et al., 2013).

Correlation studies therefore assists breeders to know and understand how different morphological traits of agronomic importance are interrelated and this may assist in prediction of correlated response and selection of traits that are useful indicators of agronomic traits to be improved such as leaf yield (Shukla et al., 2010). Identification and manipulation of traits like leaf width, leaf length, number of primary branches and plant height either through genetic improvement or by alteration of the local environment where the African nightshade cultivar is grown for example by use of greenhouses may lead to increased leaf yield.

In African nightshade vegetable, leaf size and number of berries per panicle are yield indicators and cultivars with bigger leaves may weigh more kilograms compared to genotypes with smaller

leaves hence this may generate more income for farmers especially those who grow African nightshade for commercial purposes. Seed are also very important since they are the main means for propagating African nightshade. The significant difference ($p \leq 0.05$) in plant height, internode length, number of primary branches, leaf length, leaf width, leaf size and number of berries per panicle among various African nightshade cultivars is an indication that there is diversity amongst them. These results concur with findings of Nandhini et al., (2014) who reported that there was genetic diversity among various African nightshade genotypes in both quantitative and qualitative traits.

3.8 CONCLUSION

The assessment of African nightshade cultivars using morphological traits showed that there was significant variation recorded for all the qualitative and quantitative traits among the 19 African nightshade cultivars. African nightshades cultivars in this study clustered into different sub

groups representing different species which included *S. scabrum, S. villosum, S. americanum, S. sarrachoides and S.nigrum* L based on their distinct morphological features.

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

Significant correlation results indicated that correlation studies are important in breeding programs because they can assist breeders to understand the interrelationship between various agronomic traits and use the results for selection during African nightshade breeding work.

This study also revealed that both qualitative and quantitative traits were important in the assessment of genetic diversity within African nightshade cultivars and could be used in the identification of important cultivars that could be incorporated into breeding program for further crop improvement.

3.9 RECOMMENDATIONS

African nightshade cultivars belonging to *S. scabrum* species were shown to perform better compared to the rest of the African nightshade species in most quantitative traits and can therefore be recommended to farmers for commercial production.

This study also recommends that further morphological characterization be carried out and be narrowed down to studying cultivars belonging to the same species after which superior cultivars within particular African nightshade species could be identified and be introduced into breeding

program for development of improved and better yielding genotypes that are resistant to biotic and abiotic stresses.

CHAPTER 4

MOLECULAR CHARACTERIZATION OF AFRICAN NIGHTSHADE CULTIVARS USING SSR MARKERS

ABSTRACT.

Genetic diversity study is key to the initiation of any breeding program. Evaluation and estimation of genetic diversity can be done through genotypic characterization using molecular

markers. Little research has been carried out on the estimation of genetic diversity in African nightshade cultivars using molecular markers. The main objective of this study was to evaluate the existence of genetic diversity within African nightshade cultivars using SSR markers. Molecular characterization of twenty seven African nightshade cultivars collected from the National Gene bank of the Genetic Resources Research Institute, World vegetable Centre (AVRDC) Tanzania and from Kenyan farmer was done using SSR markers. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis separated the genotypes into three main clusters indicating that there existed genetic diversity within the African nightshade genotypes under study.

Key words: Genetic diversity, Markers, SSR, African nightshade, UPGMA.

4.1 INTRODUCTION

Modern plant breeding programs are rare for indigenous vegetables such as African nightshades and other minor crops because they have been neglected and more emphasis are being given for major economic crops such as wheat, maize, rice and sorghum. There is also limited morphological and genetic information on these minor crops (FAO, 2010). Molecular characterization provides means of assessing and identifying genetic diversity available within various genotypes for use in breeding programs and for efficient conservation of germplasm

(Herraiz et al., 2015). Due to limitations experienced with morphological characterization such as susceptibility to environmental interferences, breeders are quickly shifting to the use of molecular markers which are more efficient, specific and are not influenced by the environment. However, molecular markers are very expensive, requires highly skilled personnel and equipment for analysis (Singh et al., 2011).

Polymerase chain reaction uses variety of molecular markers for analysis one of them being SSR (simple sequence repeats) which are short nucleotide sequence repeats that can be detected by PCR with the use of specific primers. SSR markers are preferred for diversity studies as compared to other molecular markers because they are highly polymorphic, reproducible, co-dominant, reliable, multi-allelic, simple and easy to score (Chauhan et al., 2015). SSR which have been used in different crops can be evaluated for and used for crops of the same species as this provides a cost effective means for development of SSR markers for species with little or no information on their sequence. Possible transferability of SSR primers across different crop species of the same genus have been reported (Yasodha et al., 2005).

African nightshade (black nightshade) have often been referred to as poisonous weed in many parts of the world and this has led to it being neglected and underutilized in European countries. However, but it serves as a source of food and medicine in Africa and Asian countries (Poczai and Hyvonen, 2011). The aim of this study was to carry out genetic diversity studies on African nightshade cultivars using SSR markers obtained from tomatoes, potatoes and eggplants all of which belong to the genus *Solanum* of the *Solanaceae* family.

4.2 MATERIALS AND METHODS

Twenty seven African nightshade cultivars were used in this study.

Table 33: Cultivar used for molecular characterization of African nightshade

Cultivar code	**Source**
GBK 050570	Gene bank of Kenya
GBK 050572	Gene bank of Kenya
GBK 050604	Gene bank of Kenya
GBK 050350	Gene bank of Kenya
GBK 050287	Gene bank of Kenya

GBK 050292	Gene bank of Kenya
GBK Genotype	Gene bank of Kenya
BG 14	AVRDC-TZ
BG 16	AVRDC-TZ
BG 17	AVRDC-TZ
BG 18	AVRDC-TZ
BG22	AVRDC-TZ
BG23	AVRDC-TZ
MW13	AVRDC-TZ
MW17	AVRDC-TZ
SS49	AVRDC-TZ
RC 01	AVRDC-TZ
RC11	AVRDC-TZ
Kisii type 1	Famers from Kisii, Kenya
Kisii type 2	Farmers from Kisii, Kenya
Kakamega 1	Farmers from Kakamega, Kenya
Kakamega 2	Farmers from Kakamega, Kenya
Kakamega 3	Farmers from Kakamega, Kenya
Nakuru county	Farmers from Nakuru, Kenya
OARA 2	Farmers from Kisii, Kenya
OARA 1	Farmers from Kisii, Kenya

The germplasm were obtained from the Gene bank of Kenya, Kenyan farmers and from World vegetable center (AVRDC Tanzania) (Table 33). The African nightshade cultivars were planted in pots filled with very fine soil in a greenhouse and DNA extraction done using Modified Doyle and Doyle (1990) methodology which was had also been used for DNA extraction of sweet potatoes (Silva et al., 2014).

4.2.1. Leaf harvesting and DNA extraction

Young, tender and healthy African nightshade cultivars leaves were harvested after 4 weeks and wrapped in foil paper and then immediately taken to the laboratory then refrigerated at -20°C so as to retain its quality. The leaves were then rinsed in distilled water to remove soil particles on their surfaces. This protocol was also used by Agbagwa et al., (2012). African nightshade cultivars leaves were weighed and 200mg of the each cultivar's leaves was 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. 50 µl 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 resuspended in 400µl of sterile DNase free water. 10µl/ml (10µl RNase in 10ml H_2O) 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.

4.2.2. DNA quality confirmation

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 28 wells, one well for loading the ladder and the remaining wells for loading the 27 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 the

remaining 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.

4.2.3. 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 thirteen different primers were used for polymerase chain reaction with each primer pair (reverse and forward) being used per reaction. The primers used were from tomatoes, eggplant and potatoes and they were adopted from Zhu *et al.* (2012). 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 (depending on the annealing temperatures of the primers which were calculated using Thermo Fisher scientific calculator based on the primer sequences) 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. The PCR products were separated by 3% agarose gel electrophoresis and the images obtained in the gels were taken using LPIx Image program. 3% agarose gel produced more refined and visible bunds compared to 1% or 2% agarose gelconcentration.

4.2.5 Primers information

Primer CA 158 was used successfully in the study of mating systems and fine scale genetic structure of *Solanum lycocarpum* ***in*** Brazil by Martins et al., (2006) and it was found to be polymorphic and presented the highest gene diversity as compared to all the other markers used

in the study. STI 001 primer was successfully used in the formation of new potato genetic identity kit by Ghislain et al., (2009) and was also found to be polymorphic.

