Genetic Characterization and nutritional analysis of Eastern and South African *Cleome Gynandra* (spider plant) accessions

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DECLARATION

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This thesis is dedicated to my family for their unwavering all rounded support, true love and patience during my study years.

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ABBREVIATIONS

ALVs	African leafy vegetables
ANOVA	Analysis of variance
DARwin	Dissimilarity analysis representation for windows
FAO	Food and Agriculture Organization of the United Nations
GBK	Genebank of Kenya
GDP	Gross domestic product
GMATo	Genome wide Microsatellite Analyzing Tool
HCD	Horticultural Crops Directorate
ITC	International Trade Centre
JKUAT	Jomo Kenyatta university of Agriculture and Technology
KARI	Kenya Agricultural Research Institute
KEPHIS	Kenya plant health inspectorate service
MOA	Ministry of Agriculture
MT	Metric tonnes
NCBI	National Center for Biotechnology Information
PCR	Polymerized chain reaction
SPAD	Soil Plant Analysis Development
SSRs	Simple sequence repeats
UPGMA	Unweighted pair-group method using arithmetic averages

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ABSTRACT

Sub-Saharan Africa is faced with a rapidly growing population, malnutrition, human diseases, increased food prices, political instability and high inflation. This calls for a multifaceted approach in bringing about food and nutritional security. African indigenous vegetables play a highly significant role in food security by providing valuable sources of energy and micronutrients to the underprivileged, in both urban and rural settings. Spider plant (*Cleome gynandra),* commonly known as African Spider Flower or Spider wisp, is an important leafy vegetable in most parts of Africa. It is highly nutritive and contains health promoting bioactive compounds important in combating malnutrition and reducing human degenerative diseases. Despite continued increase in production and consumption, there has been limited efforts towards its improvement. The aim of this study was: a) To determine the extent of phenotypic variation among selected Kenyan and South African spider plant accessions. b) To establish their nutrition composition for future improvement. c) To identify microsatellites or Simple Sequence Repeat (SSR) markers from a close relative of *C. gynandra* for which whole genome sequence is available, (*Tarenaya hassleriana)* that can be subsequently used for marker-assisted selection in *C. gynandra*. A total of 49 spider plant accessions were planted in the field at the University of Nairobi Field Station in a randomized complete block design with three replications for two seasons. Characterization of the spider plant accessions for various qualitative traits was done based on the list of modified Food and Agriculture Organization (FAO) spider plant descriptors. Days to 50% flowering, chlorophyll content, plant height, leaf length, leaf width, single leaf area, and number of leaves per plant were evaluated as quantitative traits while stem and flower colour, petiole and leaf colour, stem and petiole hairiness were evaluated as qualitative traits. Nutrition components vitamin C, beta carotene and total phenolics were determined at the

nutrition laboratories at the Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology. Broad sense heritability was estimated for the nutrition and yield related components. For bioinformatics analysis, the whole genome sequence of the close relative, *T. hassleriana* was searched for Simple Sequence Repeats (SSRs) using the Genome wide Microsatellite Analyzing Tool (GMATo) software with the objective of subsequently transferring these markers into *C. gynandra* for marker-assisted breeding. The parameters used for GMATo search were di-, tri-, tetra-, penta-, and hexa-nucleotide motifs. Estimates of Shannon-Weaver diversity index (H') for the qualitative characters were generally high (H' > 0.500) indicating a greater inter-country than intra-country diversity. Unweighted Pair Group Method with Arithmetic mean (UPGMA) clustering method using stem and flower colour traits clustered the accessions into 2 major groups, each group consisting of Kenyan and South African accessions. Analysis of variance (ANOVA) showed significant difference for all the quantitative traits at P <0.05. Positive correlation was observed between leaf yield and chlorophyll content (r=0.45), plant height (r=0.69) and number of primary branches (r=0.63). There was a wide variation in vitamin C (63-281 mg/100g) content followed by beta-carotene (0.6-7.2 mg/100g) and total phenols (3.1-10.6 mg/100g). High heritability in broad sense heritability was estimated ranging between 78% and 99% among the quantitative traits with number of leaves per plant and plant height highest at 99% while leaf width exhibited the lowest percentage at 78%. Nutrition components namely; -Vitamin C, beta carotene and phenols expressed a high heritability >80%. Bioinformatics analysis revealed a total of 76280 SSR motifs in the *T. hassleriana* genome. Dinucleotides were the most common SSRs at 88.1% (67192/76280) with AT being the most abundant motif at 39.9% (26862/67192). Statistical analysis in this study revealed a great variability among evaluated traits implying diversity

among the accessions. The presence of a considerable degree of compositional variability of the nutraceuticals among tested accessions suggests that spider plant can be a valuable source of genes for improvement of spider plant for vitamin and phenolic content. Considering all genetic parameters, selection based on vitamin C, phenols, beta carotene, plant height, number of leaves per plant and single leaf area would be effective for the improvement of nutrition quality and yield in vegetable spider plant. With the absence of *C. gynandra* whole genome sequence, cross-species transfer of molecular markers from a close relative such as *T. hassleriana* is the first step towards the development of molecular markers in *Cleome* for more efficient crop improvement.

CHAPTER 1

INTRODUCTION

1.1 Background information

Agriculture has a multifunctional role to play in economies. It is the main source of economic growth in Kenya and most of the sub-Saharan Africa. According to MOA, (2010) the Agricultural sector contributes about 30% of the gross domestic product and constitutes to over 75% of the total work force. Horticultural Crops Directorate of Kenya recorded that vegetables contributed over 40% of the total value of horticultural production between 2011 and 2013 (HCD, 2014). Production, trade and consumption of indigenous african leafy vegetables is expanding on the African continent as a whole (Schippers, 2006).

African indigenous vegetables (AIVs) are vegetables whose primary or secondary center of origin is known to be in Africa, and serve an important role in food and nutrition security (Schippers, 2000). The edible wild leafy vegetables are vital in the african agricultural and nutritional systems (Van and Gericke, 2000). Most commonly consumed indigenous vegetables on the African continent are vegetable amaranths, (*Amaranthus* species), spider plant (*Cleome gynandra*), African nightshades (*Solanum* species), cowpeas (*Vigna unguiculata*), African eggplant (*Solanum aethiopicum*), African kale (*Brassica carinata*) and jute mallow (*Corchoru solitorius*) (Schippers, 2000). These vegetables help to improve nutrition, increase income, restore agricultural biodiversity and preserve local cultures. Indigenous vegetables have the potential to contribute to poverty alleviation and nutritional security because they are easy to grow, require minimum production inputs, are rich in vitamins and minerals, phytochemicals and anti-oxidant properties. Among these indigenous vegetables, spider plant has been found to be highly nutritious and mostly consumed in Sub-Saharan Africa. Spider plant (*Cleome gynandra*)

is generally considered a weed, but it is highly nutritious, has medicinal value, and is well adapted to many African ecosystems. It is rich in vitamins and micronutrients, such as calcium, magnesium, iron besides having high levels of beta-carotene and vitamin C (Mahyao et al., 2008*)* hence contributing to a healthy diet for many rural Africans with limited food budgets. Also, spider plant has been reported to have medicinal properties like to aid constipation and facilitate birth (Olembo et al., 1995).

Cleome species is both self-and cross-pollinated which is mostly pollinated by insects (especially honey bees), spiders and the wind (Omondi, 1990). Few genetic studies have been done despite the species being widely distributed in Africa and for this case little breeding work has been reported in the literature on spider plant. Farmers use their own local selections/advances for production resulting in low productivity which cannot be commercialized. Therefore there is need for germplasm collection of different varieties for breeding programs. Different species of *cleome* exist with different characteristics. Among the different *Cleome* species that occur, *Cleome gynandra* is the most widely used as a leafy vegetable but *C. monophylla* and *C. hirta*, *C. hassleriana* which are close relatives, are also used occasionally (Vorster et al., 2002; Hart and Vorster, 2006). In Kenya, some of the landrace varieties grown lack consumption appeal for market due to lack of superior preferences like palatability due to taste, pubescence among others. In an attempt of improving this vegetable, morphological and agronomic characters have been used in characterization of the landraces conventionally (K'Opondo, 2011). This has given a great variation especially when there is an influence of the environment on the agronomic characters, hence it has not been too successful in characterizing the existing *Cleome* species in Africa. Therefore, a major potential to unveil this variation lies in characterizing at genomic level. Genetic resources play a very important role in breeding superior varieties and promoting

production of *Cleome spp*. Genetic markers have shown important and critical application in the assessment and conservation of genetic variation. Establishing unique DNA profiles for selections, will aid to identify definite varieties as well as determine their genetic relationship in an advanced breeding program. Previous research has shown some of the key traits that would aid in genetic improvement of *C. gynandra*. For instance, higher leaf yield, plant uniformity, longer vegetative phase, late flowering and drought tolerance (Omondi, 1990).

Phenotypic characterization is also necessary to establish key traits for selection. Therefore, assembling accessions in Africa and determining their phenotypic characters and nutritional composition will provide a good basis for spider plant improvement for nutrition and important agronomic traits.

1.2 Problem statement

Malnutrition is an issue of concern in Kenya (GoK, 1999) and other countries in Sub-Saharan Africa. Inadequate consumption of micronutrients normally referred to as 'hidden hunger' results in serious malnutrition problems like impaired intellectual development, anaemia, blindness, and mortality in children. This is linked to other complications like cardiovascular diseases, diabetes, obesity and decreased worker productivity (Abukutsa-Onyango, 2003). According to the Food and Agriculture Organization (FAO) of the United Nations, around 868 million people (12.5% of the world's population) are undernourished in terms of energy intake, and 2 billion people suffer from one or more micronutrients deficiencies (FAO, 2013). Agriculturalists and researchers in the past have regarded African indigenous vegetables as weeds, hence eradicating them other than conserving them.

Agricultural research has traditionally focused on staple foods, relatively giving little attention to minor or orphaned crops. Little research has been done on indigenous vegetables and in particular on *Cleome* species, especially in regard to germplasm collection. More also, the extent of diversity in spider plant is still unknown (Legwaila et al., 2011). *Cleome gynandra* has gained importance as a nutritious vegetable to reduce the underlying problem of malnutrition among the resource poor household in Africa but little improvement has been done on it. Previous research has concentrated on phenotypic characterization and seed bulking however genetic diversity and nutritional composition has not been exploited.

Important agronomic traits such as low leaf yields and unpalatability have also been a challenge in improvement of spider plant. Crop improvement in *Cleome gynandra* will require knowledge of the existing phenotypic and genetic diversity. For instance, the plant leaf yield is quantitative in nature with several leaf yield components (Chweya, 1997). Therefore, it is necessary to understand the phenotypic characters of existing *Cleome* species across Africa and identify the genetic diversity within the accessions to determine an efficient breeding strategy.

1.3 Justification

Genetic and genomic resources supporting *Cleome gynandra* breeding are quite limited, because few studies on *Cleome* have been conducted so far. There exists a lot of variation among the landraces due to natural hybridization which could be exploited for crop improvement. Evolutionary adaptations usually result in mutations and shifts in the genetic makeup of individuals (Falconer, 1989). These adaptations are often genetically controlled, highly heritable and play a big role in breeding. Marker assisted selection is the current focus in crop

improvement programs. However, little is known about simple sequences repeats that are known to be highly dominant in plant genomes, which could be resourceful in directing molecular studies aimed at spider plant breeding. Therefore, identifying such molecular markers within the genome of existing spider plant accessions will facilitate efficient selection of target traits for nutritional value and yield.

A deliberate effort to collect and conserve *C. gynandra* germplasm is important so as to facilitate systematic evaluation and breeding/selection programs that are important for genetic enhancement of valuable traits. Morphological and genetic characterization will generate new knowledge on its genetic structure enhancing identification and discrete accession evaluation. Knowledge of phenotypic characters will enable the introgression of important yield and nutritional genes into adapted *Cleome gynandra* accessions to fight the 'hidden hunger'. Also, it will help in selection of superior parents which could be used to develop mapping populations for use by breeders to determine the genetics of the yield and nutritional traits.

Malnutrition and other diseases due to micronutrients deficiencies should be overcome while consuming leafy vegetables and particularly spider plant because of their nutritional composition (Van Rensburg et al., 2004). Enough genetic variation still exists in spider plant and therefore should be exploited in a breeding program to improve important agronomic traits.

1.4 Objectives

The main objective of this study was to contribute to *Cleome* improvement through documenting the existing genetic diversity within the *C. gynandra* via phenotypic and molecular characterization.

1.4.1 Specific objectives

1. To determine the phenotypic variation among different East and South African *Cleome gynandra* accessions.
2. To determine the nutrition composition and yield related traits among the assorted *Cleome gynandra* accessions.
3. To identify simple sequence repeats (SSRs) for *Cleome gynandra* through comparative genomics.

1.4.2 Hypothesis

1. There are no agronomic and morphological differences between Kenyan and South African *Cleome gynandra* accessions.
2. There are no variations in nutrition composition and yield related traits among assorted *Cleome gynandra* accessions.
3. There are no simple sequence repeats (SSRs) in the *Tarenaya hassleriana* genome.

CHAPTER 2

LITERATURE REVIEW

2.1 Botany of spider plant

The spider plant *(Cleome gynandra)* also commonly known as spider flower plant, African spider flower or cats' whiskers, is one of the species indigenous to Africa (Schippers, 2002). It is an important leafy vegetable in both the eastern and southern parts of Africa. Locally in Kenya its known as Mwangani (Swahili), Thageti (Kikuyu), Tsisaka (Luhya), Alot-dek (Luo), Saget (Kalenjin), Chinsaga (Kisii), Mwianzo (Kamba), Jjobyu (Luganda) and Yobyu (Lusoga).

It is an annual wildflower native to Africa but has become widespread in many tropical and sub-tropical parts of the world. Spider plant originated in Africa and tropical Asia but now has a worldwide distribution (Schippers, 2002). It belongs to the botanical family Capparaceae, subfamily Cleomoideae (Kuhn, 1988; Kokwaro, 1994) with a Chromosome number of 2n = 30, 32, 34, 36 and polyploid. Spider plant is an herbaceous, erect, and annual plant that grows to a height of between 0.5 m and 1.5 m, depending on the environment, branched sometimes becoming woody with age (Chweya, 1995).

Spider plant has both green and purple stems with the latter being reported as more nutritious and resistant to insects, but more susceptible to diseases than those with the green stem (Silué, 2009). The leaves of spider plant are compound and palmate consisting of three to seven leaflets. The stem and petiole are sticky and mostly with glandular hairs and rarely glabrous. Inflorescences have terminal raceme that elongate into a fruit, which is a spindle-shaped green capsule that turns yellow when ripe, and dehisce easily when dry, to release seeds.

They have conspicuous white flowers, but pink and lilac colored flowers also occur (Van Wyk and Gericke, 2000). The seeds of spider plant are greyish to black and circular in outline, resembling shell of a snail. The seed coat is brittle with curved worm like embryos enveloped in semi-permeable cell membrane (Ochuodho, 2005).

2.2 Origin and distribution

Cleome species is thought to have originated in tropical Africa and Southeast Asia, with further spread to other tropical and subtropical countries in the Northern and Southern hemispheres (Schippers, 2002), but now has a worldwide distribution. The spread is associated with seed dispersal, and by birds due to capsule dehiscence. In the southern Africa region, the distribution extends from the Limpopo, the North-West, Gauteng, Mpumalanga, KwaZulu-Natal, Free State, the Northern Cape and Namibia. (DAFF, 2010). In Kenya, spider plant is chiefly found in Western, Rift valley, Eastern and Coastal regions. Among the main counties producing the plant are: Kisii, Nyamira, Kericho, Migori and Siaya (HCDA, 2014).

2.3 Ecologicalrequirement

Spider plant grows well in altitudes of up to 1 000 m above sea level in semiarid, sub-humid and humid climates. It requires temperatures of 18 °C to 25 °C and high light intensity but it is sensitive to cold. *Cleome* prefers well-drained medium-textured soils with a PH range of 5.5 to 7. 0 and performs poorly in heavy clay soils. The plant tolerates a degree of water stress, but prolonged water stress hastens flowering and senescence. Due to this, spider plant can grow in areas with short periods of useful rainfall. Leaf yield and quality is reduced by water stress and the plants cannot withstand flooding (Chweya and Mnzava, 1997).

2.4 Cultivation and utilization

Spider plant is a C4 plant capable of withstanding high daytime temperatures, intense sunlight and drought. It has got a Kranz-type leaf anatomy with a higher activity of phosphoenolpyruvate carboxylase (Feodorova et al., 2010). Previous research has considered spider plant to be day length-insensitive due to its tropical origin (Iltis, 1967). It grows well up to about 1000 m above sea level in semi-arid, sub humid and humid climates, and is adapted to many African ecosystems (Kumar et al., 1984). The plant is regarded as a weed, or 'volunteer' crop in most farmers' fields. In Africa, it is generally collected from the wild, although there is some limited cultivation of commercial leafy varieties in several countries in East and Southern Africa, (Edmonds and Chweya, 1997). Propagation is by seed on well-loosened fertile soil. The plants may also be grown on raised or flat beds. Germination takes 4-5 days. Thinning on the seedling is done about 3 weeks later and used as a vegetable. Leaf production for harvesting is increased by topping and removing inflorescences as soon as they appear. Tender leaves, stems, pods, and flowers are consumed as vegetables in most African and several South Asian countries. They are utilized mostly as an accompaniment to carbohydrate staples.

2.5 Constrains to spider plant production in Kenya and South Africa

Lack of knowledge on existing genetic diversity is a major challenge in improving *Cleome gynandra*. There has been neglect and stereotyping whereby the plant is regarded as a weed, and for the poor in the society (Mnzava, 1997). Another setback in spider plant improvement is lack of awareness of the merits and nutrition value of the plant hindering its full production and utilization (Mwai et al., 2007). Furthermore, exotic species, despite their inferior nutrient content are promoted in favour of indigenous vegetables like spider plant in the view that they fetch a

higher income (Adebooye and Opabote, 2004). This has resulted in indigenous vegetables being neglected and mostly used for subsistence use.

Production of *Cleome gynandra* in Africa is mainly done by smallholder farmers who use their own seed from season to season, with inadequate knowledge on how to produce, store and distribute seeds among themselves. This leads to poor quality of seed in terms of purity, viability and seed dormancy issues (Abukutsa, 2007). Further reports have indicated that the high cost of certified seeds of some indigenous vegetables has had a negative impact on massive production of these vegetables (Masayi and Netondo, 2012). Uncertain rainfall and lack of irrigation facilities in Sub Saharan Africa is a setback in spider plant cultivation as it is largely susceptible to water stress (Pachpute, 2010). Lack of adequate funding has led to limited research on indigenous vegetables especially setting up breeding programs in spider plant production. Fading traditional systems of plant resource management and culture erosion are resulting to the loss of adaptation of most indigenous vegetables (Kimiywe et al., 2007). Additionally, geographical location and cultural background remain a challenge in the adoption of these vegetables (Kimiywe et al., 2007; Uusiku et al., 2010). Other challenging factors include change in taste and preferences especially among the youth who lack interest in consuming these vegetables as they are considered traditional and outdated (Masayi and Netondo, 2012).

Pests and diseases are also a challenge in spider plant. For instance, nematodes (*Meloidogyne* spp.), flea beetles (*Podagrica* spp.), green vegetable bugs (*Nezara* spp.), pentatomids (*Acrosternum gramineum* and *Agonoselis nubilis*) and their parasitoids, locusts (*Schistocera gregaria*, cabbage sawfly (*Athalia* spp.), cotton jassids (*Empoasca* spp.) and hurricane bugs

(*Bagrada* spp.). *Cleome* seedlings may also be attacked by Slugs and snails while the seeds are eaten by birds especially the weaver bird (*Quelea quelea*). Due to lack of dense foliage in spider plant, weeds like thorn apple (*Datura stramonium*), couch grass (*Elymus repens*) and oxalis (*Oxalis sorrel*) are detrimental since the plant cannot compete with them. Spider plant is also prone to diseases like mildew fungus (*Sphaerotheca fuliginea*) and leaf spots (*Cercospora uramensis)* (Chweya, 1997; Mbugua et al., 2007). Pest and diseases may be controlled by application of insecticides and pesticides. Due to inadequate characterization, little is known about the extent and structure of genetic variation, which has hampered breeding efforts and conservation of genetic resources in spider plant improvement.