SSR 111 and SSR 60 primers were used by Hu et al., (2012) in the diversity study of Argentina tomato variety and they amplified and produced polymorphic bands. SSR 111 and SS 60 were also successfully used in the assessment of genetic variation among commercial tomato by Kwon et al., (2009) and found to be polymorphic. TSR2 primer was used in the study of genetic diversity in tomato cultivars by Yi et al., (2008) where it amplified and was polymorphic. Munoz-Falcon et al., (2009) was able to distinguish Almagro eggplant from closely related varieties using primers EM 140, EM 127, EM 155 and ESM3 since they were polymorphic. All the thirteen primers 13 primers (STI001, CA 158, SSR111, SSR 63, TSR2, EM135, EM140, EM141, STG10, EM117, EM127, EM155, ESM3) were further used by Zhu et al., (2012) in then evaluation of simple sequence repeat (SSR) markers from *Solanum* crop species for *Solanum elaeagnifolium* and they all amplified and produced a multiple bands. Amongst them only two primers (TSR2 and EM141) were monomorphic while the rest were polymorphic. In this study the SSR primers used were STI001, CA 158, SSR111, SSR 63, TSR2, EM135, EM140, EM141, STG10, EM117, EM127, EM155, ESM3 (Zhu et al., 2012) (Table 34) with the primer concentration being 0.5µl for both the forward and reverse primers.

Table 34. Cross-species simple sequence repeats (SSR) primers that amplified African nightshade cultivars

Locus	Repeat Motif	Source species	PIC $_{so}$	Calculated T_A(°C)	Provided T_A(°C)	Primer sequence (5'–3')	Source
STI001	$(AAT)_n$	Potato	0.69	49.3	58	F: CAGCAAAATCAGAACCCGAT R: GGATCATCAAATTCACCGCT	Ghislain et al. (2009)
CA 158	(GA)32	Tomato	0.85	49.3	55	F: CATGCACGTACAACCTGTTT R: TAGTTCCCTTGCTGCAGTAA	Martins et al. (2006)

SSR111	(TC)6(TCTG)6	Tomato	0.88	49.5	50	F: TTCTTCCCTTCCATCAGTTCT R: TTTGCTGCTATACTGCTGACA	Kwon et al. (2009)
SSR 63	(AT)39	Tomato	0.80	46.3	50	F: CCACAAACAATTCCATCTCA R: GCTTCCGCCATACTGATACG	Kwon et al. (2009)
TSR2	(AT)15	Tomato	0.81	51.2	50	F: TCAAGTGAGTTTATCTGCCCAC R: GCTCATCCTACACATTCATGCTC	Yi et al. (2008)
EM135	(CA)11(GA)20	Eggplant	0.75	53	58	F: ATCCTGTTGCTGCTCATTTTCCTC R: AGGAGGATCCAAGAGGTTTGTTGA	Nun me et al. (2003)
EM140	(AC)4GC(AC)5T(AC)3ATGC(AC)4AT(AC)6(AT)5(TA)13	Eggplant	0.52	50	53–48	F: CCAAAACAATTTCCAGTGACTGTGC R: GACCAGAATGCCCCTCAAATTAAA	Munoz-Falcon et al., (2009)
EM141	(AT)16(GT)19	Eggplant	0.83	52	50	F:TCTGCATCGAATGTCTACACCAAA R: AAAAGCGCTTGCACTACACTGAAT	Nun me et al., (2003)
STG10	(TG)n	Potato	0.69	53	55–50	F: CGATCTCTGCTTTGCAGGTA R: GTTCATCACTACCGCCGACT	Ghislain et al., (2009)
EM117	(AC)19(AT)11	Eggplant	0.74	50	55	F: GATCATCACTGGTTTGGGCTACAA R: AGGGGAGAGGAAACTTGATTGGAC	Nun me et al., (2003)
EM127	(AC)13(AT)13	Eggplant	0.60	49.8	55–50	F: CAGACACAACTGCTGAGCCAAAAT R: CGGTTTAATCATAGCGGTGACCTT	Munoz-Falcon et al., (2009)
EM155	(CT)38	Eggplant	0.64	49.3	50–45	F: CAAAAGATAAAAAGCTGCCGGATG R: CATGCGTGAGTTTTGGAGAGAGAG	Munoz-Falcon et al., (2009)
ESM3	(TA)9(GA)8	Eggplant	0.51	52.7	55–50	F: ATTGAAAGTTGCTCTGCTTCAC R: ACATCGTTCCGCCTCTATTG	Munoz-Falcon et al., (2009)

PIC_{SO}, polymorphism information content (PIC) in source species; Calculated TA, annealing temperature generated by Thermo Fisher Scientific calculator, provided TA ,annealing temperatures provided by Zhu et al.(2012). Source: Zhu et al. (2012).

4.2.6 Optimization of primers

The primer annealing temperatures provided by Zhu et al., (2012) did not amplify in African nightshade cultivars and new annealing temperatures were calculated using Thermo Fisher Scientific calculator (www.thermoscientific.com/pcrwebtools). This calculator calculates and generates the annealing temperature of any given primer based on the forward and reverse primer sequences and on the primer concentration used.

The first step in calculating the annealing temperatures using Thermo Fisher Scientific calculator involves identifying and selecting the type of DNA polymerase to used, this is then followed by typing the nucleotide sequences for the forward and reverse primer used on the slots given by the calculator then entering the primer concentration to be used in the PCR. The calculator will then automatically calculate and generate the annealing temperatures to be used for amplifying that specific primer during polymerase chain reaction.

4.3 DATA ANALYSIS

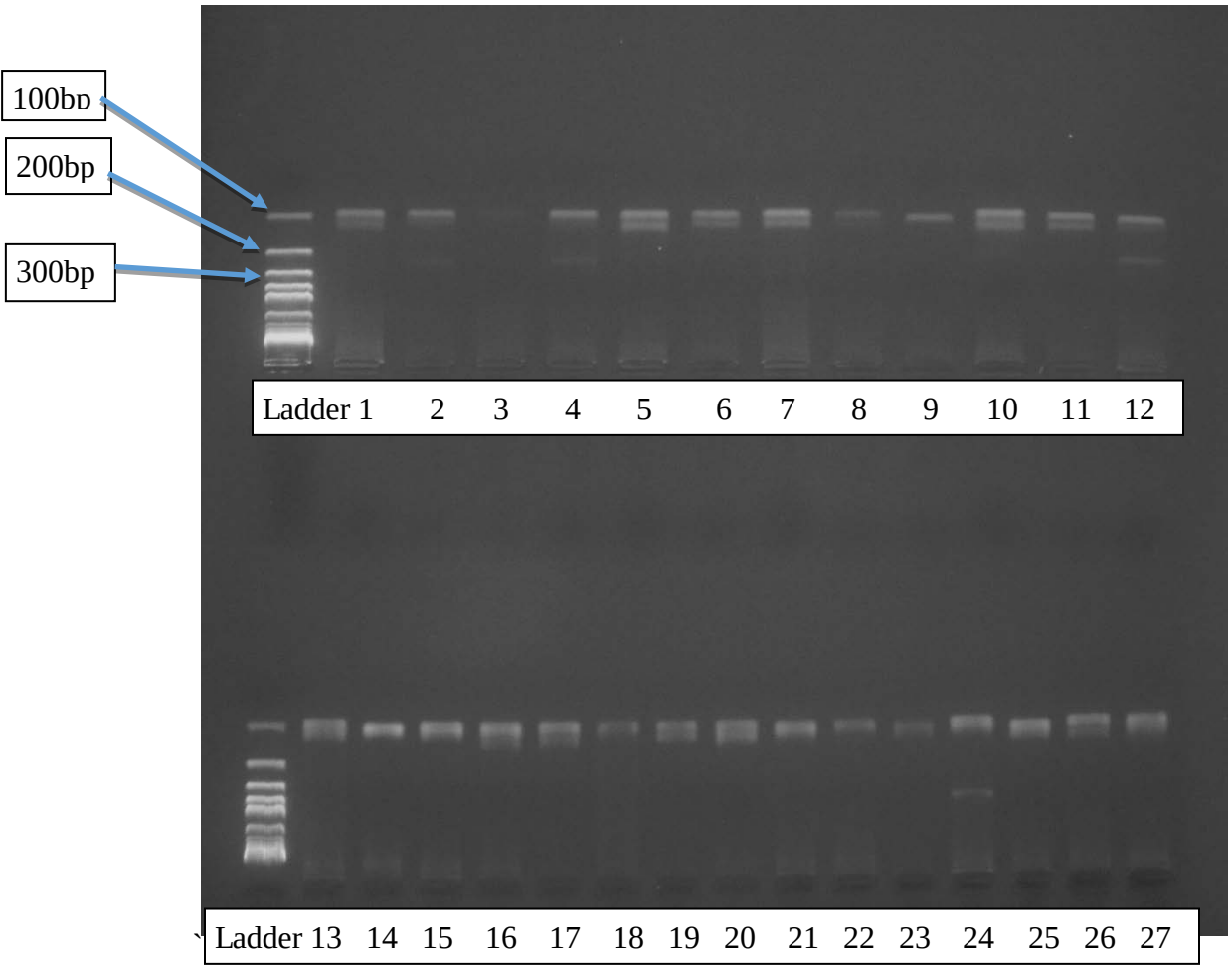
For each reproducible band visualized, the fragment size was obtained by comparing it with P1473 ladder which has a range of between 100 to 1000 base pair markers. Binary scores were assigned to each allele and scored for as 1 for band present and 0 for absent. The binary data generated was used for statistical analysis. The size of the band was determined by comparing the band with 1000 base pair ladder. Cluster analysis was performed to establish the genetic relationship among genotypes and Genetic distance dendrogram drawn using DARwin (Dissimilarity and Representation for windows) 6 software program.

The binary data was imported into DARwin software and saved as VAR (variable) file where the name of African nightshade accessions appeared in row while the scores (variables) appeared in column. The VAR file was then converted into Dis (dissimilarity) file using Dice similarity coefficient which recognizes 1 for presence and 0 for absence of bands in the file. The number of bootstraps was then selected to be 1000 iterations. The Dis file was thereafter converted into ARB file for tree construction. The tree was then constructed with hierarchical clustering using unweighted pair group method with arithmetic mean (UPGMA). The tree was then presented in a hierarchical horizontal form as a dendrogram with bootstraps values indicated on in.

4.5 RESULTS

Gel electrophoresis of amplified DNA was done and the product visualized under ultra violet rays. Five out of the thirteen markers SSR markers evaluated for African nightshade genotypes generated bands. Four of them were originating from tomatoes and only one from potatoes. No primer originating from eggplant generated bands. Out of the five markers, two primers (CA 158 and STI 001) were polymorphic while the rest (SSR111, SSR63 and TSR2) were monomorphic (Table 34).

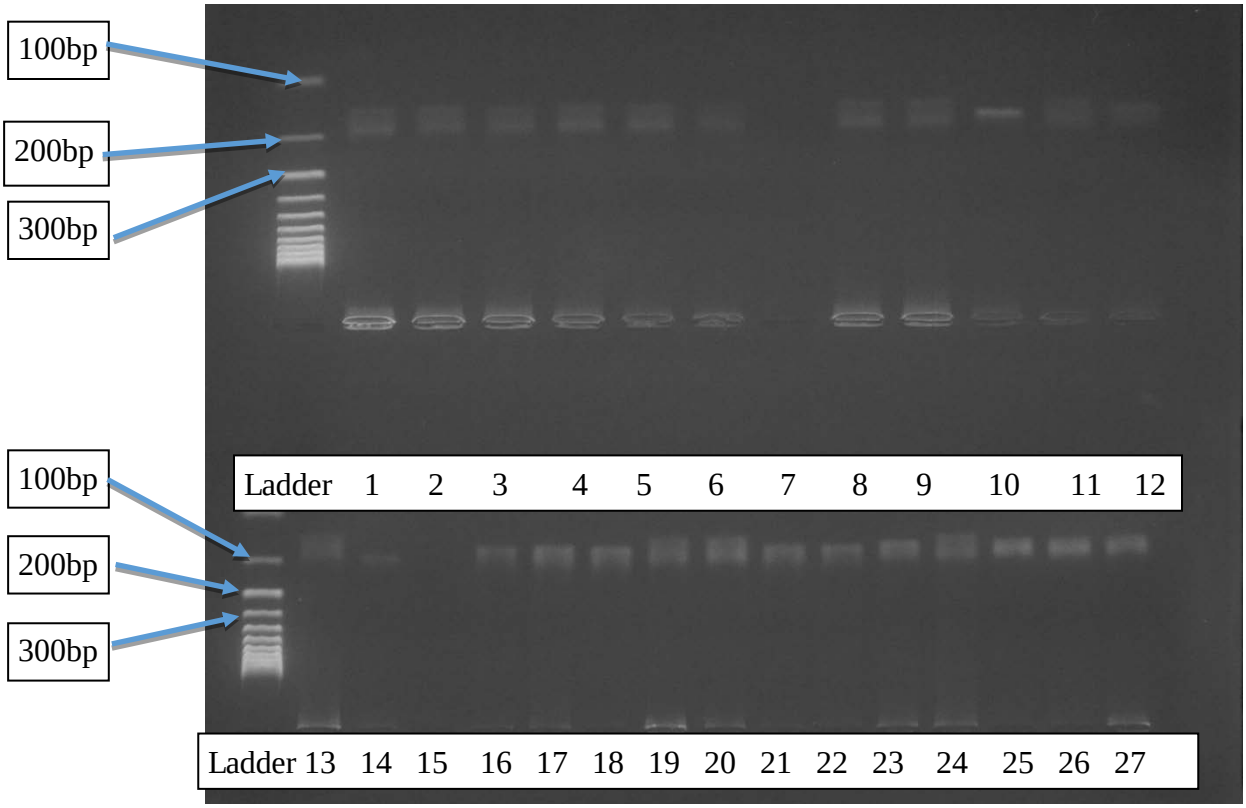
CA 158 SSR primer was polymorphic with most African nightshade cultivars producing more than one band (Figure 14).



1= GBK 050570, 2= GBK050572, 3= GBK 050604, 4= GBK050350, 5= GBK 050287, 6= GBK050292, 7= GBK Genotype, 8= Nakuru county, 9= Kisii 1, 10= Kisii 2, 11= OARA 1, 12= Improved type, 13= Kakamega 1, 14= Kakamega, 15= 2 Kakamega 3, 16= AVRDC-TZ(BG14),17= AVRDC-TZ(BG16), 18= AVRDC-TZ (BG17-1), 19= AVRDC-TZ(BG18), 20= AVRDC-TZ (BG22), 21= AVRDC-TZ(BG23), 22= AVRDC-TZ(MW17-2), 23= AVRDC-TZ (SS49), 24= AVRDC-TZ (RC01), 25= AVRDC-TZ (RC11), 26= OARA 2, 27= AVRDC-TZ (BG17 -2).

Figure 14: 3% Agarose gel image showing amplifications of 27 African nightshade cultivars by primer CA 158 from tomato

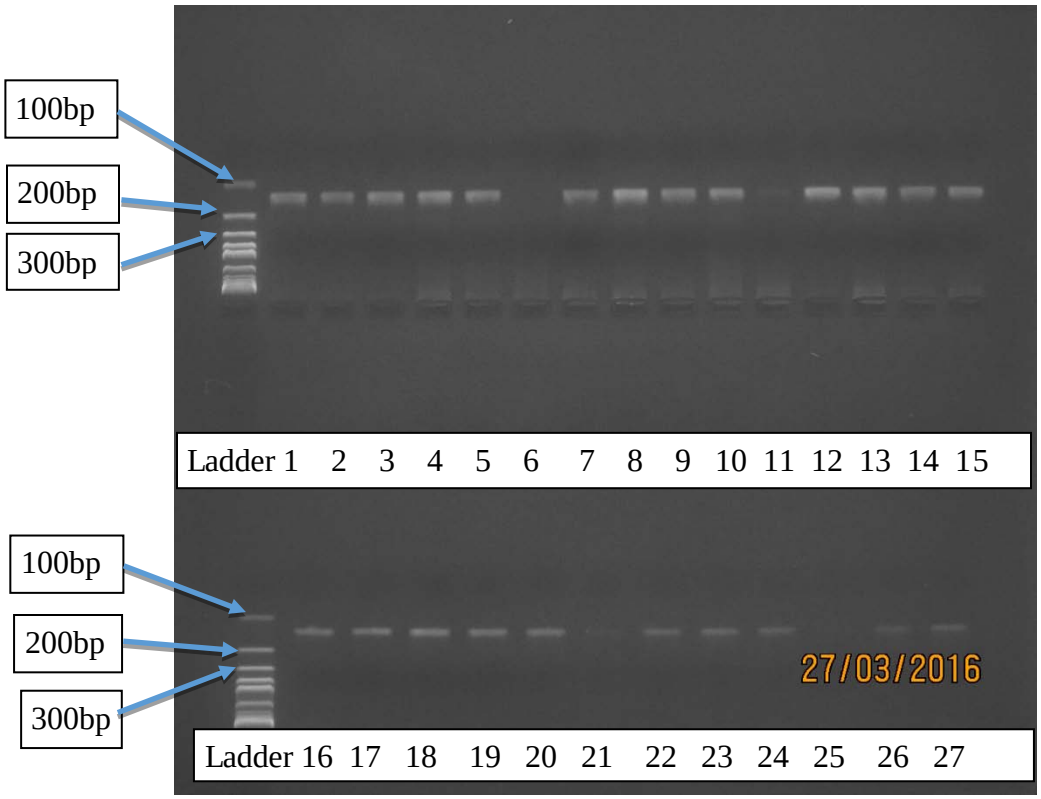
STI 001 primer was polymorphic with some African nightshade cultivars producing more than one bands (Figure 15).