2.6 Germplasm collection

Germplasm consist of genetic resources that can be used to preserve the species of a given population (Volis and Blecher, 2010). Establishing a germplasm bank is a key step in initiation of any breeding program. In order to prevent extinction of landraces and wild relatives of any cultivated crop, germplasm collection and conservation is of paramount importance (Rosenow and Dahlberg, 2000). Passport data which comprises of all basic information recorded at time of collection regarding the source or origin of the sample is very crucial in genetic research and for planning further collections as this aims at identifying new entries and duplicates (Frankel, 1989). Previous spider plant germplasm has been maintained by the gene banks. The world vegetable center (AVRDC) contributes largely in germplasm collection, conservation and distribution of spider plant genetic resources (Ojiewo and Yang, 2010).

Another source of germplasm includes farmers collection from the majorly spider plant grown areas. The farmers have deliberately and unconsciously maintained the germplasm *in situ.* This poses a risk to their extinction due to loss of natural habitat as a result of increased human activity. There is need of a conservation effort through a breeding program so as to increase and stabilize the diversity of spider plant species. Systematic evaluation and categorizing of these germplasm accessions into morphologically similar and presumably genetically similar groups is most useful and will aid in conserving the novel genes in spider plant as well as rescuing them from erosion.

2.7 Characterization of spider plant

Characterizing genetic diversity and the degree of association between and within accessions is the first step toward developing crop cultivars. In plant breeding programmes, assessment of genetic diversity and its relationship is useful for determining the uniqueness and distinctness of a phenotype, genetic constitution of genotypes and selection of parents for hybridization. In genetic terms, characterization refers to the detection of variation as a result of differences in either DNA sequences or specific genes or modifying factors (de Vincente et al., 2005). According to Perry and Battencourt (1997), the term "characterization" refers to the description of characters that are usually highly heritable, easily seen by naked eye and equally expressed in all environments.

The variation that is identified by characterization needs to be conserved and should be made available to both germplasm collectors and breeders (Huaman et al., 1997). This will aid in identifying materials in a collection or checking their authenticity. It also distinguishes homonyms or similar names and recognizes the duplicates (UPOV, 2004). It helps to identify or

select species, clones or cultivars with a desired combination of characteristics (traits) and helps to classify them. Characterization is crucial in detecting groups with correlated characteristics which may have immediate practical value or may give clues to genetic relationships among accessions while estimating the variation within a collection (Saad and Idris, 2001). Molecular characterization has been considered important to avoid duplication in genebanks protection of special cultivars.

The gene banks are also able to have a record of the stored germplasm through characterization to avoid fill up space by keeping material which is essentially the same (Lungu, 1990). Different varieties of *Cleome* exist with different characteristics. In Kenya, some of the landrace varieties grown lack consumption appeal for market due to lack of superior preferences like palatability due to taste, pubescence among others.

Morphological and agronomic characters have been used in characterization of the landraces conventionally. Morphological characterization is crucial in measuring genetic diversity through application of known morphological descriptors (FAO 1995; Van der Maesen, 1990). Traits assessed during agronomic evaluation vary according to species. Previous research has shown some of the key traits that would aid in genetic improvement of *C. gynandra* (Omondi, 1990). For instance, higher leaf yield, plant uniformity, longer vegetative phase, late flowering and drought tolerance. Morphological and genetic characterization aids in generating new knowledge on its genetic structure enhancing identification and discrete accession evaluation. For instance, morphological traits such as stem and petiole pigmentation have been used to distinguish spider plant morphotypes in western Kenya (K' Opondo, 2011). Classical breeding is imperative in introgression of important yield and nutritional genes into adapted *Cleome gynandra* accessions.

Several research studies have pointed out some measures of evaluating genetic diversity in germplasm collections of crops. They include allozyme markers, morphological characters, storage proteins, isozymes or molecular markers (Morden et al., 1989; Maquet et al., 1997; Watson and Eyzaguirre, 2002). A number of factors like the aim of the experiment, level of resolution required, available resources and technological infrastructure, and operational and time constraints determine the choice of analytical method to be used in genetic diversity (Karp et al., 1997). Some of these methods include measuring simple plant characters that are easily recorded through visual observations with the guidance of defined morphological descriptors (FAO, 1995). Molecular markers and a genetic map would be very imperative for both cultivar improvement and genomic study. Molecular markers such as SSRs have been used widely in genetic analysis and genetic mapping to detect degree of polymorphism and molecular assisted breeding (Kalwade and Devarumath, 2014; Campoy et al., 2011). SSRs markers have a high mutation rate with a high polymorphic information content (PIC), thereby giving them a wide application in molecular mapping, phylogenetic and genetic relationship studies, and marker-assisted breeding (Zhao et al., Yang et al., 2005; Sun et al., 2006).

2.8 Phenotypic variation

Morphological and phenotypic methods were among the earliest genetic markers used in germplasm management (Stanton et al., 1994). These methods rely on discriminating between individuals based on physical characteristics, like maturity cycle, growth habit, leaf shape, hairiness, nature of corolla, and panicle/pod/fruit size (Van der Maesen, 1990). Phenotypic diversity in germplasm accessions, breeding lines and segregating populations may be evaluated on pedigree, morphological and agronomic performance (Smith and Smith, 1992; Bar-Hen et al., 1995; Hamrick and Godt, 1997). Pedigree of varieties involves complete documentation of

relationships traced back to landraces and wild relatives. Selection of genetically diverse parents based on pedigree information in order to obtain transgressive segregants has been found to be effective in many crops (Gopal and Minocha, 1997). However, for spider plant accessions, pedigree records are lacking and calculation of co-ancestry among Kenyan and South African spider plants is presently not feasible. Morphological traits remain crucial in any breeding programme as they are the first step in studies of genetic relationships. Morphological characterization involves measuring simple plant characters that can be easily recorded through visual observations at different plant growth stages such as leaf area, size and colour. This is mostly done using a list of morphological descriptor that provides the simplest of formal, standardized and repeatable methods of measuring crop genetic diversity (Watson and Eyzaguirre, 2002).

Phenotypic characterization also entails characterizing complicated morphological traits of agronomic importance such as pest and disease resistance, fruit set, yield potential and biochemical properties (Ayad et al., 1995). However, this characterization is limited by a number of factors like the characters may not be significantly distinct, hence require that plants grow to full maturity prior to identification (Ratnaparkhe et al., 1995). In addition, these characters are often influenced by environmental factors, resulting in differences in expression that complicate interpretation of results. Same type of material must be used for all experiments due to different genes being expressed at different developmental stages or in different tissues. Other challenges are that in order to describe germplasm adequately one requires performing extensive trials at same environment and same season as this will give valid comparisons for various descriptors under test (Smith and Smith, 1992). More also, it is difficult to characterize a large collection of germplasm, and that which only meets the criteria of a few of the most desirable traits (Cooke

and Reeves, 1998). Despite these drawbacks, morphological and phenotypic characteristics are still important measures of genetic variation.

2.8.1 Analysis of morphological and agronomic variation in *cleome gynandra*

Morphological characterization and evaluation have been used to assess genetic diversity within and among the accessions in different crops by previous researchers. Morphological and agronomic characters remain very vital for plant breeders in development of improved cultivars in any crop production. Furthermore, these traits have long been used by farmers in selecting crops that best suits them, thereby largely contributing to their domestication (Gepts, 2004). These characters are frequently measured in each genotype with the aim of conserving, evaluating and utilizing the genetic resources in a particular crop (Franco et al., 2001).

Earlier studies have shown that a great extent of variability exists in qualitative and quantitative traits among spider populations such as plant structure, coloration on the leaves, petioles and flowers, growth habit, plant hairiness or pubescence, number of days to flowering, plant height, number of primary branches, amount of chlorophyll content and leaf size along other traits (Chweya, 1997; Schippers, 2000). Previous research has also demonstrated the presence of various morphotypes based on stem and petiole pigment. For instance, according to Masuka and Mazarura (2012), four spider plant morphs, three from Zimbabwe and one from Kenya revealed differences on the traits evaluated especially on stem pigmentation.

Wasonga (2014) characterized accessions from Kenya and South Africa thereby distinguishing the accessions from these two regions on the basis of their stem colour and pubescence. The Kenyan types were majorly purple stemmed with profuse hair, as compared to South African types that were green stemmed and less hairy. Earlier studies by K' Opondo (2011) reported

variation on the following traits; plant structure, stem pubescence, leaflet shape and leaflet apices. Similarly, there were differences on the measurable variables like plant height, petiole length and fruit breadth. Despite the merits of molecular markers in crop improvement, farmers are keen on morphological and agronomic variations which can easily be recognized by the human eye and how these traits can be integrated into their farming system to improve spider plant production, and of such should not be ignored (Hawkes, 1991). Additionally, morpho-agronomic data of genotypes is very crucial as it aims at providing valuable information that determines the most promising entities for future breeding programs. These characters provide an opportunity for plant breeders to develop new and improved farmer-preferred traits cultivars, with desirable characteristics.

The understanding of the correlations between morphological traits is of great importance in a breeding program (Shorter 1991). Morphological descriptors may aid in correlation studies where the traits have exhibited phenotypic associations. There is need to evaluate association of various characters as this is an eye opener towards spider plant improvement through a breeding program. One way to evaluate is through Trait Analysis by Association, Evolution and Linkage (TASSEL) - a software that evaluates traits associations, evolutionary patterns and linkage disequilibrium. The TASSEL software is reliable as it provides novel statistical approaches to association mapping which reduces errors in association mapping (Bradbury et al., 2007).

As the sample sizes used in breeding materials and germplasm accession used in crop improvement continue to increase, methods used to classify and order genetic variability are assuming significance. Various multivariate techniques which simultaneously analyze multiple measurements on each individual under investigation are widely used in analysis of genetic diversity irrespective of the dataset (morphological, bio chemical, or molecular marker data).

Among these algorithms, cluster analysis, principal component analysis (PCA), principal coordinate analysis (PCoA) and multidimensional scaling (MDS) are at present, most commonly employed and appear particularly useful and have been used to show the pattern of genetic relationship among accessions (Cruz and Carneiro, 2006).

2.8.1.1 Cluster analysis

"Cluster analysis" refers to "a group of multivariate techniques whose primary purpose is to group individuals or objects based on the characteristics they possess (Mutsaers et al., 1997). This implies that individuals with similar descriptions are grouped into the same cluster. The resulting cluster show high internal (within cluster) homogeneity and high external (between clusters) heterogeneity. Eventually, individuals within a cluster seem closer to each other when plotted geometrically as compared to different clusters that are further apart (Hair et al., 1995). Clustering is a useful tool for studying relationships among closely related cultivars or accessions. Previous research has shown that cultivars or accessions in cluster analysis are arranged in hierarchy by agglomerative algorithms according to the structure of a complex pairwise genetic proximity measure (Kaufman, 1990). Diversity and phylogenetic analysis on the basis of evolutionary dissimilarities may be performed by use of softwares like DARWIN Perrier (2003).

There are broadly two types of clustering methods;

(i) Distance-based methods in which a pair wise matrix is used as an input for analysis by a specific clustering algorithm (Johnson and Wichern, 1992), leading to a graphical representation (such a tree or dendrogram) where clusters may be visually identified

(ii) model-based, assumes analysis from each cluster is unsystematic, draws from some parametric model, and inferences about parameters related to each cluster and cluster association of each individual are performed equally using standard statistical methods such as maximum-likelihood or Bayesian methods (Pritchard et al., 2000). \

Distance-based methods

There are two groups of estimating distance-based clustering methods; hierarchical and non-hierarchical. Hierarchical methods apply a sequence of consecutive mergers or a series of successive divisions of group of individuals, where similar individuals are firstly grouped together. This clustering is performed by measuring standard metric distance such as the squared Euclidean. Thereafter, a clustering approach like unweighted pair group method of arithmetic averaging, (UPGMA) is applied (Franco et al., 2001). The non-hierarchical methods are based on chronological threshold and do not occupy the structure of dendrograms or trees (Everitt, 1980). In the past, different researchers have used different models for cluster analysis. For instance, Wolfe (1970) used non-hierarchical statistical methods for classifying individuals including mixture models, such as the Gaussian Model (GM) which only deals with continuous variables. On the other hand Franco et al (1997) proposed the use of hierarchical methods such as Ward or UPGMA, using Gower's distance.

Hierarchical clustering methods are more frequently used in analysis of genetic diversity in crop species. Among various agglomerative hierarchical methods, the UPGMA (Unweighted Paired Group Method using Arithmetic averages) is the most commonly adopted clustering algorithm, followed by the Ward's minimum variance method (Sneath and Sokal, 1973; Panchen, 1992) (Ward, 1963). Previous research on spider plant has shown variation on some of the traits evaluated and this has resulted in different clustering of the accessions involved. For instance, a

study by K'Opondo (2011) revealed among the four morphotypes studied, they were clustered into three groups by the dendrogram comprising of green-purple and purple -green in the first cluster, followed by the Green-purple, purple - green and purple-purple morphotype in a second cluster while the third cluster comprised of green - green morphotype. Mwase et al., (2014) has also shown clustering patterns in *Amaranthus*. Their study involved characterization of 37 accessions of *Amaranthus* into two clusters based on their growth habit. Nkouannessi (2005) through use of clustering patterns revealed three distinctive clusters among 20 accessions of cowpea genotypes from Kenya, Cameroon and South Africa using 15 qualitative and 12 quantitative traits. Considerable variations were seen depending on the geographical region of collection. According to (Patras et al., 2011) hierarchical clustering has been used to classify fruits and vegetables based on the antioxidant capacity.

2.8.1.2 Principal Component Analysis

Principal component analysis (PCA) is a statistical technique that is used to analyze the interrelationships among a large number of variables and to explain these variables in terms of a smaller number of variables, called principal components (PC), with a minimum loss of information. Principal component analysis provides variable independence and balanced weighting of traits, which leads to an effective contribution of different characters on the basis of respective variation. The initial step in PCA is to estimate Eigen values, which explain the amount of total dissimilarity that is displayed on the PC axes. The first PC summarizes most of the unpredictability present in the original data relative to all residual PCs. The second PC describes most of the variability not summarized by the first PC and uncorrelated with the first, and so on (Jolliffe, 1986). For instance, in a study aimed at profiling antioxidant properties

between fruits and vegetables, the first two components contributed to 62% of the total variability in antioxidant activity and various antioxidant groups (Patras et al., 2011).

PCA can be applied to two forms of data matrices: (i) a variance-covariance matrix, and (ii) a correlation matrix. In the use of the variance-covariance matrix, absolute changes among individuals can be studied. However, with the association matrix, only differences comparative to the consistent data can be interpreted (Wiley, 1981).

2.8.1.3 Principal coordinate analysis

Principal coordinate analysis (PCoA) is a scaling or ordination method that starts with a matrix of similarities or dissimilarities between a set of individuals and aims to produce a low-dimensional graphical plot of the data in such a way that distances between points in the plot are close to original dissimilarities. When there are relatively few characters and no missing data, the output of PCA and PCoA will be similar. When the first two or three PCs explain most of the variation, PCA and PCoA become useful techniques for grouping individuals by a scatter plot presentation (Rohlf, 1972).

2.8.1.4 Multidimensional scaling (MDS)

Multidimensional scaling (MDS), also referred to as "perceptual mapping," is a procedure that "represents a set of individuals or genotypes (*n*) in a few dimensions (*m*) using a similarity/distance matrix between them such that the inter-individual proximities in the map nearly match the original similarities/distances" (John and Wichern, 1992). There are two types of MDS: (i) non-metric MDS, which is used when the inter-individual proximities in the map nearly match the original similarities/distances, and (ii) metric MDS, helpful when the real scales

of original similarities/distances are used to get an arithmetical representation in *m* dimensions (Johnson and Wichern, 1992). Previous studies have shown that the actual arrangement of individuals consequential from PCA, PCoA, and MDS are typically related (Rohlf, 1972).

2.8.2 Measures of genetic diversity

In plant breeding programs, information concerning germplasm diversity and genetic relationships is very vital in determining the uniqueness and distinctness of a phenotype, genetic constitution of genotypes and selection of parents for hybridization among crop improvement strategies (Geleta et al., 2006). The level of genetic diversity plays a great role in determining the prospective performance of a species and its achievement from human selection in breeder's materials (Hedrick, 2000; Ayana, 2001). Therefore, many studies in genetics have been focusing on estimating the extent of this diversity in both natural and domestic populations as well as exploring the possible devices of conserving such variability in changing environments (Weir, 1996; Ayana, 2001). In general, measures of genetic variation of a sample depend mainly on the number of individuals sampled per population, the number of loci sampled, genotypic and allelic compositions of the population, mating system and effective population size (Weir, 1990). The richness and evenness of a population are fundamental in determining its genetic variation. The evenness of allele or genotype frequencies is accounted by the measures of average observed heterozygosity, expected heterozygosity and effective number of alleles. Heterozygosity is the most widespread measure of genetic variation within a population.

Diversity and differentiation are estimators of the genetic variation that are broadly used in population genetics studies. The most common measures of biodiversity are species richness, Simpson's index and Shannon-Weaver index. According to Spellerberg (1991), 'species

richness' refers to 'species diversity' which is an expression or index of some relation between number of species and number of individuals in a population. Several indices of species diversity are used in the large amount of literature on biological diversity and ecological monitoring. A commonly used index is that referred to as 'Shannon's Index' or 'H'. The Shannon-Weaver diversity index (H') is an index used in genetic studies as a convenient measure of both allelic richness and allelic evenness. However, because of log transformation it is not readily interpretable in the genetic terms (Brown and Weir 1983). A low H` indicates an extremely unbalanced frequency classes for an individual trait and a lack of genetic diversity. Perry and McIntosh (1991) described the Shannon-Weaver diversity index as:

$$H' = 1 - \sum_{i=1}^{n} \text{pi} \ln \text{pi} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .. Equation\ 1.1$$

Where

Pi is the proportion of accessions in the i^{th} class of an n-class character and n is the number of phenotypic classes of traits. Each H' value is divided by its maximum value (log n) and normalized in order to keep the values between 0 and 1. The minimum value of the index is zero for a monomorphic population. The value of the index increases with increase in polymorphism and reaches the maximum value when all phenotypic classes have equal frequencies (Yang et al., 1991).

2.8. 3 Heritability of nutrition and yield related traits

There is need to estimate the heritability of the yield and nutrition components so as to ascertain promising accessions among spider plant populations. Heritability refers to the proportion of variation in the population attributable to genetic influences. Heritability can either be broad

sense where the proportion of phenotypic variance is due to an effect for the whole genotype, including the sum of additive, dominance, and epistatic (multi-genic) effects. Heritability in narrow sense is when the proportion of phenotypic variance among individuals in a population is due to heritable genetic effects, expressed as the ratio of the additive genetic variance to the total phenotypic variance (Falconer and Mackay, 1996). Heritability of any trait is the foremost determinant of its response to selection. The approach for crop yield improvement in most breeding programmes has been indirect selection through yield related traits like days to flowering, plant height, number of leaves per plant that often have higher heritability values (Omondi, 1990). In *C. gynandra*, breeding studies have revealed a high heritability estimate for days to flowering, implying it is possible to select for late flowering genotypes so as to improve yield in *C. gynandra* (Mavengahama, 2013). High values of heritability estimates > 80% have been also reported for nutrition compounds of vegetable *Amaranthus* (Sarker et al., 2016). Accordingly, selecting genotypes that have high heritability estimates for nutrition traits is vital in improving nutrition content among spider plant populations as crop improvement is achieved through selection of a trait that has high heritability (Waqar et al., 2008).

2.9 Nutrition composition

Plants have naturally occurring, biologically active chemical compounds known as phytochemicals that have beneficial effect on health. They are linked to protection against cardiovascular diseases, some forms of cancer and other degenerative diseases (Ayoola et al., 2008). The most important action of these chemicals is that, they also function as antioxidants that react with the free oxygen molecules or free radicals in the body (Liu, 2003). Antioxidants are chemical compounds or molecules present in the biotic components and high in medicinal plants (Hae-Ryong et al., 2006). It has been reported that these compounds are necessary for

human health and nutrition (Kusum and Fazlu, 2002) as they protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite (David et al., 2004).

There is empirical evidence that indigenous leafy vegetables have high nutritive value as well as several other advantages compared to the introduced exotic varieties. They play an important role in inhibiting and scavenging these free radicals and provide protection against infectious and degenerative diseases (Canadanovic et al., 2005). These protective effects are attributed to the presence of antioxidants especially vitamins like ascorbic acid, tocopherol and β-carotene (Ganiyu, 2005). Research has shown spider plant to have a number of medicinal uses like to alleviate migraine, vomiting, diphtheria, vertigo, headache, pneumonia, septic ears, stomach ailments, induce labor during childbirth, treatment of Malaria, Piles, Rheumatism and anti tumour activity among many others (Mule et al.; Bala et al., 2010). A decoction or infusion of boiled leaves and/or roots is administered to facilitate childbirth, treat stomach ailments, constipation or thread-worm infection. The seeds and roots also have anthelmintic properties (Schippers, 2002).

There is a growing attention to the consumption of spider plant in both the eastern and southern parts of Africa as a vital component of daily diets due to the increased awareness of health protecting properties of non-nutrient bio-active compounds it possesses. It is highly nutritious with significant levels of minerals (calcium, magnesium, and iron), β-carotene, vitamins and proteins making it suitable for combating malnutrition**.** The plant contains high crude protein, lipids, and phenolic compounds (Lyimo et al. 2003; Mulokozi and Hedrén, 2004**)** with a high amino acid profile (Vanderjagt et al., 2000). Spider plant has high antioxidant capacity. According to Muchuweti et al., (2007), the antioxidant activity has been recorded at 61% due to

the presence of flavonoids and phenolic compounds (1327.33±1.66 mg/g). This antioxidant activity can help to overcome free radical and oxidant stress (Louise 2014)) and is key in strengthening the human body antioxidant defense system (Stangeland et al., 2009). The presence of polyphenols gives it a bitter taste and is assumed to constitute from 0.5% to 0.9% of the edible leaf (Chweya, 1997).

Recently, these phenolic compounds have been intensively investigated because of their potential health-promoting effects (Jahangir et al., 2009; Vallejo, 2002). Despite spider plant being endowed with such great potential of nutraceutical compounds, past research has shown that food insecurity and hidden hunger (micronutrient deficiency) affects more than half of the world population, particularly in developing countries (WHO, 2000). Further findings have identified iron, vitamin A, zinc and iodine deficiencies as the most serious health constraints worldwide (WHO, 2000), (Faber and Wenhold, 2007). Furthermore, it has been reported that the number of undernourished people has been increasing from the 1990s with an estimate of about 265 million people in sub-Saharan Africa being undernourished (FAO, 2009).

A report by (ASARECA, 2012) indicates that African Indigenous Vegetables (AIVs) have the potential to improve nutrition and generate income for small holder farmers in Eastern and Central Africa. Spider plant being rich in these nutritional and nutraceutical compounds is inevitable in combating malnutrition and thereby fighting the hidden hunger. There is need to assay the nutrition quality of various accessions of spider plant so as to identify those with high nutrition composition.

2.9.1 Genetic diversity and its effect on the quality of vegetables

Several studies have demonstrated that there is a substantial and significant variation for the antioxidant phytochemicals in vegetables. For instance, *Brassica* species, exhibit variation both within and among species, and even among crops of the same species; thus, the potential health benefits provided by cruciferous crops will depend firstly on the genotype. The phenolic compound composition may differ between cultivars, as well as among parts within the individual plant as shown in several crops like turnip greens and turnip tops (Francisco, 2010) and tronchuda cabbage according to (Fernandes et al., 2007). Biosynthesis and concentration of phenolic compounds in plants depends on genetic and environmental factors. Phenolics are distributed differently depending on the crop and on the plant part evaluated. Prior studies have shown that external and internal leaves of different *B. oleracea* crops like tronchuda cabbage and savoy cabbage are different in terms of total phenolic content (Ferreres et al., 2005; Martinez et al., 2010). Significant positive genotypic correlations with nutrition and yield related compounds have been reported in *amaranthus* (Sarker 2016). Yield positively correlated significantly with vitamin C, iron and manganese as well as like agronomic traits plant height and number of leaves per plant.

2.10 Genotypic characterization of Simple sequence repeats.

Molecular markers are crucial tools for measuring the diversity of plant species. These makers can be largely grouped into three classes founded on the method of their detection namely; hybridization-based, polymerase chain reaction- (PCR-) based, and DNA sequence-based (Botstein 1980). Hybridization-based markers like Restriction fragment length polymorphism (RFLPs) have limited polymorphism, highly expensive, labour intensive, time consuming and require larger amounts of DNA particularly in closely related lines (Bennetzen 2000).

Polymerase chain reaction (PCR) based marker systems such as Randomly amplified polymorphic DNA (RAPDs) require less plant material for DNA extraction and are more rapid however, they only detect the dominant traits of interest and their results are not reproducible or transferable between laboratories (Collard et al., 2005). DNA sequence-based markers for example SSRs, depend on the availability of short oligonucleotide repeats sequences in the genome of plants. Microsatellites, or Simple sequence repeats (SSRs) are tandem repeats nucleotides, mostly between 1 to 6 nucleotides, that occur in DNA sequences. These sequences have been detected in the genomes of numerous organisms and are distributed throughout the whole genome across both coding and non-coding DNA sequences (Li et al., 2016). Most studies have highlighted these SSRs to have potential to act as molecular markers that could assist in genetic analysis, genetic mapping and molecular assisted breeding (Kalwade and Devarumath, 2014; Campoy et al., 2011). They are highly variable and exhibit high levels of inter- and intraspecific polymorphism hence making them excellent markers with numerous applications in plant breeding (Queller et al., 1993). This makes them the marker of choice since they are extremely informative (Morgante and Olivieri, 1993). Despite the numerous merits of SSRs, their application is limited mainly to species with enriched genomic resources. This calls for an alternative approach such as cross species transferability especially in species that lack genomic information (Satya et al., 2016).

Tarenaya hassleriana plant is closely related to *Cleome gynandra,* and has been extensively studied with its genome also fully sequenced. This is mainly due to the fact that *Tarenaya hassleriana* has a relatively small genome size of about 290 Mb accompanied by an array of interesting phenotypic features (Cheng et al., 2013). The *Tarenaya hassleriana* genome has simple sequence repeats (SSRs) that have been distributed throughout the genome. These

sequences are publically available in databases such as National Centre for Biotechnology (NCBI) and of such, offer a great breakthrough in providing information on the structure of assembled genomes. The availability of enormous sequence data for a large number of genomes has enhanced research aimed at understanding the origin and functions of microsatellites as well as searching for new applications.

Many software packages are available for genome wide SSR mining in the databases. Basic local Alignment Search Tool (BLAST) that involves pattern matches have been used in the past (Temnykh et al., 2001) but more recently other advanced microsatellite specific software such as MIcroSAtellite (MISA) and Genome wide Micrrosatelite Analyzing Tool (GMATo) have been explored. Nevertheless, the existing SSR mining tools are limited in processing large genomes competently and generate poor or no statistics. GMATo is novel and very efficient than other most widely used tools for identification and analysis of microsatellites. It is used for microsatellite sequence identification from any genomes or DNA sequences irrespective of the size (Wang et al., 2013). For instance, genome-wide characterization of simple sequence repeat and primer transferability (SSR) has recently been adopted in a fruit tree, Chinese jujube *(Ziziphus jujube)* species (Xiao et al., 2015).

CHAPTER 3

PHENOTYPIC CHARACTERIZATION OF EAST AND SOUTH AFRICAN *CLEOME GYNANDRA* ACCESSIONS

3.1 Abstract

Spider plant (*Cleome gynandra)* commonly known as African Spider Flower or Spider wisp is an important leafy vegetable in most parts of Africa. It is highly nutritious with high levels of mineral nutrients and antioxidant compounds, hence its importance and popularity as a health promoting indigenous vegetable. Despite the great value of spider plant, there are limited efforts towards its improvement especially in the area of phenotypic diversity. Crop improvement in spider plant will require knowledge of the existing phenotypic and genetic diversity in the population to inform selection of desirable traits. Therefore, a study was conducted at the Field Station of the University of Nairobi, Upper Kabete Campus to determine phenotypic variation among different Kenyan and South African spider plant accessions. A total of 49 spider plant accessions were planted in the field in a randomized complete block design with three replications. Characterization based on the list of modified Food and Agriculture Organization (FAO) spider plant descriptors, was done on various qualitative traits namely; growth habit, flower colour, stem colour, stem hairiness, petiole colour, petiole hairiness, leaf colour, and leaf pubescence. Days to 50% flowering, number of leaves per plant, number of primary branches, plant height, leaf length, leaf width, single leaf area, and chlorophyll content were evaluated as quantitative traits and their heritability estimated. Unweighted pair group method with arithmetic mean (UPGMA) grouped the accessions into two groups, each comprising of Kenyan and South African accessions on the basis of their stem and flower colour. Analysis of variance (ANOVA) showed significant differences at $P < 0.05$ for all the quantitative traits evaluated. For all the accessions, leaf yield significantly correlated positively with chlorophyll content (r = 0.45), plant

height (r = 0.70) and number of primary branches (r = 0.65) implying selection based on the three is vital in breeding for greater yield in spider plant. Estimates of Shannon-Weaver diversity index (H') for the qualitative characters assessed were generally high at H' > 0.500 indicating a greater inter-country than intra-country diversity. High estimates of heritability were expressed on all the quantitative traits evaluated namely; days to flowering 91%, number of leaves per plant 99%, plant height 99%, number of primary branches 94%, chlorophyll content 94% and single leaf area 87%. This suggests that selection based on such highly heritable traits is crucial in yield improvement among spider plant populations. From this study, it can be deduced that there is significant phenotypic variation among the spider plant accessions. Further studies are recommended to establish and relate the phenotypic differences to genetic diversity which is a requisite for spider plant crop improvement.

3.2 Introduction

Cleome gynandra is among the most important traditional leafy vegetables widely used in Africa (Schippers, 2000). It is native to the following regions: Southern Africa, Western Africa, Central Africa, Eastern Africa and South East Asia (DAFF, 2010). The major areas where spider plants are found as wild in South Africa are the KwaZulu-Natal, Free State, Northern Cape, Limpopo and North West provinces (DAFF, 2010). In Kenya, the plants are mainly found in Western, Rift valley, Eastern and Coastal regions. The key counties producing the plant include Kisii, Nyamira, Kericho, Migori, and Siaya (HCDA, 2014). Despite this wide adaptation, there lacks quantitative information on the extent and structure of their phenotypic variation, which would be important for breeding and conservation (K'opondo et al., 2009; Chweya and Mnzava, 1997). Genetic diversity is particularly useful in characterizing individual accessions and cultivars, in detecting genetic materials with novel genes and thereby rescuing them from erosion, and as a

general guide in selecting parents in breeding programmes. Most of the genetic diversity of spider plant in Kenya and South Africa is traditionally maintained by farmers in situ. This poses the risk of the species extinction due to loss of natural habitat as humans continue to exploit and develop land, divert water flow, and change the environment. Secondly, as the human population continues to increase, there is pressure on the natural land being changed by human activity. The need for cultivation and conservation in spider plant improvement is pivotal in order to save its genetic information and diversity.

Categorizing germplasm accessions into morphologically similar and presumably genetically similar groups is most useful when the population structure in a collection is unknown (Marshall and Brown 1975). Cluster analysis has been used to place similar accessions into groups among a large number of accessions (Assefa et al., 1999). In spider plant, as is true for other crop plants, the earliest methods for estimating genetic diversity include Mendelian analysis of discrete morphological traits (Motlhaodi et al., 2009). Some of the important traits reported that could be used in spider crop improvement include; higher leaf yield, plant uniformity, longer vegetative phase, late flowering and drought tolerance (Omondi, 1990). Previous research has also shown some of the qualitative traits that were used to estimate the level of variation among spider accessions. For instance; stem colour flower colour, pubescence among others (K'Opondo, 2011). The main objective of this study was to estimate the extent of phenotypic diversity among 49 Kenyan and South African spider plant accessions based on qualitative and quantitative traits data, therefore identifying promising accessions for different traits that could be utilized in spider plant breeding programs.

3.3 Materials and methods

3.3.1 Germplasm assembly and collection

The study consisted of forty-nine (49) spider plant accessions comprising local landraces and wild types. Germplasm was assembled from 3 sources:

i. Gene bank of Kenya - 25

ii. Gene bank of south Africa - 9

iii. Kenyan farmers landraces - 15

The spider plant collection sites conducted by the Kenyan and South African gene banks are shown in Figure 3.1 and 3.2. Table 3.1 shows the codes of the various accessions from the respective gene banks and Kenyan farmers collection based on collection eco-regions. The accessions from South Africa were delivered to study site following the phytosanitary regulations under Kenya Plant Health Inspectorate Service (KEPHIS). There were no previous characterization data for the accessions sourced from the Kenyan and South African gene banks.

Table 3. 1: List of Kenyan and South African spider plant accessions evaluated in the study

Entry	Accession no	Species name	Region
1	1^{ke}	*C.gynandra*	Siaya
2	2^{ke}	*C.gynandra*	Bungoma
3	3^{ke}	*C.gynandra*	Kakamega
4	4^{ke}	*C.gynandra*	Kitale
5	5^{ke}	*C.gynandra*	Mbale
6	6^{ke}	*C.gynandra*	Bomet
7	7^{ke}	*C.gynandra*	Busia
8	9^{ke}	*C.gynandra*	Marakwet
9	10^{ke}	*C.gynandra*	Kisumu
10	11^{ke}	*C.gynandra*	Homabay
11	12^{ke}	*C.gynandra*	Nandi
12	13^{ke}	*C.gynandra*	Kakamega
13	14^{ke}	*C.gynandra*	Kisii
14	15^{ke}	*C.gynandra*	Mbale
15	16^{ke}	*Cgynandra*	Meru
16	1959^{sa}	*C.gynandra*	Mpumalanga
17	1988^{sa}	*C.gynandra*	Mpumalanga
18	2000^{sa}	*C.gynandra*	Mpumalanga
19	2232^{sa}	*C.gynandra*	Northern province

Table 3. 1: List of Kenyan and South African spider plant accessions evaluated in the study

Entry	Accession no	Species name	Region
20	2241[sa]	*C.gynandra*	Northern province
21	2249[sa]	*C.gynandra*	Northern province
22	2279[sa]	*C.gynandra*	Northern province
23	2289[sa]	*C.gynandra*	Mpumalanga
24	2299[sa]	*C.gynandra*	Mpumalanga
25	30316[ke]	*C.gynandra*	Western
26	31990[ke]	*C.gynandra*	Western
27	31992[ke]	*C.gynandra*	Western
28	45426[ke]	*C.gynandra*	Western
29	45446[ke]	*C.gynandra*	Central
30	45451[ke]	*C.gynandra*	Central
31	50259[ke]	*C.gynandra*	Kisii
32	50264[ke]	*C.gynandra*	Nyamira
33	50265[ke]	*C.gynandra*	Nyamira
34	50273[ke]	*C.gynandra*	Nyamira
35	50290[ke]	*C.gynandra*	Nyamira
36	50296[ke]	*C.gynandra*	Nyamira
37	50298[ke]	*C.gynandra*	Nyamira
38	50299[ke]	*C.gynandra*	Nyamira
39	50307[ke]	*C.gynandra*	Kisii
40	50319[ke]	*C.gynandra*	Nyamira
41	50325[ke]	*C.gynandra*	Kisii
42	50326[ke]	*C.gynandra*	Nyamira
43	50328[ke]	*C.gynandra*	Nyamira
44	50330[ke]	*C.gynandra*	Nyamira
45	50332[ke]	*C.gynandra*	Kisii
46	50339[ke]	*C.gynandra*	Nyamira
47	50353[ke]	*C.gynandra*	Nyamira
48	50584[ke]	*C.gynandra*	Nyamira
49	50600[ke]	*C.gynandra*	Kisii

[Ke] – Kenyan, [sa]- South African accessions

Figure 3. 1: Map of Kenya showing the collection sites for the accessions evaluated in this study

Blue colour denotes accessions collection sites.

Figure 3. 2: Map of South Africa showing the collection sites for the accessions evaluated in this study

Green colour denotes accessions collection sites.

3.3.2 Study site

Field and glasshouse experiments were conducted at the University of Nairobi's Kabete Field station, Kenya. Kabete field station is, near Nairobi, about 8 km North West of Nairobi at 36^0 41'E and $01^0$15'S and an altitude of 1737m above sea level. The area is sub humid with an average temperature of 23^0C with a bimodal rainfall pattern and an average annual precipitation ranging from 600mm to 1800 mm. There are two cropping seasons, in March until September and season two from October to December. The soil type is well drained very dark reddish, brown to dark red friable clay locally known as kikuyu red clay loam with an average PH of 6.2 (Jaetzold, 2004).

3.3.3 Experimental design and crop husbandry

3.3.3.1 Field experiments

The experiment was carried out at the Kabete Field Station University of Nairobi. The 49 spider plant accessions were grown in a randomized complete block design with three replications. Pre-germination for each accession was done for 72 hours under treatment with 0.2 % gibberellic acid (SinoHarvest, Shenzen, China). The seeds of each accession were planted by hand in three rows of ten seeding holes per row (20 plants in a plot). Row plots were 3 m in length with inter-row spacing of 30 cm and intra-row spacing of 30 cm. No artificial fertilizers were used but farm yard manure at the rate of 10.5 g/line was applied to rows and mixed with soil at planting. Hand weeding was done to keep plants free from weeds throughout the experimental period. The experiment was conducted under rain-fed conditions. The experiments were carried out in two seasons namely; March 2014 to May 2014 and October 2014 to January 2015. A record of weather conditions during these two seasons is provided (appendix 1).

3.3.3.2 Greenhouse experiment

Soil testing and analysis was done at the University of Nairobi's Soil Science Laboratories. Top (0-15 cm) of field soil in all the plots was sampled and bulked for testing before the start of the experiments. The following protocol was followed during the analysis. Soil pH (H_20) was determined using a pH meter by weighing 20 g of the pooled soil into duplicate universal bottles and adding 50 ml of distilled water. The mixture was then shaken for 5 minutes in a shaker, left to settle and pH measured using a pH meter glass electrode (Schofield and Taylor, 1955). Organic carbon was determined using Walkley-Black method (Walkey and Black, 1934). Total soil N was determined by micro Kjedahl method (Kjedahl, 1883) by weighing 10 g of air dried soil, heating with concentrated sulphuric acid to convert organic N to ammonium and determination of the ammonium in the digest by distilling with 10NNaOH and further titration with 0.01 N HCL. Soil available P was determined using Mehlich's method by weighing 5 g of soil in duplicates into universal bottles and adding 50 ml of the double acid (0.95 N HCL in $0.025NH_2S0_4$) to each sample. The samples were then placed in a reciprocating shaker for 30 min and the soils filtered through Whatman no. 42 filter papers. A 5 ml aliquot of the soil extract was then pipetted into a 50 ml volumetric flask and 25 ml of distilled water and 8 ml of ascorbic acid added (molybdenum blue method), mixed and readings taken with a spectrophotometer. Basic cations (Na, K, Mg and Ca) were determined by leaching with 1 N NH_4OAc at pH 7.0 (Warnkce and Brown, 1998). Cation exchange capacity (CEC) was determined by leaching further with KCL then distilling the leachet with 10 N NaOH and further titration with 0.01 N HCL.

The greenhouse experiment was carried out at the Kabete field station glass house. A randomized complete block design with three replications was used to evaluate the forty nine

(49) accessions of spider plant in pots in a glasshouse. The total number of pots in the greenhouse was 147. The soil used in this study was collected within Kabete field station close to the field experiment site and sterilized at 105^0 C for 72 hours. Two parts of the soil was mixed with one part of sand and one part of cow manure (ratio 2:1:1) before filling in pots. Each replication consisted of 49 pots filled with 5 kg of air-dried soil mixture. The seeds of the 49 accessions were pre-germinated in petri dishes using 0.2 percent gibberellic acid (Sino Harvest, Shenzen, China), followed by sowing sparingly in each of the separate pots. Watering was done prior to and after sowing. Thinning was done 14 days after seedling emergence and again after 21 days to leave 3 plants per pot. The plants were watered after every three days each week and kept free from weeds by uprooting any volunteer weeds from the pots.

3.4 Data collection

Data was collected on both qualitative and quantitative traits.

3.4.1 Qualitative traits

Determination of morphological data/ qualitative traits was done by tagging the plants before flowering. Tagging was done by random selection of three plants of each accession grown in each plot in the field, as well as three plants in each pot in the glasshouse. Characterization based on the list of modified spider plant descriptors (FAO, 1995) was done on various qualitative traits namely; growth habit, flower colour, stem colour, stem hairiness, petiole colour, petiole hairiness, leaf colour, and leaf pubescence. Data scoring for each character was done on the same day for all accessions after 50% flowering to avoid differences in the developmental stages of growth. The table below shows the list of descriptors used.