1= GBK 050570, 2= GBK050572, 3= GBK 050604, 4= GBK050350, 5= GBK 050287, 6= GBK050292, 7= GBK Genotype, 8= Nakuru county, 9= Kisii 1, 10= Kisii 2, 11= OARA 1, 12= Improved type, 13= Kakamega 1, 14= Kakamega, 15= 2 Kakamega 3, 16= AVRDC-TZ(BG14),17= AVRDC-TZ(BG16), 18= AVRDC-TZ (BG17-1), 19= AVRDC-TZ(BG18), 20= AVRDC-TZ (BG22), 21= AVRDC-TZ(BG23), 22= AVRDC-TZ(MW17-2), 23= AVRDC-TZ (SS49), 24= AVRDC-TZ (RC01), 25= AVRDC-TZ (RC11), 26= OARA 2, 27= AVRDC-TZ (BG17 -2).

Figure 15: 3% Agarose gel image showing amplifications of 27 African nightshade cultivars by primer STI 001 from potato

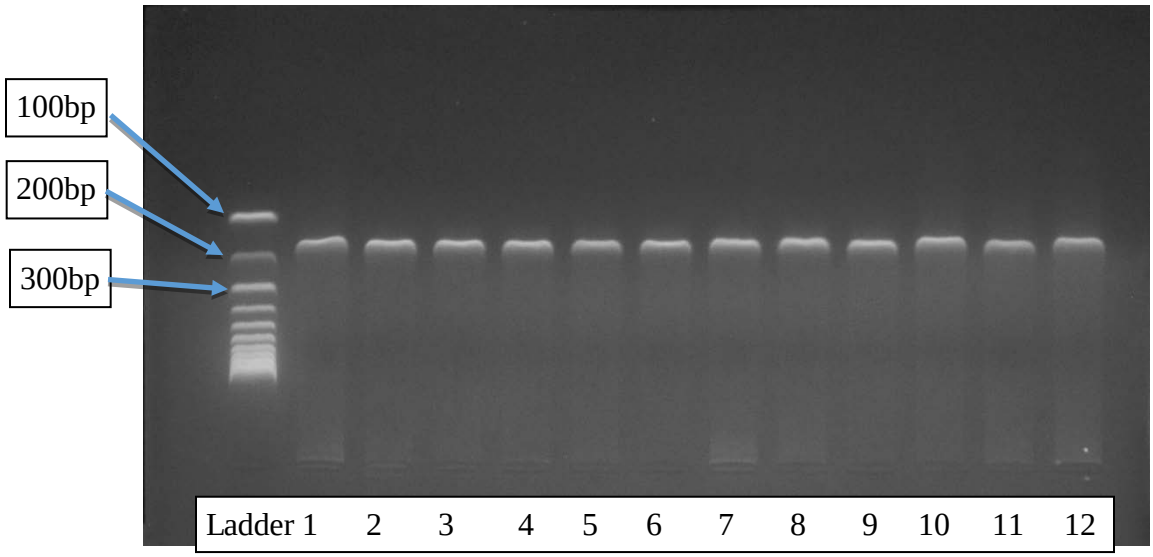
Primer TSR2 was monomorphic since it produced single bands in all African nightshade cultivars (Figure 16).



1= GBK 050570, 2= GBK050572, 3= GBK 050604, 4= GBK050350, 5= GBK 050287, 6= GBK050292, 7= GBK Genotype, 8= Nakuru county, 9= Kisii 1, 10= Kisii 2, 11= OARA 1, 12= Improved type, 13= Kakamega 1, 14= Kakamega, 15= 2 Kakamega 3, 16= AVRDC-TZ(BG14),17= AVRDC-TZ(BG16), 18= AVRDC-TZ (BG17-1), 19= AVRDC-TZ(BG18), 20= AVRDC-TZ (BG22), 21= AVRDC-TZ(BG23), 22= AVRDC-TZ(MW17-2), 23= AVRDC-TZ (SS49), 24= AVRDC-TZ (RC01), 25= AVRDC-TZ (RC11), 26= OARA 2, 27= AVRDC-TZ (BG17 -2).

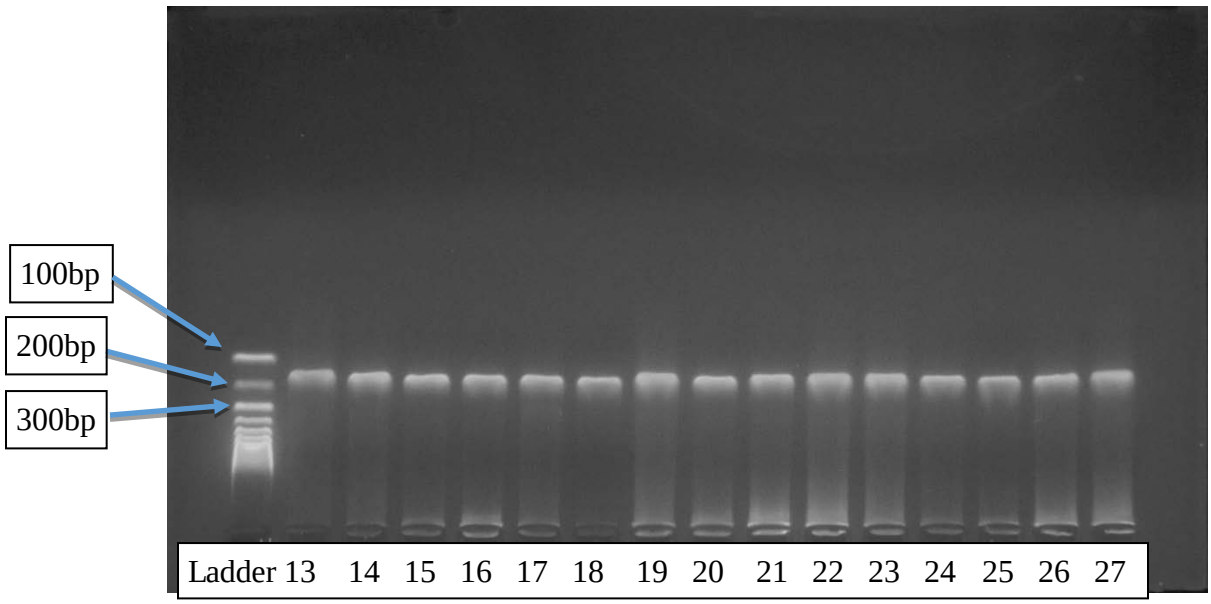
Figure 16: 3% Agarose gel image showing amplifications of 27 African nightshade cultivars by primer TSR2 001 from potato

SSR 66 primer produced single band for all African nightshade cultivars hence being monomorphic (Figure 17 and Figure 18).



1= GBK 050570, 2= GBK050572, 3= GBK 050604, 4= GBK050350, 5= GBK 050287, 6= GBK050292, 7= GBK Genotype, 8= Nakuru county, 9= Kisii 1, 10= Kisii 2, 11= OARA 1, 12= Improved type.