Table 3. 2: Character, descriptor and codes used for characterization of qualitative traits in spider plant accessions used in the study

S/No.	Character	Descriptor and code
1	Growth habit	Erect (2), semi-erect (4) and prostrate (6)
2	Flower colour	White (1), purple (2) and pink (3)
3	Stem colour	Green (1), pink (2), violet (3) and purple (4)
4	Stem hairiness	Glabrous (1), weak/sparse (3), medium (5) and profuse (7)
5	Petiole colour	Green (1), pink (2), violet (3) and purple (4),
6	Petiole hairiness	Glabrous (1), weak/sparse (3), medium (5) and profuse (7)
7	Leaf colour	Dark green (1) and light green (2),
8	Leaf hairiness	Glabrous (1), weak/sparse (3), medium (5) and profuse (7)

Source: Food and Agriculture Organization of the United Nations (FAO, 1995)
Numbers in brackets on the right-hand side are the corresponding descriptor codes listed in the FAO publication with modifications during the development of the list.

3.4.2 Quantitative traits

The forty-nine (49) accessions were characterized for various agronomic traits. This was done by taking measurements and counts of a given trait. They included; days to 50% flowering, SPAD values, plant height , number of primary branches, leaf length, leaf width, single leaf area, and number of leaves per plant. Quantitative data was collected from both the field and glasshouse experiments. All measurements for each character were made on the same day for all accessions after 50% flowering to avoid differences in the developmental stages of growth.

3.4.3 Phenotypic characterization

The functioning hypothesis of this study was that certain characteristics will consistently separate the 49 accessions into various morphotypes. This was achieved by comparing a number of variable scores, counts and measurements in order to establish whether there were differences among them. Both the agronomic and morphological data were used in this characterization. The

parameters measured included growth and yield components namely; 1) Number of days to 50% flowering which was recorded from day of planting to when 50% of the plants in each plot/pot had flowered. 2) The leaf chlorophyll content was measured using Soil Plant Analysis Development (SPAD-502, Minolta Camera Co., Ltd., Japan). It is a portable diagnostic tool, simple, non-destructive, hand-held chlorophyll meter that measures the greenness or relative content of leaves. Compared to the traditional destructive methods, the use of this equipment saves time, space and not destructive. The SPAD meter determines the relative amount of chlorophyll present in the leaf by measuring the transmittance of the leaf in two wave bands. At flower initiation stage, the average of fully expanded young leaf from three plants in each stand was taken. The SPAD value obtained was proportional to the amount of chlorophyll present in the leaf and ranges from 0 to 80 (Jarvis, 2008). 3) Plant height was measured after 50% flowering from the base of the plant to the tip of the main stem using a meter rule in centimeters.

An average of three plants randomly selected from the rows of a plot in each accession was used. 4) The number of primary branches was determined by counting the main branches from three plants tagged in each plot after 50% flowering and the value averaged. 5) Leaf length was measured in centimeters from the pulvinus to the tip of the leaf while 6) leaf width (cm) was measured at the widest part of the basal leaves. This was done at flowering by randomly selecting three basal leaves in each of the three tagged plants per plot. 7) The single leaf area (cm^2) was calculated using the formulae $SLA = 0.763L + 0.34W$, where *SLA* is single leaf area, *L* is leaf length and *W* is leaf width (Rivera et al., 2007). 8) The yield component was determined by counting the number of leaves per plant from the tagged plants in each plot. An average from the three plants was then taken.

3.5 Data analysis

3.5.1 Qualitative traits

Data from the qualitative traits was subjected to DARwin 5.0 software as described by Perrier and Jacquemoud-Collet (2006). Euclidean distance matrix and hierarchical clustering analyses of unweighted pair group method of arithmetic averaging was used to estimate the dissimilarities. Cluster analysis was performed in order to get relationships among the accessions displayed in a dendrogram.

3.5.2 Quantitative traits

Quantitative data was subjected to ANOVA to generate genotype means. Analysis of variance (ANOVA) was done for all the quantitative traits collected at 5% level of significance. The genotype means were separated using Fishers protected Least significant differences (LSD) at P < 0.05. A two tail correlation analysis was performed to estimate quantitative relationships among the traits and also to identify those traits that could be of great significance in a spider plant breeding program. Broad sense heritability was estimated estimated as a ratio of genotypic variance to the phenotypic variance and expressed in percentage (Hanson et al., 1956) as per the formula;

Heritability $(H^2) = (V_g/V_p \times 100)$ …… *Equation* 3.1

Where, V_g= Genotypic variance, V_p = Phenotypic variance

Genotypic variance($\sigma^2{}_g$) was derived by subtracting error mean sum of squares (EMS) from the genotypic mean sum of squares (GMS) and divided by the number of replications as given by the formula;

$\sigma^2{}_g = GMS - EMS/r$ …… *Equation* 3.2

Where GMS=Genotype mean sum of squares, EMS= error mean sum of squares and r = number of replications.

Phenotypic variance ($\sigma^2{}_p$) was derived by adding genotypic variance with error variance as per the formula;

$$\sigma^2{}_p = \sigma^2{}_e + \sigma^2{}_g \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation } 3.3$$

3.6 Results

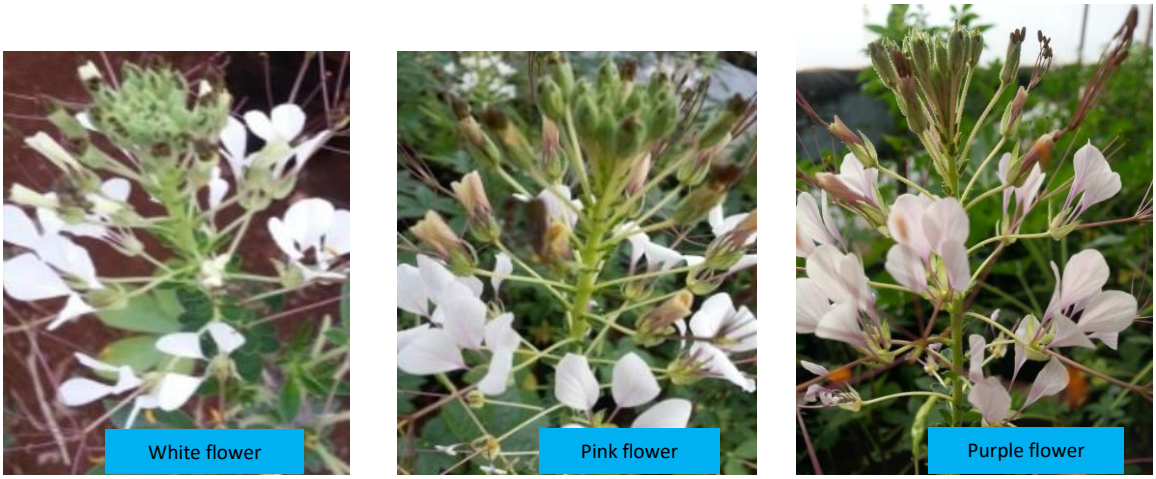
3.6.1 Qualitative traits

The 49 Spider plant accessions showed some differences for the different traits evaluated (appendix 2). However, for all the qualitative traits analyzed no seasonal effect was observed.

3.6.1.1 Morphological descriptors

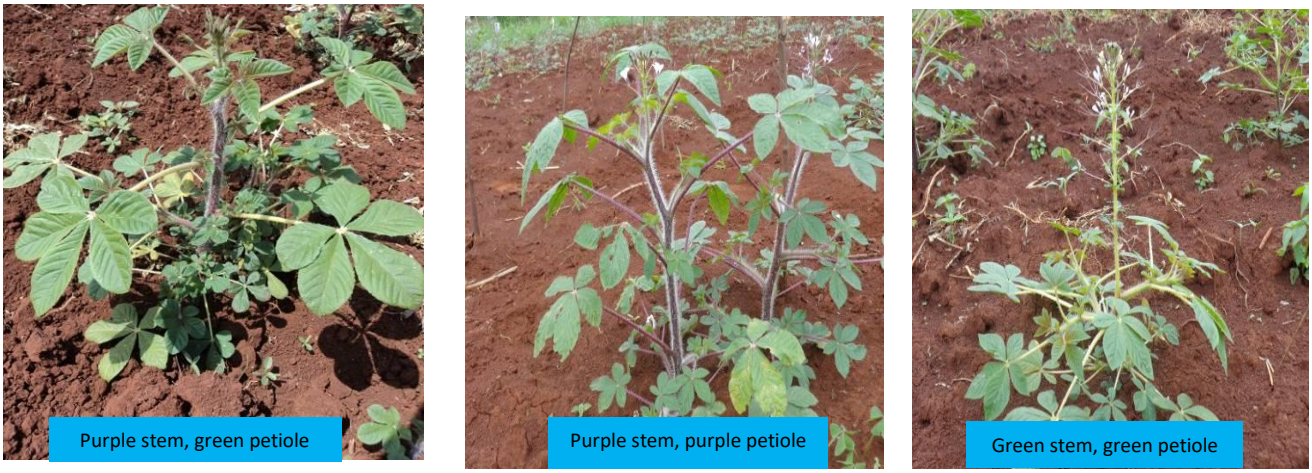
White, pink and purple flower colors were displayed among the accessions. The white flowers were largely from South African. Most of the Kenyan accessions were dominated with purple flowers. It was observed that 49% of the accessions studied in the field produced purple flowers, 26.5% pink, and 24.5 % white flowers (Figure 3.3 (i)). The stem colour varied between purple (79.6%) and green (20.4%) (Figure 3.3 (ii)). The proportions of petiole colour were 63.3% purple, 18.4% green and 18.4% pink colour. It was noted that majority of the accessions with purple stem, had purple petiole. Similarly, most accessions with green stem, presented a green petiole. The study also revealed 63.3% of the accessions of the stem hair were mainly profuse, 22.4% medium, 10.2% glabrous and 4% sparse (Figure 3.3 (iii)). Petiole hairiness varied from medium (63.3 %), to sparse (18.4%) profuse (10.1%) and glabrous (8.2%). Leaf colour was either dark green (67.3%) or light green (32.7%). The proportion of leaf hairiness of the study accessions ranged from sparse (57.1%), medium (30.6%) glabrous (10.2%) and profuse (2%).

For the growth habit, nearly 90% of all accessions were erect. Majority of the South African accessions had a semi erect growth. (Figure 3.3(iv)).



South African accession 2000 Kenyan accession 50259 Kenyan accession 10

Figure 3. 3 (i): The three colours displayed among the accessions.



Kenyan accession 31992 Kenyan accession 13 South African accession 2299

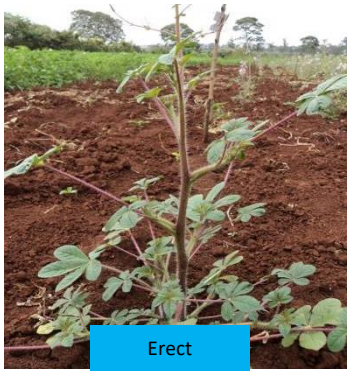
Figure 3.3 (ii): Stem and petiole pigmentation of various accessions

South African accession 1988

Kenyan accession 50298

Kenyan accession 50290

Figure 3.3 (iii): Hair characteristic in the accessions



Kenyan accession 50353

South African accession 1988

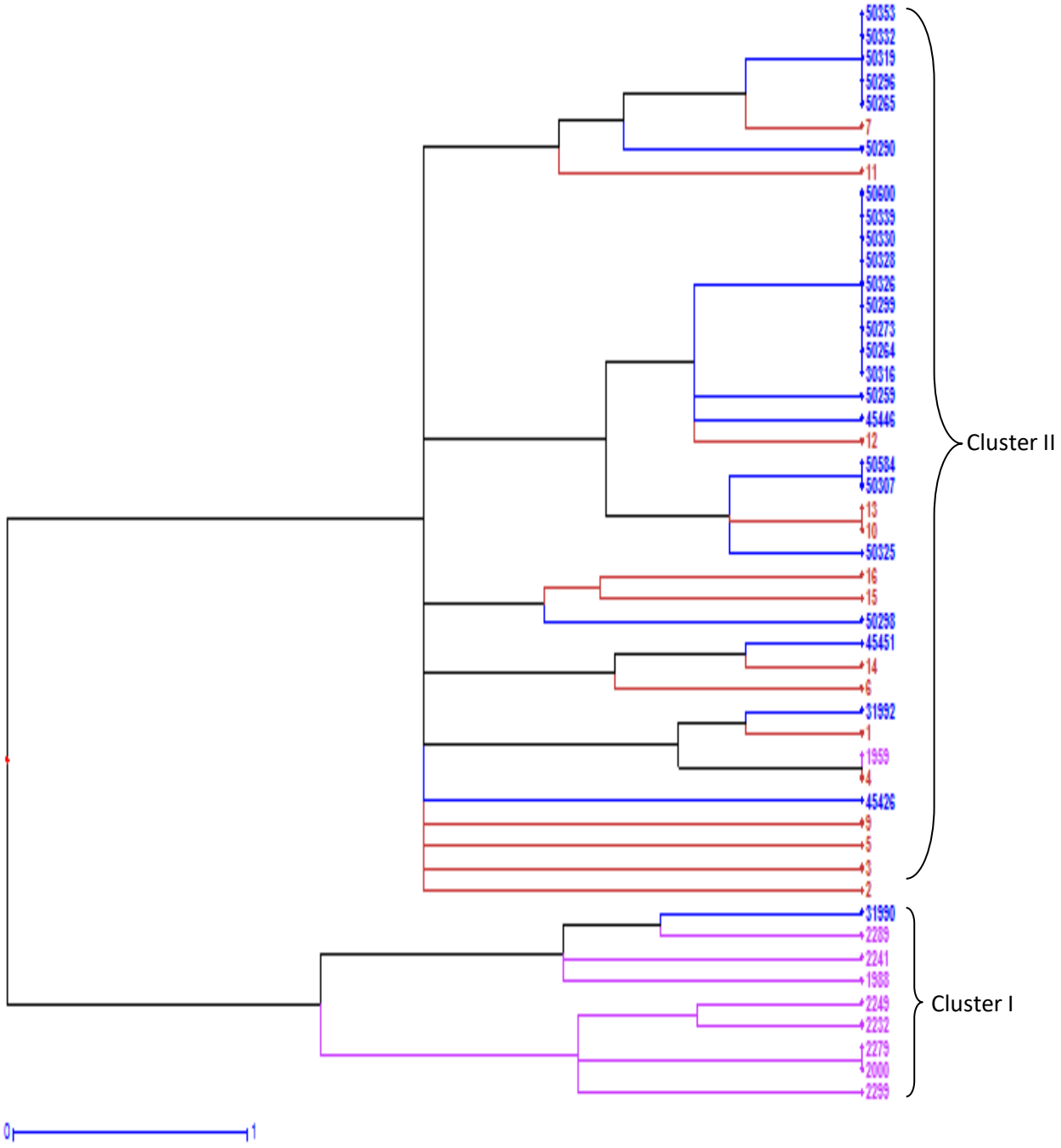
Figure 3.3 (iv): The two growth habits revealed in the accessions.

Table 3. 3: Morphological descriptors recorded for the 49 field grown spider plant accessions for the combined season

Entry	Accession no	Origin	Flower colour	Stem colour	Petiole colour	Stem hairiness	Petiole hairiness	Leaf colour	Leaf hairiness	Growth habit
1	1	Kenya	purple	Purple	green	profuse	medium	dark green	Medium	Erect
2	2	Kenya	white	Purple	pink	profuse	medium	light green	Sparse	Erect
3	3	Kenya	pink	Purple	pink	profuse	medium	dark green	Sparse	Erect
4	4	Kenya	pink	Purple	pink	profuse	medium	light green	Medium	Erect
5	5	Kenya	white	Purple	purple	profuse	profuse	light green	Profuse	Erect
6	6	Kenya	pink	Purple	pink	profuse	profuse	dark green	Medium	Erect
7	7	Kenya	pink	Purple	purple	profuse	medium	dark green	Medium	Erect
8	9	Kenya	white	Purple	purple	profuse	profuse	dark green	Sparse	Erect
9	10	Kenya	purple	Purple	purple	medium	medium	light green	Sparse	Erect
10	11	Kenya	pink	Purple	purple	medium	medium	dark green	Medium	Erect
11	12	Kenya	pink	Purple	purple	profuse	medium	light green	Sparse	Erect
12	13	Kenya	purple	Purple	purple	medium	medium	light green	Sparse	Erect
13	14	Kenya	purple	Green	purple	profuse	profuse	dark green	Medium	Erect
14	15	Kenya	pink	Purple	purple	medium	sparse	dark green	Sparse	Erect
15	16	Kenya	pink	Purple	pink	medium	sparse	light green	Sparse	Erect
16	1959	S. Africa	pink	Purple	pink	profuse	medium	light green	Medium	Erect
17	1988	S. Africa	white	green	green	sparse	sparse	light green	Sparse	Semi erect
18	2000	S. Africa	white	green	green	glabrous	glabrous	light green	Glabrous	semi erect
19	2232	S. Africa	white	green	green	glabrous	glabrous	dark green	Glabrous	Erect
20	2241	S. Africa	white	green	pink	sparse	sparse	light green	Sparse	Erect
21	2249	S. Africa	white	green	pink	glabrous	glabrous	light green	Glabrous	Erect
22	2279	S. Africa	white	green	green	glabrous	glabrous	light green	Glabrous	Semi erect
23	2289	S. Africa	white	green	pink	medium	sparse	dark green	Sparse	Erect
24	2299	S .Africa	white	green	green	glabrous	sparse	light green	Glabrous	Erect
25	30316	Kenya	purple	purple	purple	profuse	medium	dark green	Sparse	Erect
26	31990	Kenya	purple	green	green	medium	sparse	light green	Sparse	Erect
27	31992	Kenya	pink	purple	green	profuse	medium	dark green	Medium	Erect
28	45426	Kenya	purple	purple	green	profuse	sparse	dark green	Sparse	Erect
29	45446	Kenya	white	purple	purple	profuse	medium	dark green	Sparse	Erect
30	45451	Kenya	pink	purple	purple	profuse	profuse	dark green	Medium	Erect
31	50259	Kenya	pink	purple	purple	profuse	medium	dark green	Sparse	Erect
32	50264	Kenya	purple	purple	purple	profuse	medium	dark green	Sparse	Erect
33	50265	Kenya	purple	purple	purple	profuse	medium	dark green	Medium	Erect
34	50273	Kenya	purple	purple	purple	profuse	medium	dark green	Sparse	Erect
35	50290	Kenya	purple	purple	purple	profuse	medium	dark green	Medium	Semi erect
36	50296	Kenya	purple	purple	purple	profuse	medium	dark green	Medium	Erect
37	50298	Kenya	purple	purple	purple	medium	sparse	light green	Sparse	Semi erect
38	50299	Kenya	purple	purple	purple	profuse	medium	dark green	Sparse	Erect
39	50307	Kenya	purple	purple	purple	medium	medium	dark green	Sparse	Erect
40	50319	Kenya	purple	purple	purple	profuse	medium	dark green	Medium	erect
41	50325	Kenya	pink	purple	purple	medium	medium	dark green	Sparse	erect
42	50326	Kenya	purple	purple	purple	profuse	medium	dark green	Sparse	erect
43	50328	Kenya	purple	purple	purple	profuse	medium	dark green	Sparse	erect
44	50330	Kenya	purple	purple	purple	profuse	medium	dark green	Sparse	erect
45	50332	Kenya	purple	purple	purple	profuse	medium	dark green	Medium	erect
46	50339	Kenya	purple	purple	purple	profuse	medium	dark green	Sparse	erect
47	50353	Kenya	purple	purple	purple	profuse	medium	dark green	Medium	erect
48	50584	Kenya	purple	purple	purple	medium	medium	dark green	Sparse	erect
49	50600	Kenya	purple	purple	purple	profuse	medium	dark green	Sparse	erect

3.6.1.2 Cluster analysis

Cluster analysis revealed two major clusters (Cluster I and II) for the study accessions (Fig 3.4). The phenogram was generated based on Euclidean Distance Coefficient and UPGMA clustering method. This revealed a phenotypic relationship among the accessions for the eight morphological descriptors studied. Cluster I included 9 accessions chiefly from the South African region with an exception of one Kenyan accession 1959. Similarly, cluster II had 40 accessions majorly comprising the Kenyan accessions (Fig 3.4). There was a close relationship between South African accessions 2249 and 2232 which were collected from Northern Province. Some variation was also noted among the Kenyan accessions involving a mixture of gene bank and farmers landraces. Most of the Kenyan gene bank accessions namely 50339,50330,50328 50326,50299 and 50273 from Nyamira region clustered closely together with accession 30316 from western despite being collected from the two different regions. Additionally, Kenyan accession 45451 from central and Kenyan accession 14 from Kisii grouped together suggesting a close resemblance in their genetic traits. Most of the Kenyan farmer's landraces were seen to cluster together according to their area of collection. More so, they overlapped with the gene bank accession from that particular region hence expressing some similarities. The present study also noted that Kenyan accession 31990 from western region was more closely related to the South African accessions than to other Kenyan accessions (Fig 3.4).



Kenyan gene bank accessions Kenyan farmers' landraces South African gene bank accessions

Figure 3.4: Phenogram showing relationship among accessions

3.6.1.3 Diversity index

Estimates of Shannon-Weaver (H') for the qualitative characters evaluated in the study accessions were generally high. All traits showed high (H'>0.500) levels of polymorphism. The indices ranged from 0.980 (petiole colour) to 0.997 (Growth habit) with an average of 0.993 (Table 3.4).

Table 3. 4: Diversity index values

Qualitative character	Shannon-Weaver index (H')
flower colour	0.987
Growth habit	0.997
leaf colour	0.988
petiole hair	0.987
petiole colour	0.980
stem colour	0.983
stem hair	0.985
Average diversity index	0.993

3.6.1.4 Principal component analysis

The present study revealed that the first four components contributed to 95% of the total variation among the 49 accessions (Table 3.5). It was observed that Stem hairiness and petiole hairiness were the main traits that contributed positively to the first Principal component. Leaf colour had a negative loading to this component at -0.09. Stem hairiness contributed the most to the second principal component. Petiole hair, petiole colour and stem colour had negative loadings to this component at -0.01, -0.89 and -0.06 respectively (Table 3.5).

Table 3. 5: Principal component analysis values

Qualitative character	***principal component***					
	PC 1	***PC 2***	***PC 3***	***PC 4***	***PC 5***	***PC 6***
% variation	74.29	9.91	7.01	4.02	2.57	1.52
% cumulative	74.29	84.20	91.21	95.23	97.80	99.32
Eigen value	7.30	0.97	0.69	0.40	0.25	0.15
flower colour	0.14	0.13	0.39	0.56	**0.68**	0.15
Growth habit	0.05	0.03	-0.03	0.05	0.08	-0.21
leaf colour	-0.09	0.04	-0.02	0.16	-0.29	0.91

Table 3. 5: Principal component analysis values

Qualitative character	*principal component*					
	PC 1	*PC 2*	*PC 3*	*PC 4*	*PC 5*	*PC 6*
petiole hair	**0.50**	-0.01	**-0.67**	**0.52**	-0.14	-0.08
petiole colour	0.32	**-0.89**	0.04	-0.19	0.20	0.16
stem colour	0.38	-0.06	0.63	0.25	**-0.60**	-0.18
stem hair	**0.69**	**0.43**	0.03	-0.53	0.15	0.19

Values in bold indicate the most relevant descriptors that contributed most to the particular component.

3.6.2 Quantitative characteristics

There were variations in the counts and measurable traits amongst the accessions under study. These were; days to flowering, leaf length and width, number of primary branches, number of leaves per plant, plant height, single Leaf area and amount of chlorophyll present through SPAD. The average number of days to 50% flowering per accession varied from 32 days to 50 days with a grand mean of 41.8. The earliest to flower was a South African accession 2000 at 32 days after emergence (Table 3.6). Accession number 2279 recorded the longest flowering period of 50 days. The coefficient of variation for the number of days to 50% flowering was 2.2%. Both the leaf length and leaf width varied significantly (P <0.05) among the study accessions. Accession 11 had the shortest length of 3.6 cm while accession 50296 had the longest length of 10.5 cm. The mean of the leaf length was 5.8 cm with a coefficient of variation of 7.4%. The leaf width ranged from 7.9 cm for accession 50298 to 18.25 cm for 50296 with a mean of 12 cm and a coefficient of variation of 8.1%. A mean of eight primary branches per plant was recorded in this study (Table 3.6). The lowest and highest number of branches was four and twelve respectively among the accessions. The coefficient of variation was 7.8%. Results indicated that there was a high significance for the number of leaves among the accessions. Kenyan accession 45451 yielded most leaves (247), while the South African accession (2000) had the least number of leaves counted (19). The coefficient variation was 4% and a grand mean of 97 leaves per plant. Plant height varied from the tallest accession 50296 with a height of 113 cm to the shortest being

21 cm for accession 2249. Generally, Kenyan accessions were taller than the South African accessions with a grand mean of 68.4 and a coefficient variation of 4%. There were highly significant (P <0.01) differences among the accessions in leaf area. A mean of 8.5 cm^2 and a coefficient variation of 6.5% were recorded. Accession 15 had the smallest area of 6 cm^2 while the largest leaf area was 14 cm^2 for accession 50296. The South African accessions showed relatively much lower SPAD value than the Kenyan accessions. The lowest and highest value recorded was 24 and 62 respectively. The grand mean for chlorophyll content was 55 with a coefficient variation of 3.3% for all the studied accessions

Table 3. 6: Table of means for the quantitative traits of 49 spider plant accessions from Kenya and South Africa grown in the University of Nairobi Field at Kabete, for the two combined seasons

Entry	*Accession No.*	*Origin*	*DTF*	*LL*	*LW*	*NPB*	*NLPP*	*PH*	*SLA*	*SPAD*
1	1	Kenya	39.7	5.1	14.2	6.3	105.0	38.3	8.7	56.4
2	2	Kenya	45.7	6.4	11.5	5.7	58.8	31.5	8.8	57.6
3	3	Kenya	45.3	6.7	17.0	7.0	112.2	41.0	10.9	50.3
4	4	Kenya	43.8	5.2	13.8	7.5	75.8	36.7	8.7	56.9
5	5	Kenya	45.3	5.0	13.2	7.5	53.8	46.8	8.3	57.6
6	6	Kenya	39.7	5.0	11.7	7.5	57.2	47.8	7.8	56.9
7	7	Kenya	45.0	5.9	16.0	7.7	91.3	51.2	9.9	53.0
8	9	Kenya	45.5	6.2	14.3	6.5	94.3	39.0	9.6	56.6
9	10	Kenya	41.5	5.6	11.7	5.7	68.0	40.7	8.3	58.3
10	11	Kenya	45.0	3.6	10.6	5.5	100.5	40.1	6.3	56.8
11	12	Kenya	46.0	4.6	11.9	7.5	61.0	42.2	7.6	58.8
12	13	Kenya	37.8	5.0	13.1	6.2	64.8	38.3	8.3	55.9
13	14	Kenya	39.8	4.7	11.3	7.8	83.3	63.2	7.4	53.3
14	15	Kenya	38.7	3.7	9.3	5.7	55.7	37.8	6.0	46.7
15	16	Kenya	45.2	7.8	15.6	9.0	75.7	75.5	11.2	61.6
16	1959	S. Africa	43.8	5.8	12.0	6.7	89.2	34.3	8.5	54.3
17	1988	S. Africa	34.2	6.8	12.6	5.7	50.7	45.2	9.5	49.9
18	2000	S. Africa	32.8	6.5	13.6	4.2	19.7	30.8	9.6	53.1
19	2232	S. Africa	39.8	5.3	10.0	4.7	56.0	26.3	7.4	24.3
20	2241	S. Africa	45.3	7.8	15.4	7.8	41.0	42.3	11.2	44.0
21	2249	S. Africa	39.3	6.2	13.2	4.2	20.3	21.2	9.2	42.9
22	2279	S. Africa	53.2	6.3	12.3	6.7	23.2	22.1	9.0	39.6
23	2289	S. Africa	44.0	7.2	13.9	6.3	31.3	41.7	10.2	43.0
24	2299	S. Africa	40.0	5.1	9.8	7.3	78.0	30.3	7.2	43.7
25	30316	Kenya	37.7	5.9	10.3	10.0	146.0	83.0	8.0	57.1
26	31990	Kenya	41.0	5.7	13.2	8.5	109.3	91.3	8.8	55.4
27	31992	Kenya	42.0	8.0	11.0	9.2	201.0	91.7	9.9	59.5
28	45426	Kenya	43.0	7.4	11.5	10.0	97.0	97.0	9.5	58.4
29	45446	Kenya	42.3	8.8	13.0	8.3	68.0	111.7	11.1	57.3
30	45451	Kenya	47.7	7.2	16.1	9.2	247.5	109.3	10.9	53.9
31	50259	Kenya	44.0	5.7	10.6	9.2	182.0	101.3	7.9	58.1
32	50264	Kenya	40.2	4.4	10.5	11.7	172.2	108.8	6.9	60.8
33	50265	Kenya	44.3	5.5	11.6	10.3	78.7	92.2	8.2	60.1
34	50273	Kenya	40.3	5.1	9.5	8.7	92.8	89.7	7.1	57.5

Table 3. 6: Table of means for the quantitative traits of 49 spider plant accessions from Kenya and South Africa grown in the University of Nairobi Field at Kabete, for the two combined seasons

Entry	*Accession No.*	*Origin*	*DTF*	*LL*	*LW*	*NPB*	*NLPP*	*PH*	*SLA*	*SPAD*
35	50290	Kenya	40.3	4.8	10.4	9.3	105.2	93.2	7.1	62.2
36	50296	Kenya	40.0	10.5	18.3	10.7	109.5	113.0	14.2	57.9
37	50298	Kenya	40.0	4.4	7.9	11.5	140.0	82.7	6.1	59.5
38	50299	Kenya	41.7	4.8	9.4	10.5	101.3	99.8	6.9	58.9
39	50307	Kenya	40.0	6.0	12.0	8.3	140.7	93.8	8.7	58.4
40	50319	Kenya	40.3	6.2	12.5	9.5	101.0	77.8	9.0	62.5
41	50325	Kenya	40.0	6.8	12.1	10.0	94.8	95.0	9.3	57.9
42	50326	Kenya	44.3	5.6	11.1	12.5	165.8	101.2	8.1	57.5
43	50328	Kenya	40.2	5.4	8.6	8.7	96.5	75.7	7.1	58.6
44	50330	Kenya	39.8	5.7	10.4	9.8	172.0	104.3	7.8	60.8
45	50332	Kenya	40.5	4.2	8.8	9.2	115.8	110.5	6.2	62.5
46	50339	Kenya	42.7	5.2	12.8	12.5	149.8	101.7	8.3	61.6
47	50353	Kenya	42.3	4.5	8.4	11.0	146.8	86.0	6.3	57.8
48	50584	Kenya	39.7	4.8	9.8	9.7	88.8	78.7	7.0	57.9
49	50600	Kenya	38.8	5.9	8.8	9.5	78.8	98.8	7.5	56.2
	Mean		**41.8**	**5.8**	**12.0**	**8.0**	**97.0**	**68.4**	**8.5**	**55.1**
	LSD (p<0.05)		**1.5**	**0.7**	**1.6**	**1.0**	**6.3**	**4.4**	**0.9**	**2.9**
	CV %		**2.2**	**7.4**	**8.1**	**7.8**	**4.0**	**4.0**	**6.5**	**3.3**

LSD- Least significant difference, CV-coefficient of variation, DTF- days to 50% flowering, SPAD- chlorophyll content, PH- plant height (cm),, NPB- number of primary branches, LL- single leaf length, LW- leaf width, SLA- single leaf area (cm2), NLPP- number of leaves per plant.

Table 3. 7: Means for the quantitative traits of 49 spider plant accessions from Kenya and South Africa grown in the University of Nairobi Field at Kabete during the first season

Entry	*Accession No.*	*Origin*	*DT*	*LL*	*LW*	*NPB*	*NLPP*	*PH*	*SLA*	*SPAD*
1	1	Kenya	40.7	5.3	14.4	6.3	103.0	37.7	8.9	56.2
2	2	Kenya	47.0	6.5	11.8	5.7	60.0	31.0	9.0	57.3
3	3	Kenya	46.3	6.9	17.2	7.0	112.3	40.3	11.1	51.2
4	4	Kenya	45.0	5.2	13.9	7.3	75.3	36.3	8.7	56.8
5	5	Kenya	46.0	5.0	13.2	7.7	54.0	46.0	8.3	57.2
6	6	Kenya	40.7	5.2	11.5	7.7	54.7	47.0	7.9	57.2
7	7	Kenya	45.7	6.1	16.3	7.7	89.7	52.3	10.2	52.8
8	9	Kenya	46.3	6.3	14.5	6.3	95.3	39.3	9.7	55.7
9	10	Kenya	42.3	5.8	11.7	5.7	67.0	41.0	8.4	58.9
10	11	Kenya	45.7	3.5	10.9	5.7	100.7	40.2	6.4	56.0
11	12	Kenya	47.7	4.7	12.3	7.3	62.3	42.0	7.7	59.0
12	13	Kenya	38.3	5.1	13.3	6.0	64.7	37.3	8.4	55.9
13	14	Kenya	41.0	4.7	11.3	8.0	83.3	62.7	7.4	53.3
14	15	Kenya	39.0	3.6	9.2	5.7	55.0	37.3	5.9	46.4
15	16	Kenya	46.0	7.5	15.3	9.3	75.0	77.0	10.9	61.6
16	1959	S. Africa	45.0	5.8	12.2	6.7	89.3	33.7	8.6	54.2
17	1988	S. Africa	34.0	7.1	12.3	5.3	50.3	45.0	9.6	50.4
18	2000	S. Africa	33.3	6.6	13.7	4.0	19.0	31.0	9.7	53.5
19	2232	S. Africa	40.7	5.4	10.4	4.3	57.3	26.0	7.6	21.1
20	2241	S. Africa	46.0	8.0	15.6	8.0	40.0	41.0	11.4	42.8
21	2249	S. Africa	40.0	6.4	13.3	4.3	17.7	21.3	9.4	44.2
22	2279	S. Africa	50.3	6.5	12.8	6.7	21.0	21.8	9.4	38.3
23	2289	S. Africa	45.3	7.5	14.0	6.7	31.0	41.0	10.5	43.0
24	2299	S. Africa	40.0	5.3	10.0	7.0	76.7	29.7	7.5	44.0
25	30316	Kenya	37.7	5.9	10.6	10.7	147.3	82.7	8.1	56.7
26	31990	Kenya	40.7	5.7	13.3	8.7	110.3	91.7	8.9	55.4
27	31992	Kenya	42.7	8.1	11.6	9.3	200.0	93.3	10.2	59.6

Table 3. 7: Means for the quantitative traits of 49 spider plant accessions from Kenya and South Africa grown in the University of Nairobi Field at Kabete during the first season

Entry	*Accession No.*	*Origin*	*DT*	*LL*	*LW*	*NPB*	*NLPP*	*PH*	*SLA*	*SPAD*
28	45426	Kenya	43.3	7.1	11.9	10.3	95.0	95.0	9.4	58.9
29	45446	Kenya	43.3	8.7	13.0	8.3	67.7	112.0	11.1	57.8
30	45451	Kenya	48.7	7.2	16.2	9.3	243.7	109.0	11.0	52.7
31	50259	Kenya	44.7	5.7	10.8	9.3	182.7	104.7	8.0	58.4
32	50264	Kenya	41.3	4.5	10.7	12.0	173.3	107.3	7.0	61.0
33	50265	Kenya	45.3	5.5	11.4	10.3	77.3	92.7	8.1	60.2
34	50273	Kenya	42.0	5.2	9.6	8.7	91.7	89.0	7.2	57.6
35	50290	Kenya	41.3	4.8	10.2	9.3	102.3	92.3	7.1	62.2
36	50296	Kenya	41.3	10.5	18.3	11.7	109.7	113.3	14.3	57.5
37	50298	Kenya	40.7	4.3	8.1	11.7	142.0	81.3	6.1	58.0
38	50299	Kenya	42.0	4.8	9.3	10.3	105.3	98.7	6.8	59.5
39	50307	Kenya	40.7	6.0	12.0	8.3	139.3	92.7	8.7	58.8
40	50319	Kenya	41.0	6.3	12.5	9.3	101.3	77.7	9.1	61.2
41	50325	Kenya	40.7	6.9	12.0	10.7	96.3	93.3	9.4	58.8
42	50326	Kenya	45.3	5.6	11.1	12.7	166.7	98.0	8.1	55.2
43	50328	Kenya	40.7	5.3	8.8	8.7	96.3	75.0	7.0	58.6
44	50330	Kenya	40.7	5.7	10.6	10.3	170.7	105.7	8.0	61.5
45	50332	Kenya	41.3	4.3	8.9	9.3	116.7	111.0	6.3	63.1
46	50339	Kenya	43.3	5.3	12.9	12.7	144.3	101.7	8.4	61.5
47	50353	Kenya	43.3	4.6	8.5	11.0	145.0	85.7	6.4	56.8
48	50584	Kenya	40.0	4.9	9.7	9.3	86.7	76.3	7.0	58.4
49	50600	Kenya	39.3	5.9	8.9	9.7	81.0	98.0	7.5	56.7
	Mean		**43.0**	**5.9**	**12.1**	**8.0**	**97.0**	**68.1**	**8.6**	**55.0**
	LSD (p<0.05)		**1.6**	**0.78**	**1.92**	**0.9**	**5.9**	**4.65**	**1.06**	**2.37**
	CV %		**2.3**	**8.2**	**9.8**	**6.5**	**3.8**	**4.2**	**7.6**	**2.7**

LSD- Least significant difference, CV-coefficient of variation, DTF- days to 50% flowering, SPAD- chlorophyll content, PH- plant height (cm),, NPB- number of primary branches, LL- single leaf length, LW- leaf width, SLA- single leaf area (cm2), NLPP- number of leaves per plant

Table 3. 8: Table of means for the quantitative traits of 49 spider plant accessions from Kenya and South Africa grown in the University of Nairobi Field at Kabete during the second season

Entry	*Accession No.*	*Origin*	*DT*	*LL*	*LW*	*NB*	*NLPP*	*PH*	*SLA*	*SPAD*
1	1	Kenya	38.7	5	13.9	6.3	107	39	8.6	56.5
2	2	Kenya	44.3	6.2	11.3	5.7	58	32	8.6	57.9
3	3	Kenya	44.3	6.5	16.8	7	112	41.7	10.7	49.4
4	4	Kenya	42.7	5.3	13.7	7.7	76	37	8.7	57
5	5	Kenya	44.7	5	13.2	7.3	54	47.7	8.3	58
6	6	Kenya	38.7	4.8	11.9	7.3	60	48.7	7.7	56.6
7	7	Kenya	44.3	5.7	15.8	7.7	93	50	9.7	53.1
8	9	Kenya	44.7	6.1	14	6.7	93	38.7	9.4	57.4
9	10	Kenya	40.7	5.5	11.7	5.7	69	40.3	8.2	57.7
10	11	Kenya	44.3	3.6	10.4	5.3	100	40	6.3	57.6
11	12	Kenya	44.3	4.5	11.5	7.7	60	42.3	7.4	58.7
12	13	Kenya	37.3	5	12.9	6.3	65	39.3	8.2	56
13	14	Kenya	38.7	4.7	11.2	7.7	83	63.7	7.4	53.3

Table 3. 8: Table of means for the quantitative traits of 49 spider plant accessions from Kenya and South Africa grown in the University of Nairobi Field at Kabete during the second season

Entry	*Accession No.*	*Origin*	*DT*	*LL*	*LW*	*NB*	*NLPP*	*PH*	*SLA*	*SPAD*
14	15	Kenya	38.3	3.9	9.4	5.7	56	38.3	6.1	46.9
15	16	Kenya	44.3	8	16	8.7	76	74	11.5	61.6
16	1959	S. Africa	42.7	5.7	11.8	6.7	89	35	8.4	54.4
17	1988	S. Africa	34.3	6.5	12.8	6	51	45.3	9.3	49.4
18	2000	S. Africa	32.3	6.5	13.6	4.3	20	30.7	9.6	52.6
19	2232	S. Africa	39	5.1	9.6	5	55	26.7	7.2	27.5
20	2241	S. Africa	44.7	7.6	15.2	7.7	42	43.7	11	45.1
21	2249	S. Africa	38.7	6	13	4	23	21	9	41.7
22	2279	S. Africa	51.3	6.1	11.7	6.7	25	22.3	8.7	40.9
23	2289	S. Africa	42.7	6.8	13.8	6	32	42.3	9.9	43
24	2299	S. Africa	40	4.8	9.7	7.7	79	31	6.9	43.4
25	30316	Kenya	37.7	5.9	10	9.3	145	83.3	7.9	57.6
26	31990	Kenya	41.3	5.7	13	8.3	108	91	8.8	55.5
27	31992	Kenya	41.3	7.9	10.3	9	202	90	9.6	59.5
28	45426	Kenya	42.7	7.7	11.1	9.7	99	99	9.6	57.9
29	45446	Kenya	41.3	8.8	13	8.3	68	111.3	11.2	56.8
30	45451	Kenya	46.7	7.1	15.9	9	251	109.7	10.8	55
31	50259	Kenya	43.3	5.7	10.5	9	181	98	7.9	57.8
32	50264	Kenya	39	4.3	10.3	11.3	171	110.3	6.8	60.5
33	50265	Kenya	43.3	5.6	11.8	10.3	80	91.7	8.3	59.9
34	50273	Kenya	38.7	5	9.4	8.7	94	90.3	7	57.5
35	50290	Kenya	39.3	4.7	10.5	9.3	108	94	7.2	62.2
36	50296	Kenya	38.7	10.5	18.2	9.7	109	112.7	14.2	58.2
37	50298	Kenya	39.3	4.6	7.8	11.3	138	84	6.1	61
38	50299	Kenya	41.3	4.8	9.5	10.7	97	101	6.9	58.2
39	50307	Kenya	39.3	6.1	12	8.3	142	95	8.7	58
40	50319	Kenya	39.7	6.1	12.4	9.7	101	78	8.9	63.8
41	50325	Kenya	39.3	6.7	12.1	9.3	93	96.7	9.2	56.9
42	50326	Kenya	43.3	5.6	11.1	12.3	165	104.3	8	59.7
43	50328	Kenya	39.7	5.6	8.4	8.7	97	76.3	7.1	58.5
44	50330	Kenya	39	5.6	10.1	9.3	173	103	7.7	60.2
45	50332	Kenya	39.7	4.1	8.7	9	115	110	6.1	61.9
46	50339	Kenya	42	5.1	12.6	12.3	155	101.7	8.2	61.6
47	50353	Kenya	41.3	4.5	8.3	11	149	86.3	6.2	58.8
48	50584	Kenya	39.3	4.7	9.9	10	91	81	6.9	57.4
49	50600	Kenya	38.3	5.9	8.7	9.3	77	99.7	7.4	55.7
	Mean		**41**	**5.8**	**11.8**	**8.2**	**98**	**69**	**8.4**	**55.2**
	$LSD_{(p<0.05)}$		**1.46**	**0.61**	**1.12**	**1.2**	**6.8**	**4.1**	**0.7**	**3.4**
	CV %		**2.2**	**6.5**	**5.9**	**8.9**	**4.3**	**3.7**	**5.1**	**3.8**

Table 3. 8: Table of means for the quantitative traits of 49 spider plant accessions from Kenya and South Africa grown in the University of Nairobi Field at Kabete during the second season

Entry	*Accession No.*	*Origin*	*DT*	*LL*	*LW*	*NB*	*NLPP*	*PH*	*SLA*	*SPAD*

LSD- Least significant difference, cv-coefficient of variation, DTF- days to 50% flowering, SPAD- chlorophyll content, PH- plant height (cm), NPB- number of primary branches, LL- single leaf length, LW- leaf width, SLA- single leaf area (cm2), NLPP- number of leaves per plant.