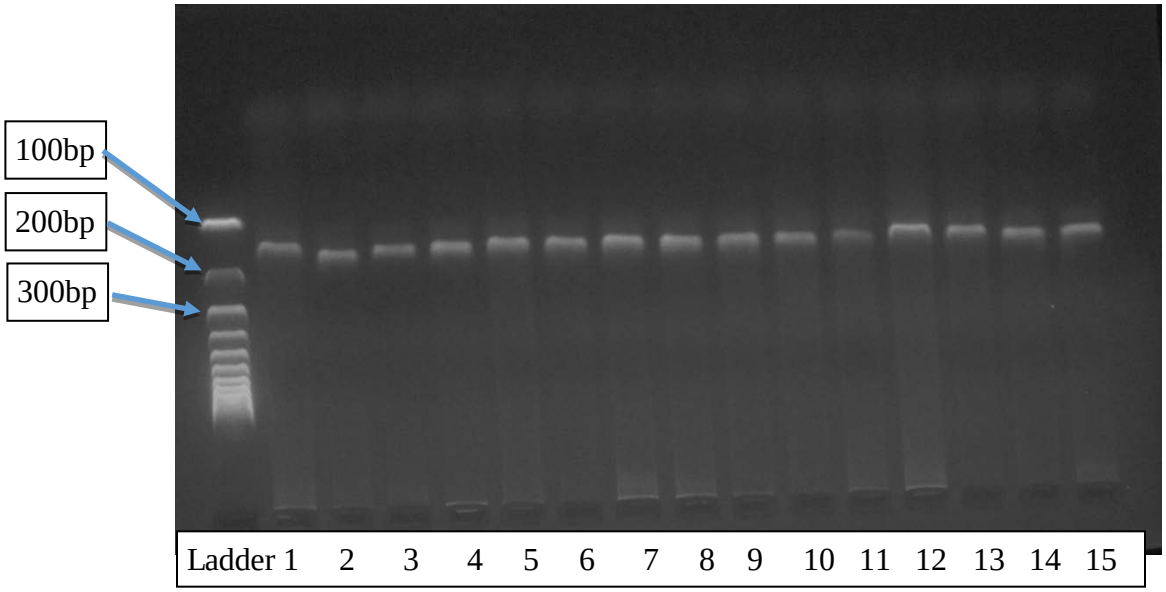
Figure 17: 3% Agarose gel image showing amplifications of 12 African nightshade cultivars by primer SSR 63 from tomato



13= Kakamega 1, 14= Kakamega, 15= 2 Kakamega 3, 16= AVRDC-TZ(BG14),17= AVRDC-TZ(BG16), 18= AVRDC-TZ (BG17-1), 19= AVRDC-TZ(BG18), 20= AVRDC-TZ (BG22), 21= AVRDC-T Z(BG23), 22= AVRDC-TZ(MW17-2), 23= AVRDC-TZ (SS49), 24= AVRDC-TZ (RC01), 25= AVRDC-TZ (RC11), 26= OARA 2, 27= AVRDC-TZ (BG17 -2).

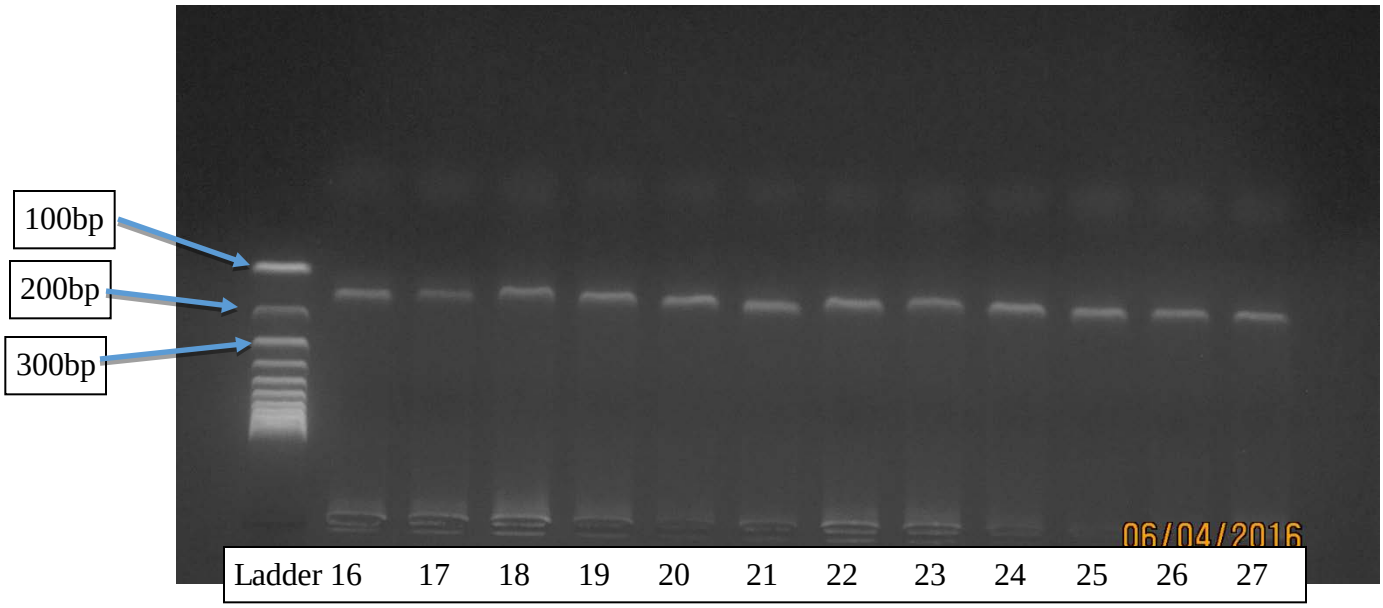
Figure 18: 3% Agarose gel image showing amplifications of 15 African nightshade cultivars by primer SSR 63 from tomato

Primer SSR 111 produced single bands for all African nightshade cultivars therefore was monomorphic (Figure19 and 20)



1= GBK 050570, 2= GBK050572, 3= GBK 050604, 4= GBK050350, 5= GBK 050287, 6= GBK050292, 7= GBK Genotype, 8= Nakuru county, 9= Kisii 1, 10= Kisii 2, 11= OARA 1, 12= Improved type, 13= Kakamega 1, 14= Kakamega, 15= 2 Kakamega 3.

Figure 19: 3% Agarose gel image showing amplifications of 15 African nightshade cultivars by primer SSR 111 from tomato.



16= AVRDC-TZ(BG14),17= AVRDC-TZ(BG16), 18= AVRDC-TZ (BG17-1), 19= AVRDC-TZ(BG18), 20= AVRDC-TZ (BG22), 21= AVRDC-T Z(BG23), 22= AVRDC-TZ(MW17-2), 23= AVRDC-TZ (SS49), 24= AVRDC-TZ (RC01), 25= AVRDC-TZ (RC11), 26= OARA 2, 27= AVRDC-TZ (BG17 -2).

Figure 20: 3% Agarose gel image showing amplifications of 12 African nightshade cultivars by primer SSR 111 from tomato.

The clustering and sub clustering seen in the dendrogram indicated that there was rich genetic diversity among African nightshade cultivars being studied. The dendrogram revealed 3 main clusters. Clusters A, B and C (Figure 21). Cluster A consisted of 8 cultivars (GBK 050570, AVRDC-TZ (BG 18), Managu from Nakuru County, GBK Genotype, GBK O5O292, GBK 050287, Kisii type 2 and AVRDC-TZ (BG22), Cluster C consisted of only 2 cultivars (AVRDC-TZ (MW) and AVRDC TZ (BG 17-1) while the remaining 17 cultivars AVRDC TZ (BG 23), AVRDC TZ (BG17-2), AVRDC TZ (BG 16), BG14, RC11, RC 1,SS 49, GBK 050350, GBK 050604, GBK 050572, Kiss type 1, Kakamega type 1,Kakamega 2 and Kakamega 3,OARA type 1and OARA type 2 and improved type fell into cluster B. The highest bootstrap (64%) value was recorded in sub cluster consisting of AVRDC TZ (BG16), AVRDC TZ (BG 14) and AVRDC TZ (RC11) whereas the lowest bootstrap value was 14% (Figure 21).