3.6.2.1 Correlation among the traits

There was positive significant correlation of leaf length, leaf width and leaf area with number of days to 50% flowering. In the combined season, yield had a positive significant correlation with plant height (r = 0.69) and number of primary branches (r = 0.63). Single leaf area correlated positively with leaf length, width and days to 50% flowering at r = 0.92, r = 0.88 and r = 0.21 respectively. SPAD value had a significant correlation with number of leaves per plant (r = 0.45) number of primary branches (r = 0.54) and plant height (r = 0.59). All the correlations were similar for both seasons for all the accessions studied (Table 3.9; Table 3.10).

Table 3. 9: Correlation in combined seasons at the Kabete field station

	DTF	**LL**	**LW**	**NPB**	**NLPP**	**PH**	**SLA**	**SPAD**
DTF	-							
LL	0.12*	-						
LW	0.28**	0.62**	-					
NPB	0.09	0.02	-0.20**	-				
NLPP	0.1	-0.01	-0.11	0.63**	-			
PH	-0.07	0.16*	-0.19*	0.82**	0.69**	-		
SLA	0.21**	0.92**	0.88**	-0.08	-0.06	0.01	-	
SPAD	-0.04	-0.07	-0.11	0.54**	0.45**	0.59**	-0.1	-

*implies significance difference at P<0.05; ** implies significance difference at p<0.001 (2-tailed) ;,DTF- days to 50% flowering, SLA- single leaf area (cm^2), LL- leaf length (cm), LW- leaf width (cm), NLPP- number of leaves per plant, NPB- number of primary branches, PH- plant height (cm), SPAD- soil plant analysis development

Table 3.10 Correlation coefficients for the quantitative traits in season 1(April - July 2014) at the Kabete field station

	DTF	**LL**	**LW**	**NPB**	**NLPP**	**PH**	**SLA**	**SPAD**
DTF	-							
LL	0.11	-						
LW	0.31**	0.63**	-					
NPB	0.09	0.04	-0.17*	-				
NLPP	0.11	-0.04	-0.1	0.65**	-			

PH	-0.05	0.13	-0.19*	0.83**	0.70**	-		
SLA	0.22*	0.92**	0.88**	-0.06	-0.07	-0.01	-	
SPAD	-0.03	-0.09	-0.11	0.53**	0.42**	0.59**	-0.11	-

*implies significance difference at P<0.05; ** implies significance difference at p<0.001 (2-tailed) ;,DTF- days to 50% flowering, SLA- single leaf area (cm^2), LL- leaf length (cm), LW- leaf width (cm), NLPP- number of leaves per plant, NPB- number of primary branches, PH- plant height (cm), SPAD- soil plant analysis development

Table 3.11 Correlation coefficients for the quantitative traits in season 2 (Oct – Dec 2014) at the Kabete field station

	DTF	**LL**	**LW**	**NPB**	**NLPP**	**PH**	**SLA**	**SPAD**
DTF	-							
LL	0.11	-						
LW	0.25*	0.60**	-					
NPB	0.08	0.01	-0.23*	-				
NLPP	0.1	0.01	-0.12	0.62**	-			
PH	-0.09	0.20*	-0.19*	0.80**	0.68**	-		
SLA	0.19*	0.92**	0.87**	-0.11	-0.05	0.03	-	
SPAD	-0.03	-0.05	-0.1	0.57**	0.47**	0.59**	-0.08	-

*implies significance difference at P<0.05; ** implies significance difference at p<0.001(2-tailed) ;,DTF- days to 50% flowering, SLA- single leaf area (cm^2), LL- leaf length (cm), LW- leaf width (cm), NLPP- number of leaves per plant, NPB- number of primary branches, PH- plant height (cm), SPAD- soil plant analysis development

3.6.3 Heritability

Estimates of heritability in broad sense for all the traits studied were similar in both seasons. They ranged from 78% to 99% for the measured parameters namely; number of leaves per plant, number of primary branches, leaf length, leaf width, plant height, single leaf area, and soil plant analysis development. High percentages of broad sense heritability were estimated for number of leaves per plant, plant height at 99%, and SPAD value at 96%. Leaf width exhibited the lowest percentage at 78% followed by single leaf area and leaf length at 86%, 89% respectively. The genotypic variance was also found to be higher than environmental variance (Table 3.11).

Table 3. 12: Estimate of broad sense heritability expressed for 49 *C. gynandra* accessions.

Traits		**VE**	**VG**	**VP**	**HBS %**
DTF	Season 1	1.0	11.2	12.2	91.8
	Season 2	1.0	10.1	11.1	91.0
LL	Season 1	0.2	1.7	1.9	89.3

	Season 2	0.2	1.7	1.9	89.3
LW	Season 1	1.4	5.0	6.4	78.0
	Season 2	1.4	5.3	6.7	79.2
NPB	Season 1	0.3	4.8	5.1	94.1
	Season 2	0.3	3.8	4.1	92.7
NLPP	Season 1	13.4	2294.1	2307.5	99.4
	Season 2	13.4	2314.6	2328.0	99.4
PH	Season 1	8.2	908.6	916.8	99.1
	Season 2	8.2	905.8	914.0	99.1
SLA	Season 1	0.4	2.6	3.0	86.5
	Season 2	0.4	2.6	3.0	86.5
SPAD	Season 1	2.1	54.4	56.5	96.3
	Season 2	2.1	45.1	47.2	95.5

VE = Environmental variance VG = Genotypic variance VP = Phenotypic variance, HBS = Broad sense heritability, NLLP = Number of leaves per plant, NPB=Number of primary branches, LL = Leaf length, LW =Leaf width, PH =Plant height, SLA= Single leaf area, SPAD = soil plant analysis development

3.7 Discussion

Previous research has indicated that spider plant belongs to the genus *Cleome* with over 200 species that are highly polymorphic (K'opondo, 2011). During the research work, the weather conditions were favorable in both seasons. However, overhead irrigation was supplemented at the onset of flowering. This presented an ideal condition for yield performance among the study accessions. This study revealed variations among the genotypes based on the morphological characters that were measured. Similarly, the analysis of variance for the quantitative traits showed significant differences implying the existence of variability for these traits among the 49 accessions of spider plant studied. About 49% of the accessions had purple flowers of which, all happened to be of Kenyan origin. The other half was dominated by pink and white flowers. All South Africa accessions had white flowers with an exception of one accession 1959 that had a pink flower.

Variations were also noted among the stem and petiole pigmentation. Pigmentation variation is associated with accumulation of anthocyanins in the plant tissues that are very important in health promoting compounds (Beggs et al., 1994). In his study, Schippers (2002), indicated that the purple stemmed cultivars are more nutritious and resistant to insects, but more susceptible to diseases than the green stemmed. The stem colour observed in this study was either green or purple. Majority of the South African accessions presented a green stem with a green petiole contrarily to the Kenyan accessions that were majorly purple stemmed. This shows a high potential for nutrition value especially in the purple stemmed accessions from Kenya. The purple stem seemed to have profuse pubescence as compared to the green stem that was glabrous or sparsely haired. This could have a negative effect in terms of taste and preferences in its adaptability where the hairs are not preferred (Masayi and Netondo, 2012). Therefore, sparsely haired purple stems should be regarded for high nutrition during selection to ensure palatability of the improved varieties. Makgakga (2011) reported that purple stemmed plants are usually hairier than green stemmed plants. However, the hairs have been reported to improve the adaptability of the plant in various environs.

Earlier studies have reported that growth habit is crucial in the cropping system of spider plant and a significant trait in harvesting of the plant (Mnzava, 1997). Three main growth habits namely erect, semi erect and prostrate have been described in spider plants, by the Food and Agriculture Organization (FAO, 1995). Two growth habits were observed among the forty-nine studied accessions. About 90% of the accessions had an erect growth versus a proportion of 10% with a semi erect growth. This implies that farmers could use the semi-erect types in mixed cropping whereas the erect types are ideal for intercrop adaptability (Masuka and Mazarura,

2012). The results in this study also conformed to previous study by K'Opondo (2011) who characterized majority of the spider plant morphotypes with an erect growth.

Through the morphological characterization of the eight descriptors used in this study, all the accessions were grouped into two major clusters namely; Kenyan accessions and South African accessions. The clustering patterns based on UPGMA revealed variations in the phenotypic makeup of the two accession groupings (cluster I and II). The two clusters clearly differentiate the accessions from Kenya and South Africa suggesting that crop improvement programs on spider plant should be country specific to target preferred traits in each country.

Late flowering enables a genotype to have a longer vegetative phase during growth period (Omondi, 1990). In this study, accessions that took long to reach the 50% flowering yielded more leaf count. Other traits that contributed to increased leaf count were plant height and number of primary branches.

From the correlation results plant height and number of primary branches were positively correlated with number of leaves per plant implying that the higher the number of the branches and the taller the plant, the higher the number of leaves. There was significant positive correlation between leaf length, leaf width and leaf area. However there was a negative correlation between leaf size and leaf yield. The accessions with large single leaf areas had few leaves per plant but with high SPAD value. Leaf yields may be improved through selection of accessions that showed high leaf count as well large single leaf area. The heritability of any trait depends on the genotype as well as the environment in which it is grown (Nguyen and Sleper, 1983). In this study, the genotypic variance was higher than the environmental variance implying that much of the phenotypic variation among the accessions was attributed to variation in

genotype while there was little influence from the environment. The high estimates of heritability displayed in the studied traits like number of leaves per plant and plant height at 99% signify that they can greatly aid in selection for yield improvement in spider plant in a breeding program.

3.8 Conclusion

Significant phenotypic variation exists in *Cleome gynandra.* Morphological and agronomic characterization differentiated the study accessions from the two regions. An overlap in morphological statistics was also observed indicating similarities between accessions from the same region for most of the characters studied. The new knowledge generated on the morphological structure of these accessions has a great potential in developing a mapping population for a spider plant breeding program. Generally, the Kenyan accessions seemed to perform better than South African accessions for most traits evaluated in this study. This could be possibly due to the fact that on contrary, domestication of spider plant in South Africa is still mainly limited to research stations (DAFF, 2010), as compared to Kenya where domestication is by farmers who select those traits that are of merit to them through continued cultivation (K'Opondo, 2011). Besides, the accessions from Kenya could have performed well because of adaptability to the Kenyan environment.

This study also revealed some of qualitative and quantitative traits that could be of importance in evaluating elite accessions of breeding programme for yield namely days to 50% flowering, leaf width and length, single leaf area, number of primary branches and number of leaves per plant. The outstanding accessions for these traits in this study were Kenyan accessions: 3, 7, 8, 16, 45426, 45446, 45451, 50296, and 50325. South Africa accessions: 1959, 2000, 2279 and

2289. This study concludes that number of leaves per plant, plant height and number of primary branches with high heritability should be taken into consideration in selection for yield in *C. gynandra.*

3.9 Recommendation

Gene interaction is critically influenced by their environment (Phillips, 2006). The use of molecular markers would complement this study by identifying greater polymorphism**.** Furthermore, integration of molecular markers to genetically group the accessions would be a milestone in establishing true genetic diversity. More characterization needs to be done under different environments to evaluate yield performance across localities. Additionally, further evaluation should also be done using more accessions and looking at more characteristics other than the ones used in this study so as to widen the scope of diversity. Improving a nutritious vegetable with nutraceutical value which is locally available among the most vulnerable population in Africa promises to significantly improve food security and result in improved health among the rural and urban populations of Kenya.

CHAPTER 4

NUTRITION AND YIELD EVALUATION AMONG ASSORTED SPIDER PLANT ACCESSIONS.

Abstract

Today most scientific research reveals that human health is directly connected to nutrition. The need for a broad range of nutrients is seen from the current efforts on bio fortification in some key crops due to an increasing rise in cases of malnutrition and micro-nutrient deficiency diseases across Africa. Indigenous leafy vegetables have been found to be highly nutritious, most convenient natural sources that can be consumed to overcome malnutrition in Africa. Spider plant being one of the indigenous African leafy vegetables has recently been attracting research attention not only in terms of its inherent nutrition quality but also its healing power. Despite it being a micronutrient-rich crop, little attention has focused on its production and improvement as compared to other energy and protein-giving foods. Spider plant contains high levels of beta-carotene, vitamin C, and moderate levels of minerals. The concentration of these nutrition components vary due to genotypic and environmental influences. The objective of this study was to identify genotypes with high nutrition content and good yield and yield related traits. A total of nineteen assorted spider accessions were grown in pots using a randomized complete block design with three replications in a glasshouse at the field station of University of Nairobi, Upper Kabete Campus. Vitamin C, beta carotene and phenol were the nutritional components evaluated. Morphological characterization for various spider plant accessions was done as per the list of modified Food and Agriculture Organization (FAO) spider plant descriptors. Stem and flower colour, petiole and leaf colour, stem and petiole hairiness were evaluated as qualitative traits while number of leaves per plant, plant height, leaf width, leaf length, single leaf area and

chlorophyll content were the quantitative traits evaluated. Heritability for the nutrition and yield related traits were also estimated. The study accessions were very divergent with regard to nutrition and yield related traits showing significant differences at $P < 0.05$. The average values showed a wide variation in vitamin C with accession 9^{ke} recording the highest amount of vitamin C with 279 mg/100g whereas the lowest recorded was 63 mg/100g. Beta carotene ranged between 0.6 - 7.2 mg/100g while the total phenols ranged between 3.1-10.6 mg/100g respectively. We observed a positive correlation between beta carotene and flower(r = 34.2) stem colour(r = 35.6) and petiole colour (r = 32.7) implying spider plant accessions can be differentiated, not just as a function of their morphological attributes but also as a function nutritional quality. Leaf yield varied significantly in terms of number of leaves and leaf size with some low yielders displaying a large single leaf among the genotypes. The mean values for the number of leaves ranged between 9 and 157 while the single leaf area ranged from 6.7 cm^2 to 11.4 cm^2. High estimates of heritability > 80% were expressed for the nutrition and yield related characters. The presence of a considerable degree of compositional variability of these nutraceuticals among the tested accessions, suggests that these accessions can be a valuable source of genes for improved vitamin and phenolic content in spider plant. The nutrition components vitamin C, beta carotene, phenols and yield related traits namely; number of leaves per plant, plant height and single leaf area were found to be crucial traits that will greatly aid in selecting for nutrition and yield improvement in a spider plant breeding program.

4.1 Introduction

Nutrition plays a central role in alleviating food insecurity and ill health in developing countries. Food insecurity and malnutrition is an issue of concern in Kenya and other countries in Sub-Saharan Africa. Poverty and the insufficient supply of nutritious foods are hindrances to an adequate and balanced diet which is essential for health. Studies have also shown the potential synergetic effect of indigenous African leafy vegetables as nutraceuticals (micro-nutrient dense and medicinal) (IPGRI**,** 2006). Nutritional studies of spider plant (*Cleome gynandra*) have reported that it is nutritionally superior when compared to exotic vegetables like cabbage because it contains higher levels of β-carotene, vitamin C, protein, iron, calcium and magnesium that are crucial in counteracting deficiency related diseases (Mbugua et al., 2011). Therefore, integrating micronutrient-rich foods such as indigenous vegetables into diets is the most practical and sustainable way to alleviate micronutrient deficiency (Ali and Tsou, 1997). Surveys have shown that spider plant is among the traditional leafy vegetables whose consumption is on the increase in Kenya (IPGRI, 2003). However, due to lack of improved varieties it has led to low yields of the crop (Ekpong, 2009; Madisa et al., 1997). Presently, there are many genotypes of spider plant that are available (IPGRI, 1997). However, majority of these varieties are yet to be characterized in terms of their nutritional composition. Studies of genetic variability, heritability and correlation between properties can show the scope to which certain traits are genetically determined and which of them is the most significant in the selection and development of new cultivars (Milatovic et al., 2010). The present study was therefore designed to determine the levels of β- carotene, vitamin C content and total phenolic, and their correlations with morphological traits; flower, stem and petiole colour as well as stem, petiole and leaf hairiness. Heritability of yield related components namely; number of leaves per plant, leaf length, leaf

width, plant height, single leaf area and SPAD value together with nutrition components; vitamin C, beta carotene and phenols were also estimated.

4.2 Materials and methods

4.2.1 Study site and plant materials

The glasshouse experiments were carried out in October 2014 to January 2015 at the field station of University of Nairobi, Upper Kabete Campus. A total of 19 accessions were grown in 57 pots using a randomized complete block design with three replications. They consisted of 5 assorted accessions and 14 accessions from the World Vegetable Centre (AVRDC) (Table 4.1). The seeds were sparingly sown in each separate pot and watered after every three days .Thinning was done 14 days after seedling emergence leaving three plants per pot which were kept free from any volunteer weeds. Data on both the quantitative and qualitative traits was recorded. All the leaves in each pot were harvested separately for analysis at the 6^{th} week after planting just before the onset of flowering. The leaf samples were freeze dried and ground into moderately fine powder as per (AOAC 2000). Vitamin C, Beta carotene and total Phenols analysis was done at the laboratories in the Department of Food Science and Technology, JKUAT.

Table 4. 1: List of the accessions used in this study

Entry	Acc.No	source
1	9^{ke}	Kenya
2	13^{ke}	Kenya
3	50339^{ke}	Kenya
4	50265^{ke}	Kenya
5	2000^{sa}	South Africa
6	IP 5^{avrdc}	AVRDC
7	IP 3^{avrdc}	AVRDC
8	GPS^{avrdc}	AVRDC
9	ST $73\text{-}3^{avrdc}$	AVRDC
10	$RW\text{-}SF\text{-}3^{avrdc}$	AVRDC
11	GS^{avrdc}	AVRDC
12	GKK 285^{avrdc}	AVRDC
13	$ML\text{-}SF\text{-}13^{avrdc}$	AVRDC
14	HTT^{avrdc}	AVRDC
15	IP 8^{avrdc}	AVRDC
16	UG $SF\text{-}23^{avrdc}$	AVRDC
17	IP 11^{avrdc}	AVRDC
18	$SITE\text{-}94^{avrdc}$	AVRDC
19	$ST\text{-}93\text{-}1GS^{avrdc}$	AVRDC

^{ke} =Kenyan accessions, ^{sa}= South African accessions, ^{avrdc} = accessions from world vegetable center

4.3 Chemical analysis

4.3.1 Vitamin C analysis

The ascorbic acid content in the samples was determined by HPLC method according to (Vikram et al. 2005). About 0.02 g of the freeze died leaf sample was weighed and extracted with 30ml of 0.8% metaphosphoric acid. This was followed by centrifuging at 3000 rpm for 10 minutes at 4°C. The supernatant was filtered and diluted with 10 ml of 0.8% metaphosphoric acid. This was passed through 0.45 µ filter and 20 µL injected into the HPLC machine. Various concentrations of ascorbic acid standards were also made to make a calibration curve (appendix 3). HPLC analysis was done using Shimdzu UV-VIS detector. The mobile phase was 0.8% metaphosphoric acid, at 1.2 mL/min flow rate and wavelength of 266.0 nm.

4.3.2 Beta carotene

Beta carotene content was analyzed using column chromatography and UV Spectrophotometer; by acetone and petroleum ether extraction method as described by Rodriguez-Amaya and Kimura (2004). Approximately 0.02 g of the freeze dried leaf sample was weighed and extracted using acetone. The extraction with acetone continued until the residue no longer gave colour. The combined extract was made to a volume of 100 mL with acetone. Exactly 25 mL of the extract was evaporated to dryness using rotary evaporator. The residue was dissolved with 10 mL petroleum ether and the solution introduced into a chromatographic column. This was eluted with petroleum ether and beta carotene collected in a flask. The beta carotene elute was made to a volume of 25 mL with petroleum ether and the absorbance was read at 440 nm in a UV-Vis spectrophotometer (Shimadzu model UV –1800). Beta carotene standard was also prepared to make a calibration curve (appendix 4).

4.3.3 Determination of Phenols

Total phenolic content was estimated spectrophotometrically using Folin Ciocalteu reagent, as described by Spanos and Wrolstad (1990) with slight modification, using gallic acid as a standard. About 0.25g of the dry sample was weighed and extracted with 25ml of 95% ethanol at 40°C for 10 minutes. The samples were filtered through Whatman No. 4 filter paper and then evaporated to dryness. Exactly 0.02g of freeze dried leaf sample was weighed in triplicate and each dissolved in 5ml of methanol. These were then shaken for 45 minutes at 40°C then centrifuged at 1000g for 10 minutes. Approximately 0.2ml of the supernatant was mixed with 0.6ml of H_2O followed by 0.2ml of Folin Ciocalteu phenol reagent for 5 minutes. Precisely 1 ml of saturated sodium carbonate (8%w/v) was added and the total volume made up to 3ml with

distilled water. The samples were then kept in the dark for 30 minutes and absorbance read at 765nm. A reference standard of 10-100 ppm of gallic acid in methanol was used (appendix 5). The amount of total phenolics was expressed as milligram gallic acid equivalents (mg GAE) per 100 g sample.

4.3.4 Yield and yield related components

The yield component was estimated by counting the (i) number of leaves per plant and taking the average of three tagged plants in each pot. (ii) Plant height was measured in centimeters from the base of the plant to the tip of the main stem. (iii) Leaf width was measured in centimeters at the widest part of the basal leaf while (iv) leaf length (cm) was measured from pulvinus to the tip of the leaf. Formulae $SLA = 0.763L + 0.34W$, was used to calculate the (v) single leaf area (cm^2) where *SLA* is single leaf area, *L* is leaf length and *W* is leaf width (Rivera et al., 2007). (vi) Leaf chlorophyll content was measured using a SPAD meter (Soil Plant Analysis Development SPAD-502, Minolta Camera Co., Ltd., Japan).

4.3.5 Data analysis

Data obtained from various laboratory analyses of these samples was subjected to the general analysis of variance (ANOVA) to generate genotype means which were separated using Fishers protected least significant model at $P < 0.05$. To obtain the correlation coefficients between the nutritional compounds and the morphological traits, the genotype means were further analyzed using the TASSEL software under the general linear model. Heritability in broad sense was also estimated as a ratio of genotypic variance to the phenotypic variance and expressed as a percentage (Hanson et al., 1956) as per the formula;

$$(H^2) = \left(V_g/V_p \times 100\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 4.1$$

Where, V_g = Genotypic variance, V_p = Phenotypic variance

Genotypic variance ($\sigma^2{}_g$) was derived by subtracting error mean sum of squares (EMS) from the genotypic mean sum of squares (GMS) and divided by the number of replications as given by the formula;

$$\sigma^2{}_g = GMS - EMS/r \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 4.2$$

Where GMS=Genotype mean sum of squares, EMS= error mean sum of squares and r = number of replications

Phenotypic variance ($\sigma^2{}_p$) was derived by adding genotypic variance with error variance as per the formula;

$$\sigma^2{}_p = \sigma^2{}_e + \sigma^2{}_g \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 4.3$$

4.4 Results

4.4.1 Nutrition and yield related components

Table 4.2 shows that there were significant differences at P <0.05 among the genotypes for the nutritive traits (vitamin C, total phenolics, beta-carotene) and yield related traits (number of leaves per plant, leaf length and width, plant height, single Leaf area and amount of chlorophyll through SPAD).

Table 4. 2: Mean sum of squares of accessions grown in the screen house with regard to nutrition and yield related traits among the spider plant accessions.

Source of variation		Nutrition components(mg/100g)			Yield related components(mg/100g)					
	d.f.	Vit C	β carotene	Phenols	NLPP	LL	LW	PH	SLA	SPAD
rep	2	0.65	0.07	0.68	0.02	0.09	1.64	0.39	0.08	0.86
accession no.	18	9986.9*	5.9*	9.9*	4655.36*	3.46*	11.30*	1144.69*	4.45*	118.8*
Residual	36	4.82	0.15	0.48	4.48	0.24	0.72	2.61	0.29	0.93
Total	56									

* = term refers to significant at 5% probability level

Various accessions responded differently in terms of their nutrition composition. (Appendices 6, 7 and 8). For Vitamin C, the Kenyan accession 9^{ke} recorded the highest vitamin C content of 281 mg/100g while AVRDC accession had the least content of vitamin C (63mg/100g) (Table 4.3). Nine accessions; 9^{ke}, 13^{ke}, 503339^{ke}, 50265^{ke}, 2000^{ke}, $IP8^{avrdc}$, $IP11^{avrdc}$, $SITE\text{-}94^{avrdc}$ and $ST\text{-}93\text{-}IGS^{avrdc}$ were found to contain high Vitamin C ranging from 100 to 281mg/100. The Beta carotene ranged from 0.6 -7.2 mg/100g with a grand mean of 2.2 mg/100g (Table 4.3). Among the accessions, 50265^{ke}, $SITE\text{-}94^{avrdc}$, $IP6^{avrdc}$ and 2000^{sa} were found to have high levels of beta-carotene. The total phenolic compounds were significantly different among the *C. gynandra* genotypes and ranged from 3.1 to 10.6 mg/100g with IP^{avrdc} recording the highest content of phenolics among the accessions.

Table 4. 3: Means for vitamin C, Beta carotene and phenols of the assorted spider plant accessions.

	Nutrition components (mg/100g)			Yield components					
Accession No.	**Vitamin C**	**Beta carotene**	**Phenols**	**NLPP**	**LL**	**LW**	**PH**	**SLA**	**SPAD**
9[ke]	279.7	2.4	9.4	94.3	6.2	14.0	43.0	9.5	55.0
13[ke]	201.0	1.9	7.9	66.3	5.1	12.9	43.0	8.3	53.7
50339[ke]	173.7	1.5	6.9	157.3	5.4	12.6	101.0	8.4	59.3
50265[ke]	178.0	7.2	7.7	80.3	5.7	11.8	96.0	8.4	55.3
2000[sa]	139.7	2.9	5.1	26.0	6.4	13.6	36.0	9.5	50.0
IP 5[avrdc]	89.0	2.1	8.4	10.7	6.3	11.5	39.0	8.7	44.4
IP 3 [avrdc]	74.0	1.0	10.6	8.7	6.1	11.7	49.0	8.6	64.0
GPS[avrdc]	70.0	0.7	5.2	21.7	6.3	10.3	35.3	8.3	51.2
ST 73-3[avrdc]	97.0	2.2	7.8	18.3	5.4	11.2	38.9	7.9	49.3
RW-SF-3[avrdc]	84.3	2.0	7.7	14.0	6.6	11.4	54.9	8.9	53.9
GS [avrdc]	65.7	2.1	6.5	12.0	6.3	13.0	21.0	9.3	39.4
GKK 285[avrdc]	67.0	1.5	6.2	11.3	6.8	17.4	33.7	11.1	40.5
ML-SF-13[avrdc]	67.0	0.6	3.1	9.0	4.5	9.5	45.3	6.7	49.2
HTT[avrdc]	82.7	1.2	6.1	11.3	6.0	12.2	45.7	8.7	56.3
IP 8[avrdc]	102.3	2.7	4.8	33.0	9.7	11.8	35.0	11.4	53.0
UG SF-23[avrdc]	63.0	2.2	4.7	11.3	6.1	11.7	49.7	8.6	58.7
IP 11[avrdc]	105.0	2.2	6.1	9.7	7.4	16.2	48.3	11.2	59.4
SITE-94[avrdc]	113.0	3.2	5.2	17.3	5.9	12.1	44.3	8.6	53.3
ST-93-1GS[avrdc]	116.0	2.0	5.5	15.7	5.3	9.9	40.0	7.4	50.5
Mean	114.1	2.2	6.6	33.1	6.2	12.4	47.3	8.9	52.4
%CV	1.9	17.6	10.5	6.4	7.9	6.9	3.4	6.1	1.8
LSD	3.6	0.6	1.2	3.5	0.8	1.4	2.7	0.9	1.6

LSD = Least significant difference, CV = coefficient of variation

4.4.2 Heritability of yield and nutrition components

Broad sense heritability was estimated for both the yield and nutrition components. Components of variation studied included environmental variance, genetic variance and phenotypic variance of the measured nutrition and yield related parameters. In this study, results showed that high broad sense heritability estimates were detected ranging from 82% to 99% (Table 4.4). For the

nutrition components, broad sense heritability estimates was lowest for phenols at 86% and highest for vitamin C at 99% while beta carotene had 92%. High magnitude of broad sense heritability was estimated for number of leaves per plant at 99%, leaf length and width 82%, 83% respectively, plant height 99% single leaf area 82% and SPAD value at 97%. It was also noted that, the genetic variance was greater than the environmental variance (Table 4.4).

Table 4. 4: Estimates of broad sense heritability for nutrition and yield components in 19 *C. gynandra* accessions

	Yield components						Nutrition components		
	NLPP	**LL**	**LW**	**PH**	**SLA**	**SPAD**	**Vit C**	**βcarotene**	**Phenols**
VE	4.48	0.24	0.72	2.61	0.29	0.93	4.82	0.15	0.48
VG	1550.29	1.08	3.53	380.69	1.39	39.29	3327.36	1.92	3.14
VP	1554.77	1.31	4.25	383.3	1.68	40.22	3332.18	2.07	3.62
HBS %	99	82	83	99	82	97	99	92	86

VE = Environmental variance VG = Genotypic variance VP = Phenotypic variance, HBS = Broad sense heritability, NLLP = Number of leaves per plant, LL = Leaf length, LW =Leaf width, PH =Plant height, SLA= Single leaf area, SPAD = soil plant analysis development

4.4.3 Correlations of nutrition compounds with morphological traits

Among the nutrition compounds (vitamin C, beta carotene, phenol) evaluated, only significant correlations between beta carotene and some morphological traits (appendix 9) were observed (Table 4.5). A significant positive correlation r = 0.342 between flower color and beta carotene was detected. Similarly, stem and petiole colour had a significant positive correlation with beta carotene r = 0.356, r = 0.327 respectively.

Table 4. 5: Correlation between specific compounds with morphological characteristics

	Vitamin c		Phenols		Beta carotene	
Morphological traits	**p value**	**r^2**	**p value**	**r^2**	**p value**	**r^2**
Flower colour	0.258	0.027	0.392	0.046	0.004**	0.342
stem colour	0.522	0.010	0.68	0.012	0.005**	0.356
petiole colour	0.234	0.029	0.361	0.052	0.006*	0.327
Stem hairiness	0.204	0.036	0.27	0.082	0.351	0.055
Petiole hairiness	0.185	0.039	0.277	0.08	0.066	0.189
Leaf colour	0.266	0.026	0.305	0.065	0.871	0.002
Leaf hairiness	0.846	0.001	0.987	0.00	0.017*	0.266

**= highly significant, *=significant

4.5 DISCUSSION

Indigenous African leafy vegetables have recently been attracting research attention not only in terms of their inherent nutrition quality but also the healing power of some of these plants. Previous studies have shown spider plant to have more phenolic compounds over many other vegetables using the Folin- Ciocalteou method (Muchuweti et al., 2007). Vitamin C, including ascorbic acid and dehydroascorbic acid, is one of the most important nutritional quality factors in many horticultural crops and has many biological activities in the human body. The content of vitamin C in fruits and vegetables can be influenced by various factors such as genotypic differences, pre-harvest climatic conditions and cultural practices, maturity and harvesting methods and postharvest handling procedures. Vitamin C recorded in this study was within the ranges of 127-484 mg/100g early reported by Chweya and Mnzava (1997) in Spider plant.

The beta carotene range reported in this study was higher compared to the highest range (4.53 mg/100g) recorded in kales (Lefsrud et al., 2007). This underlines the superior nutritional quality of spider plant compared to the ‘fashionable’ exotic vegetables that have been embraced at the expense of indigenous vegetables.

High amounts of total phenolic compounds were observed in all *C. gynandra* genotypes given the fact that this crop has a natural bitter taste when consumed. The Kenyan accessions seemed to generally have a higher phenolic content than the South African and AVRDC accessions. This could have been attributed by their purple stem as compared to the South Africans that were green stemmed with the former being associated with higher nutrition value (Schippers, 2002). Understanding the genetic variability of *C. gynandra* genotypes in nutritional composition will help to identify the source of plant materials for use in various genetic studies and its improvement. For instance, breeding aimed at lowering the bitterness; while enhancing the anti-oxidants. Evolutionary adaptations usually result in mutations and shifts in the genetic makeup of individuals (Falconer, 1989). Such evolutionary adaptations are often genetically controlled, highly heritable and amenable to breeding. The accessions IP3 ^{avrdc}, IP 5^{avrdc} and 9^{ke} that displayed high levels of total phenolics could be good sources of useful anti-oxidants as it has been reported that the antioxidant capacity of phenolics found in *C. gynandra* is far more pronounced than that of the vitamins (Dai and Mumper, 2010). Therefore, there is need to exploit this indigenous vegetable more so as to give breeders an insight on traits that can guide their selection for yield and nutrition.

The studied accessions were very divergent with regard to nutrition and yield related components. None of the accessions combined excellent performance in nutrition content and high yield. However, as compared to other accessions under study, Kenyan accessions 9^{ke}, 50265^{ke} and 50339^{ke} were outstanding in terms of high nutrition content as well as high number of leaves. In regard to high vitamin C, accession IP11, IP8 and GKK expressed a big leaf area. The study reported some of the low yielding accession such as accession IP 3^{avdrc} and IP 5^{avdrc} in

terms of number of leaves but with high phenol content. Similarly, accession GKK 285[avrdc] had a large leaf area but with little amount vitamin C content. This upholds the need to improve accessions with good nutritional merits for better yield. Significant correlations observed in this work indicated high associations $P < 0.005$ between beta carotene, flower and stem color. This implies beta carotene could be playing a big role in determining the flower and stem color. This is consistent with the fact that the β-ring carotene hydroxylase gene plays a role in carotenoid biogenesis for pigment formation (Wang et al., 2016).

Indirect selection for yield may be obtained through information on character association between yield and yield related characters (Binodh et al., 2008). The estimates of heritability are fundamental in selection as they inform on transmission of parameter (s) from parents to offspring. All the traits in this study showed high estimates of broad sense heritability of >80% among the study accessions. This suggests that any selection in *C. gynandra* based on the phenotype of these characters will be effective in yield improvement. This was a similar finding in a study involving an indigenous vegetable *Corchorus olitorius* where high estimates of broad sense heritability were recorded for number of leaves per plant (96.99%) and plant height (95.61%) among other measured parameters (Nwangburuka and Denton, 2012). Accordingly, such kind of high heritability suggests that selection based on these characters will be significant in predicting for vegetative yield in *C.gynandra.* The genotypic variance was greater than environmental variance. This suggests sufficient variation among the genotypes and less influence from the environment. Consequently, this provides an opportunity to select desirable traits effective in yield improvement among the accessions in a spider plant population. However, heritability in a narrow sense is more dependable in predicting transmissibility of

variation from generation to generation than broad sense since the latter is limited in plant improvement program because the genetic variation includes the fixable additive and non-fixable dominance and epistatic variation (Falconer and Mackay, 1996).

4.6 Conclusion.

This study reveals potential of spider plant in providing the much-needed dietary nutraceutical potential for curbing malnutrition and other related micro-nutrient deficient diseases. Diversification of diets through increased utilization and consumption of these vegetables would go a long way in alleviating hidden hunger and malnutrition. Accordingly, selection of indigenous vegetables such as spider plant should go beyond yield, diseases resistance and horticultural traits to include nutrient content. Correlation analysis exhibited significant correlation among some of the morphological traits suggesting that some traits could be used to predict the other and consequently aid in their improvement simultaneously. This work concludes that high heritability estimate for nutrition components vitamin C, beta carotene and phenols should be considered in nutrition improvement in *C. gynandra*. Similarly, number of leaves per plant, plant height and single leaf are indispensable in improving yield in spider plant.

4.7 Recommendation

There is need to promote the production, utilization and conservation of indigenous leafy vegetables through educational programs and market linkages to communities. Of paramount importance, is the diversification of spider plant, among farmers by increasing its potential through improved seed production, breeding and selection. Lastly there is need to investigate, isolate and elucidate the compounds responsible for antioxidant and antimicrobial activity in the

spider plant accessions using spectroscopic and chromatographic studies and carry out metabolomic fingerprinting of these varieties to access the profiles of secondary metabolites in them. Indigenous foods are important for the food security of many rural households and are likely to contribute micronutrients and other nutritional qualities. However, this needs to be documented and supported through more research. Increasing the limited knowledge of indigenous food nutritional qualities could enable increased promotion and marketing of such products. Nutrition education should be an integral component of community development activities so as to promote increased awareness and consumption of the varied diet required in meeting dietary nutrient needs.

CHAPTER 5

COMPARATIVE GENOMICS AND SSR IDENTIFICATION FOR *CLEOME GYNANDRA*

Abstract

Simple sequence repeats (SSRs) have been cited to be the genetic markers of choice as they are easy to score, dominant and are relatively informative as compared to other markers. Additionally, advances in bioinformatics tools have enabled mile breaking developments in SSR markers in a cost effective manner. This breakthrough may also be attributed to the free public availability of genome databases such as National Centre for Biotechnology Information (NCBI). Cross-species transferability is a fast and economic method to enrich SSR database, especially for minor crops where little genomic information is available. Despite the lack of genomic sequences in *Cleome*, the availability of the whole genome sequences of *Tarenaya hassleriana* a close relative, provided resource to assess SSRs and to make inferences to other *Cleome* species. Although microsatellites or simple sequence repeats (SSRs) have been acknowledged as one of the most powerful choices of markers for molecular biology, they have not been exploited in the study of *Cleome gynandra* populations. This study sought to determine the presence and frequency of SSR markers in *T. hassleriana* genome. The full genome sequences were downloaded from the NCBI genome database and GMATo software was used to search for the occurring SSRs. Only perfect SSRs di-, tri-, tetra-, penta-, and hexa-nucleotide motifs with numbers of uninterrupted repeat units more than 5 were targeted. A total of 76280 SSRs were identified in *Tarenaya hassleriana* genome. Dinucleotides and trinucleotide accounted for 88.1% and 9% of all repeats respectively. Among the dinucleotide repeats, AT was most abundant whereas, AAG was the most common trinucleotide. Transferability of molecular markers from a

close relative such as *T. hassleriana* will enhance development of molecular markers in *Cleome* for further utilization in spider plant improvement.