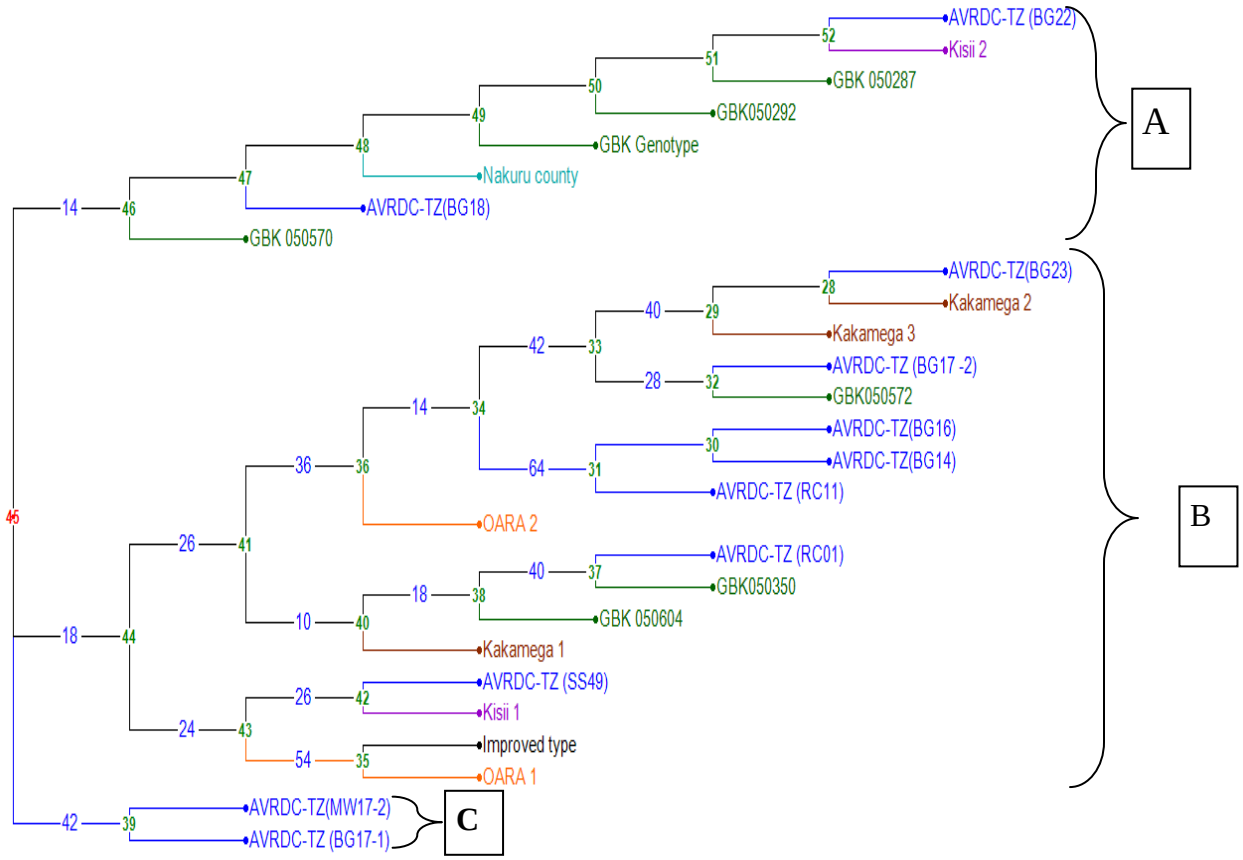


Figure 21: UPGMA dendrogram analysis of 27 African nightshade cultivars using SSR primer pairs with bootstrap values (1000 iterations) of between 0-100 shown on the branches.

4.6 DISCUSSION

The results from the analysis which described primer CA 158 to be polymorphic are in line with those of *Solanum lycocarpum* by Martins et al. (2006) and in *S. elaeagnifolium* by Zhu et al., (2012). Subsequently, STI 001 primer was similarly reported to be polymorphic by Ghislain et al. (2009) and Zhu et al., (2012). Primers SSR111, SSR63 and TSR2 produced single bands with African nightshade cultivars whereas in *S. elaeagnifolium*, SSR 111 and SSR 63 had multiple bands while TSR2 was monomorphic (Zhu et al., 2012). SSR 111 AND SSR 60 primers were also described to be polymorphic by Kwon et al. (2009) and Hu et al. (2012).

Primer STG10 from potato did not amplify in African nightshade even though it was stated to be polymorphic by Ghislain et al., (2009). Primers EM135, EM140, EM141, EM117, EM127, EM155, and ESM3 from eggplants did not produce any bands in African nightshade cultivars despite having been reported to be polymorphic by Zhu et al., (2012) and Munoz-Falcon et al., (2009). This might be because the evolutionary distance between African nightshade cultivars and tomato was close followed by that of tomato. Evolutionary distance between African nightshade and eggplant might be too big to allow primer transferability between them. Rossetto (2001) reported that for heterologous PCR amplification to succeed, the evolutionary distance between the source of the primer and the species being evaluated should be close since the more the genomic similarity between the pair the more the conservation of SSR flanking regions and hence transferability of the primer pair. Demir et al., (2010) also reported low polymorphic frequencies in solanaceous plants among cultivars and intraspecific lines and stated that this could be because they are mainly self-pollinated.

When transferring SSR primer pairs across different species the limitation encountered is that one may get either a false positive or a false negative results were either the SSR nucleotide

repeat is absent from the fragment amplified or the SSR nucleotide repeat is not amplified even though it is present. This might cause variations of sequences at the primer target site thereby preventing the primer from annealing hence resulting to no amplification thus leading to inaccurate results being obtained (Rossetto, 2001).

African nightshade cultivars within a cluster consisted of the more genetically similar the cultivars than those among different clusters. Similar results were obtained by (Osei et al., 2013). Bootstrap values are a measure of the confidence limit of the phylogenetic branches and values of at least 50% are considered to be statistically significant. Low bootstrap values in some branches could have been achieved as a result of redundancy in genetic dissimilarity dataset brought about by the limited number of primers used in the generation of the dendrogram. These results are in line with those of Chemutai et al., (2016). Evaluation of genetic diversity within cultivars is important for preservation of genetic resources needed for breeding work more so in the prediction of the combining ability among parental lines for development of new African nightshade genotypes or for the improvement of already existing cultivars in terms of yield and resistance to abiotic and biotic stresses (Demir et al., 2010).

4.7 CONCLUSION

The assessment of African nightshade cultivars using SSR primers showed that there was genetic diversity recorded because cultivars clustered into three different groups (A, B and C). The clusters and sub-clusters seen in the dendrogram indicated that there was rich genetic diversity within the African nightshade cultivars studied.

When separating African nightshade cultivars PCR products after amplification the best agarose concentration to use in gel formation during electrophoresis was 3% for the production of high resolution bands.

Thermo Fisher Scientific calculator can effectively be used to calculate annealing temperature of primers whose sequences and concentrations are known but the annealing temperature is not known.

4.8 RECOMMENDATION

Primers from eggplant, tomatoes and potatoes have not been evaluated and reported to be successful in the evaluation of African nightshade cultivars. It is therefore necessary for SSR primers specific for African nightshades to be designed for further molecular work to avoid low primer amplification and polymorphism.

CHAPTER FIVE

5.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the findings of this study shows that there is rich diversity both within and among African nightshade species which can be exploited for further breeding work. From these studies, it has been shown that cultivars belonging to the *S. scabrum* species such as AVRDC-TZ (SS 49), AVRDC-TZ (BG 14), AVRDC-TZ (BG 22), AVRDC-TZ (BG 23), Managu from Nakuru county and improved type were the best performers since they produced numerous branches which could translate into higher leaf yield and can therefore be recommended for commercial production.

For effective African nightshade genetic diversity assessment to be achieved, it is recommended to combine both morphological and genotypic characterization of cultivars and this could also assist in the identification of genotypes which have been stored in the gene bank under different names whereas they are actually the same genotype but collected from different sources.

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APPENDICES

Appendix 1: Analysis of variance for plant height of African nightshade cultivars grown in Kisii season 1

Variate: Plant_height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	137.9	69.0	0.36	
Rep.*Units* stratum					
Cultivar	18	17189.1	954.9	5.01	<.001
Residual	36	6857.8	190.5		
Total	56	24184.9			

Appendix 2: Analysis of variance for internode length of African nightshade cultivars grown in Kisii season 1

Variate: Internode_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.9016	0.9508	1.19	
Rep.*Units* stratum					
Cultivar	18	296.3720	16.4651	20.60	<.001
Residual	36	28.7709	0.7992		
Total	56	327.0445			

Appendix 3: Analysis of variance for leaf length of African nightshade cultivars grown in Kisii season 1

Variate: Leaf_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	4.908	2.454	0.47	
Rep.*Units* stratum					
Cultivar	18	1250.853	69.492	13.24	<.001
Residual	36	189.011	5.250		
Total	56	1444.772			

Appendix 4: Analysis of variance for leaf width of African nightshade cultivars grown in Kisii season 1

Variate: Leaf_width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.782	0.391	0.37	
Rep.*Units* stratum					
Cultivar	18	504.495	28.028	26.51	<.001
Residual	36	38.058	1.057		
Total	56	543.335			

Appendix 5: Analysis of variance for leaf size of African nightshade cultivars grown in Kisii season 1

Variate: Leaf size

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	0.5005	0.2502	1.52	
Rep_No.*Units* stratum					
Cultivar	18	11.5814	0.6434	3.90	<.001
Residual	36	5.9373	0.1649		
Total	56	18.0191			

Appendix 6: Analysis of variance for number of primary branches of African nightshade genotypes grown in Kisii season 1

Variate: number of primary branches

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	20.632	10.316	1.85	
Rep_No.*Units* stratum					
Cultivar	18	910.246	50.569	9.07	<.001
Residual	36	200.702	5.575		
Total	56	1131.579			

Appendix 6: Analysis of variance for number of berries per panicle for African nightshade cultivars grown in Kisii season 1

Variate: Number of berries per panicle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0628	0.0314	0.22	
Rep.*Units* stratum					
Cultivar	18	78.8523	4.3807	30.16	<.001
Residual	36	5.2297	0.1453		
Total	56	84.1447			

Appendix 6: Analysis of variance for plant height of African nightshade cultivars grown in Kisii season 2

Variate: Plant_height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	86.75	43.38	0.48	
Rep.*Units* stratum					
Cultivar	18	24048.49	1336.03	14.85	<.001
Residual	36	3239.39	89.98		
Total	56	27374.64			

Appendix 7: Analysis of variance for internode length of African nightshade cultivars grown in Kisii season 2

Variate: Internode_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.5564	0.2782	0.65	
Rep.*Units* stratum					
Cultivar	18	262.5947	14.5886	34.32	<.001
Residual	36	15.3045	0.4251		
Total	56	278.4556			

Appendix 8: Analysis of variance for number of primary branches of African nightshade cultivars grown in Kisii season 2

Variate: Number of primary branches

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.326	0.163	0.10	
Rep.*Units* stratum					
Cultivar	18	1092.014	60.667	36.57	<.001
Residual	36	59.730	1.659		
Total	56	1152.070			

Appendix 9: Analysis of variance for leaf length of African nightshade cultivars grown in Kisii season 2

Variate: Leaf_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	7.214	3.607	1.00	
Rep.*Units* stratum					
Cultivar	18	1280.570	71.143	19.66	<.001
Residual	36	130.287	3.619		
Total	56	1418.070			

Appendix 10: Analysis of variance for leaf size of African nightshade cultivars grown in Kisii season 2

Variate: Leaf_length_width_ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.018996	0.009498	1.49	
Rep.*Units* stratum					
Cultivar	18	12.591294	0.699516	109.61	<.001
Residual	36	0.229753	0.006382		
Total	56	12.840043			

Appendix 11: Analysis of variance for leaf width of African nightshade cultivars grown in Kisii season 2

Variate: Leaf_width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.7159	1.3580	1.63	
Rep.*Units* stratum					
Cultivar	18	492.0437	27.3358	32.90	<.001
Residual	36	29.9108	0.8309		
Total	56	524.6704			

Appendix 12: Analysis of variance for number of berries per panicle of African nightshade cultivars grown in Kisii season 2

Variate: Number of berries per panicle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.15112	0.07556	1.15	
Rep.*Units* stratum					
Cultivar	18	83.89728	4.66096	70.85	<.001
Residual	36	2.36820	0.06578		
Total	56	86.41660			

Appendix 13: Combined Analysis of variance for internode length of African nightshade cultivars grown in Kisii season 1 and 2

Variate: Internode_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	2.0312	1.0156	1.69	
Rep_No.*Units* stratum					
Cultivar	18	545.8475	30.3249	50.43	<.001
Seasons	1	0.0789	0.0789	0.13	0.718
Cultivar x Seasons	18	13.1192	0.7288	1.21	0.274
Residual	74	44.5022	0.6014		
Total	113	605.5790			

Appendix 14: Combined Analysis of variance for plant height of African nightshade cultivars grown in Kisii season 1 and 2

Variate: Plant_height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	39.5	19.7	0.14	
Rep_No.*Units* stratum					
Cultivar	18	38222.2	2123.5	15.28	<.001
Seasons	1	372.9	372.9	2.68	0.106
Cultivar x Seasons	18	3015.3	167.5	1.21	0.279
Residual	74	10282.4	139.0		
Total	113	51932.4			

Appendix 15: Combined Analysis of variance for leaf length of African nightshade cultivars grown in Kisii season 1 and 2

Variate: Leaf_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	3.355	1.678	0.38	
Rep_No.*Units* stratum					
Cultivar	18	2521.802	140.100	31.60	<.001
Seasons	1	2.002	2.002	0.45	0.504
Cultivar x Seasons	18	9.620	0.534	0.12	1.000

Residual	74	328.065	4.433		
Total	113	2864.845			

Appendix 16: Combined Analysis of variance for leaf width of African nightshade cultivars grown in Kisii season 1 and 2

Variate: Leaf_width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	1.3595	0.6797	0.72	
Rep_No.*Units* stratum					
Cultivar	18	994.7029	55.2613	58.33	<.001
Seasons	1	0.2018	0.2018	0.21	0.646
Cultivar x Seasons	18	1.8360	0.1020	0.11	1.000
Residual	74	70.1070	0.9474		
Total	113	1068.2072			

Appendix 17: Combined Analysis of variance for number of berries per panicle of African nightshade cultivars grown in Kisii season 1 and 2

Variate: Number of berries per panicle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	0.1042	0.0521	0.50	
Rep_No.*Units* stratum					
Cultivar	18	159.5940	8.8663	85.13	<.001
Seasons	1	0.0763	0.0763	0.73	0.395
Cultivar x Seasons	18	3.1556	0.1753	1.68	0.062

Residual	74	7.7076	0.1042
Total	113	170.6376	

Appendix 18: Combined Analysis of variance for number of primary branches of African nightshade cultivars grown in Kisii season 1 and 2

Variate: Number of primary branches

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	7.945	3.973	1.08	
Rep_No.*Units* stratum					
Cultivar	18	1713.340	95.186	25.76	<.001
Seasons	1	221.680	221.680	59.99	<.001
Cultivar x Seasons	18	288.920	16.051	4.34	<.001
Residual	74	273.444	3.695		
Total	113	2505.329			

Appendix 19: Combined Analysis of variance for leaf size of African nightshade cultivars grown in Kisii season 1 and 2

Variate: Leaf size

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	0.044404	0.022202	2.42	
Rep_No.*Units* stratum					
Cultivar	18	24.496466	1.360915	148.18	<.001
Seasons	1	0.020011	0.020011	2.18	0.144
Cultivar x Seasons	18	0.122710	0.006817	0.74	0.757
Residual	74	0.679611	0.009184		
Total	113	25.363201			

Appendix 20: Analysis of variance for plant height of African nightshade cultivars grown in Muguga season 1

Variate: Plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	325.6	325.6	1.98	
Rep.*Units* stratum					
Cultivar	18	11043.3	613.5	3.73	0.004
Residual	18	2963.9	164.7		
Total	37	14332.8			

Appendix 21: Analysis of variance for internode length of African nightshade cultivars grown in Muguga season 1

Variate: Internode Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	51.15	51.15	3.15	
Rep* Units* stratum					
Cultivar	18	437.78	24.32	1.50	0.200
Residual	18	292.42	16.25		
Total	37	781.35			

Appendix 22: Analysis of variance for number of primary branches of African nightshade cultivars grown in Muguga season 1

Variate: Number of primary branches

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Rep stratum	1	155.18	155.18	7.70	
Rep.*Units* stratum					
Cultivar	18	991.61	55.09	2.73	0.020
Residual	18	362.58	20.14		
Total	37	1509.37			

Appendix 23: Analysis of variance for leaf length of African nightshade cultivars grown in Muguga season 1

Variate: Leaf_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	6.262	6.262	1.00	
Rep.*Units* stratum					
Cultivar	18	873.294	48.516	7.76	<.001
Residual	18	112.600	6.256		
Total	37	992.156			

Appendix 24: Analysis of variance for leaf length of African nightshade cultivars grown in Muguga season 1

Variate: Leaf width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	13.980	13.980	2.55	
Rep.*Units* stratum					
Cultivar	18	321.990	17.888	3.27	0.008
Residual	18	98.504	5.472		
Total	37	434.474			

Appendix 25: Analysis of variance for leaf size of African nightshade cultivars grown in Muguga season 1

Variate: Leaf size

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Rep stratum	1	1.2774	1.2774	5.63	
Rep.*Units* stratum					
Cultivar	18	9.4789	0.5266	2.32	0.041
Residual	18	4.0811	0.2267		
Total	37	14.8374			

Appendix 26: Analysis of variance for number of berries per panicle of African nightshade cultivars grown in Muguga season 1

Variate: Number of berries per panicle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.0269	0.0269	0.10	
Rep.*Units* stratum					
Cultivar	18	50.8257	2.8237	10.82	<.001
Residual	18	4.6961	0.2609		
Total	37	55.5487			

Appendix 27: Analysis of variance for plant height of African nightshade cultivars grown in Muguga season 2

Variate: Plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep No stratum	2	10.081	5.041	1.03	
Rep No.*Units* stratum					
Cultivar	18	28380.379	1576.688	322.84	<.001
Residual	36	175.819	4.884		
Total	56	28566.279			

Appendix 28: Analysis of variance for internode length of African nightshade cultivars grown in Muguga season 2

Variate: Internode_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	2.627	1.313	0.86	

Rep_No.*Units* stratum					
Cultivar	18	394.935	21.941	14.37	<.001
Residual	36	54.962	1.527		
Total	56	452.524			

Appendix 29: Analysis of variance for leaf length of African nightshade cultivars grown in Muguga season 2

Variate: Leaf length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep No stratum	2	0.573	0.286	0.22	
Rep No.*Units* stratum					
Cultivar	18	2001.483	111.193	85.68	<.001
Residual	36	46.720	1.298		
Total	56	2048.776			

Appendix 30: Analysis of variance for leaf width of African nightshade cultivars grown in Muguga season 2

Variate: Leaf width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep No stratum	2	4.136	2.068	1.57	
Rep No.*Units* stratum					
Cultivar	18	624.846	34.714	26.43	<.001
Residual	36	47.284	1.313		
Total	56	676.266			

Appendix 31: Analysis of variance for leaf size of African nightshade cultivars grown in Muguga season 2

Variate: Leaf size

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	0.5005	0.2502	1.52	
Rep_No.*Units* stratum					

Cultivar	18	11.5814	0.6434	3.90	<.001
Residual	36	5.9373	0.1649		
Total	56	18.0191			

Appendix 32: Analysis of variance for number of primary branches of African nightshade cultivars grown in Muguga season 2

Variate: Number of primary branches

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep No stratum	2	7.423	3.711	1.16	
Rep No.*Units* stratum					
Cultivar	18	2063.816	114.656	35.95	<.001
Residual	36	114.812	3.189		
Total	56	2186.051			

Appendix 33: Analysis of variance for number of berries per panicle of African nightshade cultivars grown in Muguga season 2

Variate: Number of berries per panicle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	0.1711	0.0855	0.36	
Rep_No.*Units* stratum					
Cultivar	18	86.1791	4.7877	20.16	<.001
Residual	36	8.5476	0.2374		
Total	56	94.8978			

Appendix 34: Combined Analysis of variance for plant height of African nightshade cultivars grown in Muguga season 1 and 2

Variate: Plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep No stratum	1	226.98	226.98	2.67	
Rep No.*Units* stratum					
Cultivar	18	26230.59	1457.26	17.15	<.001

Season	1	2430.33	2430.33	28.60	<.001
Cultivar x Season	18	3481.68	193.43	2.28	0.017
Residual	37	3143.87	84.97		
Total	75	35513.46			

Appendix 35: Combined Analysis of variance for internode length of African nightshade cultivars grown in Muguga season 1 and 2

Variate: Internode_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep No stratum	1	20.030	20.030	2.18	
Rep No.*Units* stratum					
Cultivar	18	554.440	30.802	3.36	<.001
Season	1	4.133	4.133	0.45	0.506
Cultivar x Season	18	180.740	10.041	1.09	0.395
Residual	37	339.569	9.178		
Total	75	1098.912			

Appendix 36: Combined Analysis of variance for leaf width of African nightshade cultivars grown in Muguga season 1 and 2

Variate: Leaf width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep No stratum	1	7.368	7.368	2.54	
Rep No.*Units* stratum					
Cultivar	18	701.532	38.974	13.44	<.001
Season	1	0.582	0.582	0.20	0.657
Cultivar x Season	18	61.667	3.426	1.18	0.324
Residual	37	107.267	2.899		
Total	75	878.416			

Appendix 37: Combined Analysis of variance for leaf size of African nightshade cultivars grown in Muguga season 1 and 2

Variate: Leaf size

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep No stratum	1	0.9366	0.9366	5.43	
Rep No.*Units* stratum					
Cultivar	18	15.5566	0.8643	5.01	<.001
Season	1	0.4819	0.4819	2.80	0.103
Cultivar x Season	18	3.0095	0.1672	0.97	0.511
Residual	37	6.3782	0.1724		
Total	75	26.3628			

Appendix 38: Combined Analysis of variance for number of berries per panicle of African nightshade cultivars grown in Muguga season 1 and 2

Variate: Number of berries per panicle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	1	0.0032	0.0032	0.01	
Rep_No.*Units* stratum					
Cultivar	18	105.0354	5.8353	21.92	<.001
Season	1	1.2884	1.2884	4.84	0.034
Cultivar x Season	18	2.7318	0.1518	0.57	0.898
Residual	37	9.8483	0.2662		
Total	75	118.9070			

Appendix 39: Combined Analysis of variance for number of primary branches of African nightshade cultivars grown in Muguga season 1 and 2

Variate: Number of primary branches

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	1	55.21	55.21	3.84	
Rep_No.*Units* stratum					
Cultivar	18	2233.96	124.11	8.64	<.001
Season	1	9.96	9.96	0.69	0.410
Cultivar x Season	18	131.73	7.32	0.51	0.936
Residual	37	531.46	14.36		
Total	75	2962.33			

Appendix 40: Combined Analysis of variance for leaf length of African nightshade cultivars grown in Muguga season 1 and 2

Variate: Leaf length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep No stratum	1	0.981	0.981	0.29	
Rep No.*Units* stratum					
Cultivar	18	2081.094	115.616	33.88	<.001
Season	1	18.266	18.266	5.35	0.026
Cultivar x Season	18	106.440	5.913	1.73	0.078
Residual	37	126.281	3.413		
Total	75	2333.062			

Appendix 41: Combined Analysis of variance for plant height of African nightshade cultivars grown in both Mugugа and Kisii

Variate: Plant_height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	33.280	16.640	1.83	
Rep_No.*Units* stratum					
Cultivar	18	53023.480	2945.749	324.46	<.001
Location	1	629.591	629.591	69.35	<.001
Cultivar x Location	18	772.941	42.941	4.73	<.001
Residual	74	671.833	9.079		
Total	113	55131.125			

Appendix 42: Combined Analysis of variance for leaf length of African nightshade cultivars grown in both Mugugа and Kisii

Variate: Leaf_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep No stratum	2	0.0094	0.0047	0.01	
Rep No *Units* stratum					
Cultivar	18	3511.3126	195.0729	209.67	<.001
Location	1	53.8134	53.8134	57.84	<.001
Cultivar x Location	18	29.8433	1.6580	1.78	0.044
Residual	74	68.8470	0.9304		
Total	113	3663.8258			

Appendix 43: Combined Analysis of variance for leaf width of African nightshade cultivars grown in both Muguga and Kisii

Variate: Leaf_width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr
Rep No stratum	2	2.6685	1.3343	1.86	
Rep No.*Units* stratum					
Cultivar	18	1174.9545	65.2753	90.77	<.001
Location	1	8.8533	8.8533	12.31	<.001
Cultivar x Location	18	10.5186	0.5844	0.81	0.680
Residual	74	53.2163	0.7191		
Total	113	1250.2112			

Appendix 44: Combined Analysis of variance for leaf size of African nightshade cultivars grown in both Muguga and Kisii

Variate: Leaf size

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_No stratum	2	0.40843	0.20422	2.31	
Rep_No.*Units* stratum					
Cultivar	18	23.65714	1.31429	14.83	<.001
Location	1	0.02004	0.02004	0.23	0.636
Cultivar x Location	18	1.13136	0.06285	0.71	0.791
Residual	74	6.55610	0.08860		
Total	113	31.77308			

Appendix 45: Combined Analysis of variance for number of primary branches of African nightshade cultivars grown in both Muguga and Kisii

Variate: Number of primary branches

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep No stratum	2	10.163	5.082	2.60	
Rep No.*Units* stratum					
Cultivar	18	2726.500	151.472	77.48	<.001
Location	1	226.419	226.419	115.82	<.001
Cultivar x Location	18	266.738	14.819	7.58	<.001
Residual	74	144.669	1.955		
Total	113	3374.490			

Appendix 46: Combined Analysis of variance for number of berries per panicle of African nightshade cultivars grown in both Muguga and Kisii

Variate: Number of berries per panicle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep No stratum	2	0.2234	0.1117	0.84	
Rep No.*Units* stratum					
Cultivar	18	168.4408	9.3578	70.70	<.001
Location	1	3.0854	3.0854	23.31	<.001
Cultivar x Location	18	2.5251	0.1403	1.06	0.408
Residual	74	9.7943	0.1324		
Total	113	184.0690			