5.1 Introduction

Simple sequence repeats (SSRs) are tandem repeats nucleotides, mostly between 1 to 6 nucleotides, that occur in DNA sequences, across both coding and non-coding DNA sequences (Li et al., 2016). They can be found in any genome (both eukaryote and prokaryote) and in any region (protein coding regions and non-coding regions). Genomic SSRs have been widely used in genetic studies and breeding, because they are co dominant, easy to score, and highly abundant, as they can easily be assayed by polymerase chain reaction (Zaki et al., 2012). However their use has been limited mainly to species with enriched genomic resources. This calls for a different resourceful approach to search SSR markers transferable to closely related species especially in species that have inadequate or no genomic information (Satya et al., 2016). Comparative genomics in *Brassica* have revealed that microsatellite features in related species are largely comparable (Shi et al., 2014). Consequently, primer design on the basis of sequence of one species could be used to advance SSR markers for other closely related species.

Tarenaya hassleriana formerly known as *Cleome hassleriana* (Iltis and Cochrane, 2007) is closely related to *Cleome gynandra,* and both belong to the family Cleomaceae. *Tarenaya hassleriana* has been extensively studied with its genome fully sequenced and is also accompanied by an array of interesting phenotypic features (Cheng et al., 2013). However, genomic information studies and whole genome sequence supporting *Cleome gynandra* are lacking. For that reason, together with the close relation of *Tarenaya hassleriana* and *Cleome gynandra,* this study used *Tarenaya hassleriana* whose genome sequences are publically

available in the database for bioinformatics analysis. Similarly, the *Cleome* genus is closely related to *Arabidopsis thaliana* (Marshall, 2007), and of such, can be used for comparative genomics. Such genomic comparison may involve analysis of simple sequences repeats (SSRs) motifs across the two genomes. The *Tarenaya hassleriana* genome has simple sequence repeats (SSRs) that have been distributed throughout the genome. Previous research has used SSRs as genetic markers to study populations and genetic diversity (Lu et al., 2005) with recent research showing their application in most of the genetic diversity studies in african leafy vegetables (Van Biljon et al., 2010). Most studies have highlighted these SSRs to have potential to act as molecular markers that could assist in genetic analysis, genetic mapping and molecular assisted breeding (Kalwade and Devarumath, 2014; Campoy et al., 2011). As compared to other genomic markers, SSRs have been shown to be more dominant with high levels of polymorphism. For example, in the citrus plant, SSRs were found to have high polymorphism as compared with Cleaved Amplified Polymorphic Sequences Single Nucleotide Polymorphism (CAPS-SNP) markers (Khan et al., 2002). They have proven to be more powerful than Random Amplification of Polymorphic DNA (RAPDs) in distinguishing the relationships between cowpea local cultivars and breeding lines (Diouf and Hilu, 2005). Furthermore, SSRs markers have been successfully used in diversity studies of cowpea accessions in Ghana (Asare et al., 2010).

Little has been done to assess the same in *Cleome gynandra*. In genetic diversity studies on African leafy vegetables, *Cleome gynandra* has received the least attention (only 3% of all studies) while most of the studies covered *Vigna* (58%), *Solanum* (24%), and *Amaranthus* (15%) (Omondi et al., 2016). This study aimed to explore the abundance of SSRs in *Tarenaya hassleriana* with the aim of significantly transferring these markers to *Cleome gynandra* since

the *T. hassleriana* has its genome well studied with the genomic information easily accessible. The close relation of two plants gives enough justification to use them for genomic studies as there is enough evidence that cross-transferability of SSRs is more effective among closely related species (Satya et al., 2016). The information on SSR motifs will be compared to already published *Arabidopsis thaliana* motif data so as, to give comparative data between the two plants. This study will provide further room for utilization of these SSRs with a view to explore more molecular based approaches to improve plant breeding in *Cleome gynandra*. Such would be necessary especially in alienating food shortages as more genetically improved plants/crops would be available, despite changing climatic conditions.

5.2 Materials and methods

5.2.1 *Tareneya hassleriana* genome sequences.

Tarenaya hassleriana genome sequences were used for this analysis. Whole genome sequences of *Tarenaya hassleriana* were downloaded from NCBI genome database *http://www.ncbi.nlm.nih.gov/genome/?term=Cleome%20hassleriana* on 18th March 2016. Genome wide Microsatellite Analyzing Tool (GMATo) software was used to analyze these sequences and search for SSRs motifs. The GMATo software is a one-step tool for SSR characterization and genome mining. For easy processing, the software first formats the DNA sequences followed by chunking of long DNA sequences into small segments at several Mb. All microsatellite motifs consisting of A, T, G and C nucleotide of DNA are generated using Perl meta-characters and regular expression pattern. Each DNA chunk in every motif is searched avidly using Perl powerful pattern matching function. SSR loci information is generated through

the returned values at each chunk as well as the final SSR loci data at a chromosome after merging data from chunks.

5.2.2 SSR Identification

SSR motifs were identified in the *Tareneya hassleriana* genome using GMATo. Only perfect SSRs including di-, tri-, tetra-, penta-, and hexa-nucleotide motifs with numbers of uninterrupted repeat units more than 5 were targeted. These set parameters are consistent with other studies (Xiao et al., 2015; Tan et al., 2014) and were adopted. The SSRSs were obtained through a genome wide mining technique. The resulting data was transferred to a Microsoft Excel worksheet for further analysis.

5.3 Results

The genome-wide characterization of simple sequence repeats (SSRs) in the *Tarenaya hassleriana* genome detected a total of 76280 SSRs. Dinucleotide repeats were the most common type of SSR in genomic regions, followed by trinucleotide repeats. AT motif was the most frequent motif in the *Tarenaya hassleriana* genome, representing 39.9% (26862/67192) of all SSRs, followed by TA at 29.3% (19702/67192) (Table 5.1). Among all repeats, dinucleotides were the most common accounting for 88.1% (67192/76280), trinucleotides at 9%, while tetra-, penta-, and hexa-nucleotide motifs accounting for less than 3%.

Table 5. 1: Summary of SSRs motifs in *Tarenaya hassleriana*

	Number of repeats											
Motifs	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**>15**	**Total**
AT	4475	3910	3643	3193	2151	1557	1215	966	718	644	4390	26862
TA	4172	3213	2837	2124	1405	935	697	518	413	332	3056	19702
AAG	942	642	543	368	181	112	116	84	69	74	221	3352
AAT	836	674	517	339	174	104	112	95	79	67	288	3285
AC	841	584	467	289	164	107	111	77	78	50	238	3006
AG	879	624	454	308	147	96	95	79	54	58	205	2999
AGA	731	453	311	156	96	43	28	15	9	6	2	1850
ATA	592	368	239	152	80	43	35	11	9	7	4	1540
ATAC	388	235	147	95	61	29	16	8	6	4	6	995
ATC	278	127	115	70	43	23	14	15	6	2	4	694
ATG	185	127	74	67	31	34	20	15	13	10	52	628
ATT	165	100	57	49	18	18	19	15	14	16	34	505
CA	145	91	64	58	15	33	16	9	6	11	54	502
CAT	123	83	49	43	36	24	11	18	7	9	60	463
TTA	168	72	44	36	10	4	6	2	0	2	12	356
TGTA	60	65	46	22	15	12	7	6	0	4	7	244
TG	97	63	28	11	9	6	6	3	6	1	7	237
TCT	63	44	33	24	16	7	5	5	4	3	5	209
TC	88	38	20	16	11	8	5	3	4	1	9	203
GT	84	50	21	21	3	3	3	1	7	1	4	198
GC	54	25	23	6	4	8	5	5	3	8	26	167
GA	31	16	18	12	5	3	3	5	3	7	34	137
CCG	24	16	6	2	0	0	0	0	0	0	0	24

Frequency of different type of motif in Di-, Tri-, Tetra-, SSRs from *Tarenaya hasseleriana*
DNA nucleotides A= Adenine, T= Thymine, G= Guanine, C= Cytosine

Arabidopsis thaliana genome is approximately 157 Mb while that of *Tarenaya hassleriana* is 290 Mb(Table 5.2) (Cheng et al., 2013). Comparison between *Tarenaya hassleriana* and *Arabidopsis thaliana* revealed dinucleotide repeats to be the most prevalent in *Tarenaya hassleriana* while mononucleotides to be the most prevalent type in *Arabidopsis thaliana*. Both plants have the same most common dinucleotide (AT) repeats and the same most common

trinucleotides (AAG) but have little in common for the other types of repeats (Lawson and Zhang, 2006).

Table 5.2: Comparison between *Cleome* and *Arabidopsis* motifs

Motifs	*Tarenaya hassleriana* SSRs	*Arabidopsis thaliana* SSRs (Lawson and Zhang, 2006)
AT	26682	6608
AG	2999	5769
AAG	3352	7654
AAC	54	2233
AAAT	32	1872
AAAG	0	1100

Total number of different type of motif in Di-, Tri-, Tetra-, SSRs from *Cleome hasseleriana* and *Arabidopsis thaliana*

5.5 Discussion

Among the collection of DNA marker systems available, SSRs based markers have been the preferred marker system for plant genome analysis (Morgante and Oliveri, 1993). High-throughput assay speed and cost efficiency have led to their rise as the most broadly used marker system. This is due to their co-dominant inheritance, reproducibility, good genome coverage, multiallelic nature and interspecific transferability (Eujayl et al., 2004; Gautami et al., 2009). SSRs markers could be used for several applications in spider plant genetic analysis and molecular breeding. For instance, germplasm diversity analysis, genetic mapping, cultivar characterization and identification, quantitative trait loci (QTL) identification, and eventually marker-assisted selection.

SSRs can be identified within DNA database entries (Akkaya et al. 1992). The present study was carried out to promptly identify loci containing SSRs and to create a pool of microsatellite markers for species of the genus *Cleome* taking advantage of publicly available sequences for *T.*

hasseleriana. The dominance of AT motif reported in this study is consistent with Toth et al., (2000) that reported eukaryotic genomes being AT rich. In a previous study, AT was the most abundant repeat motif in the genome of cucumber (Cavagnaro et al., 2010). More interesting was the low frequency of CCG motif, which had an abundance of 24 SSRs. This is consistent with the fact that the CCG motif is rare in dicotyledons as compared to monocotyledons because of their higher GC content (Kantety et al., 2002). Previous research has shown that the dominance of one SSR motif category over other categories is very crucial in the chances of fixation of mutations against selection pressure thus their utility as markers due to their polymorphism level (Metzgar et al., 2000). The motif characteristics in *Tarenaya hassleriana* reported here are consistent with those of other plant genomes reported in other studies (Guang et al., 2012; Cai et al., 2009). Such a high proportion of SSRs may indicate that the *Tarenaya hassleriana* genome may have a long evolution history or may have a higher genomic mutation rate, as described elsewhere in yeast (Sia et al., 1997).

The SSRs commonly used for marker development are those belonging to di-, tri- and tetra-nucleotides (Kumpatla and Mukhopadhyay, 2005). Among the motifs, dinucleotide repeats are reported to be more polymorphic among SSRs (Morgante and Olivieri, 1993; Li et al., 2002) with trinucleotide repeats being over-represented in coding sequences but occurring less frequently than mono- and dinucleotide repeats in non-coding regions (Tòth et al., 2000; Gao et al., 2003) in plant species. The genus *Arabidopsis*, which includes the extensively studied *Arabidopsis thaliana*, offers a handy set of model species for studying many aspects of population divergence. *Arabidopsis thaliana* has long been a model genetic and molecular system for plant biology. Earlier research on *A. thaliana* microsatellite loci has shown that they

are abundant and highly variable (Casacuberta et al., 2000; Clauss et al., 2002). Previous work has revealed that SSR markers have been used to design primers that have high amplification fidelity of about 90% in *A. thaliana* (Symonds and Lloyd, 2003; Clauss et al., 2002; Innan et al., 1997).

The high occurrence of some SSR in the present study, may elucidate and advise on primer designs that would successfully amplify targeted regions of this genome, as SSRs can easily be assayed through PCR (Zaki et al., 2012). Transferability of SSR primers across species has been reported in many crops including their wild relatives for genetic differentiation and evolutionary studies **(**Tabbasam and Zafar, 2014**).** For instance, successful cross species transfer of SSR markers has been shown across different species of *Trifolium*, a forage legume (Verma et al., 2015). Accordingly, information of SSRs in *Tarenaya* genome could play a vital role in future studies that seek to design primers for *Cleome* studies. These SSRs markers will directly aid in studies of the genetic diversity and relatedness in *Cleome gynandra.*

5.6 Conclusion and recommendations

This is the first attempt to survey SSR markers for *Cleome gynandra*. This work provides an insight into SSR distribution in *Tarenaya hassleriana* genome. There is a major scientific opportunity to translate and transfer the genomic advances from *Tarenaya hasslereiana* to the less studied *Cleome gynandra*. This would be helpful for development of DNA markers that are of significance in trait specific genes in *Cleome*. This study clearly demonstrates the polymorphism of these markers and their potential use in detection of genetic variations in different *C. gynandra* accessions and their application in plant breeding. The novel SSR loci for

species *T. hassleriana* reported here, offer a great potential of transferability to *Cleome gynandra* populations. Such will facilitate future research and provide a foundation for their further utilization in marker assisted breeding.

Basing on data reported here, strong recommendations include designing of primers targeting abundantly occurring SSRs in the *T. hassleriena* genome. These SSRs will provide utility in application of genomic molecular techniques in spider plant improvement. In summary, the present study presents the start of development of SSRs markers and a bold step towards development of markers in *Cleome gynandra.* This will largely enhance the *C. gynandra* genetics and breeding applications.

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7.0 APPENDIX.

Appendix 1: Weather conditions at Kabete field station between May 2014 and May 2015 cropping season

	Temperature (^{o}C)		Rainfall (mm)	Relative humidity (%)
Month	Mean Max	Mean Min	Total	Mean
May	23.5	14.8	72.8	55.1
June	23.3	14.1	101.5	64.4
July	21.6	12.5	10.0	61.0
August	N/A	12.4	28.9	54.3
September	22.3	12.2	23.9	52.0
October	N/A	14.5	136.2	51.7
November	N/A	14.4	95.5	58.6
December	N/A	13.8	88.6	55.2
January	25.7	12.8	27.7	41.1
February	N/A	13.6	50.8	40.8
March	14.2	N/A	30.1	40.4
April	N/A	15.3	323.9	55.5
May	N/A	14.0	298.3	63.6

Source: Kenya Meteorological Department, Kabete Agro-met Station (June 2015).

Appendix 2: Analysis of variance

Table 1a: Analyses of variance for the agronomic traits in *Cleome gynandra* season one (April – July 2014).

		mean of squares							
Source of variation	d.f.	**DTF**	**LL**	**LW**	**NPB**	**NLPP**	**PH**	**SLA**	**SPAD**
Rep	2	4.3	0.6	1.7	1.6	27.9	6.5	0.4	1
Genotype	48	34.7*	5.2*	16.3*	14.7*	6895.6*	2734.0*	8.1*	165.4*
Residual	96	1	0.2	1.4	0.3	13.4	8.2	0.4	2.1
Total	146								

***** = term refers to significant at 5% probability level significant , DTF- days to 50% flowering, SLA- single leaf area (cm^2), LL- leaf length (cm), LW- leaf width (cm), NLPP- number of leaves per plant, NPB- number of primary branches, PH- plant height (cm), SPAD- soil plant analysis development.

Table 1b: Analyses of variance for the agronomic traits in *Cleome gynandra* in season two (Oct –Dec 2014).

		Mean of squares							
Source of variation	d.f.	**DTF**	**LL**	**LW**	**NPB**	**NLPP**	**PH**	**SLA**	**SPAD**
rep	2	5	0.5	0.5	0.4	1.5	3.2	0.6	3.6
genotype	48	31.1*	5.1*	16.5*	11.9*	6961.3*	2723.8*	7.9*	139.5*
Residual	96	0.8	0.1	0.5	0.5	17.6	6.4	0.2	4.3
Total	146								

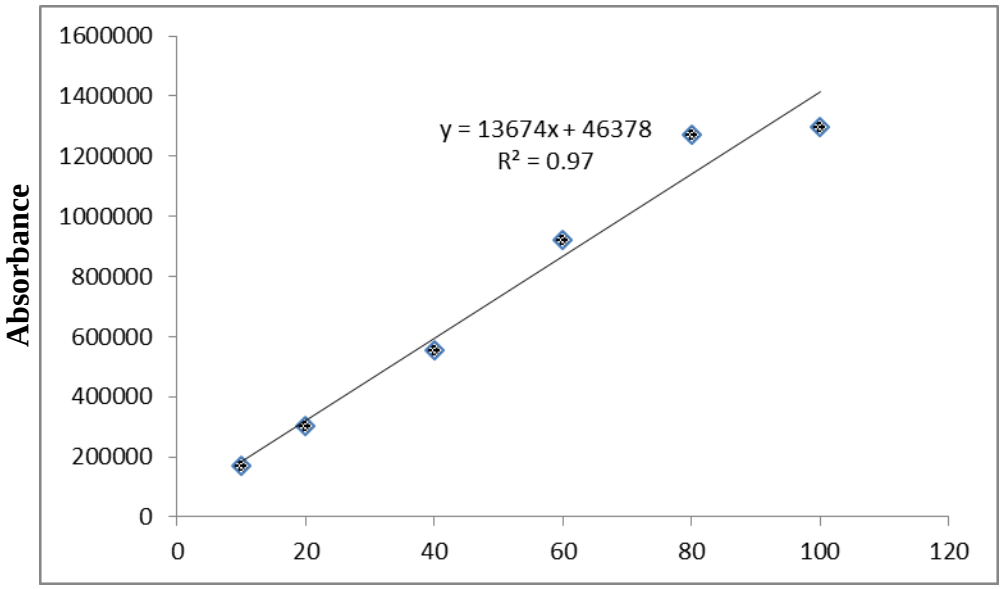
***** = term refers to significant at 5% probability level significant, DTF- days to 50% flowering, SLA- single leaf area (cm^2), LL- leaf length (cm), LW- leaf width (cm), NLPP- number of leaves per plant, NPB- number of primary branches, PH- plant height (cm), SPAD- soil plant analysis development.

Table 1c: Combined analyses of variance for the agronomic traits in *Cleome gynandra* in the two seasons

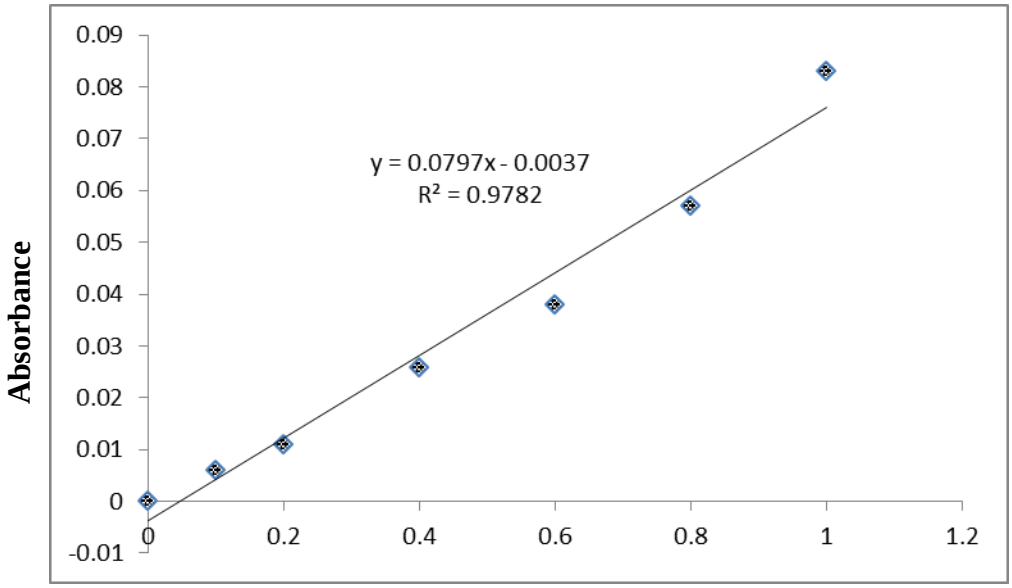
		Mean of squares							
Source of variation	d.f.	**DTF**	**LL**	**LW**	**NPB**	**NLPP**	**PH**	**SLA**	**SPAD**
rep	2	9.1	1	1.2	1.6	16	4.7	0.9	3.2
genotype	48	64.6*	10.3*	32.6*	26.1*	13840.2*	5451.1*	15.9*	300.8*
Season	1	161.6*	1.0*	4.1*	2.1*	54	33.4*	2.1*	4.8
genotype.season	48	1.2	0.1	0.2	0.4	16.7	6.7	0.1	4.1
Residual	194	0.9	0.2	0.9	0.4	15.5	7.3	0.3	3.2
Total	293								

***** = term refers to significant at 5% probability level, DTF- days to 50% flowering, SLA- single leaf area (cm^2), LL- leaf length (cm), LW- leaf width (cm), NLPP- number of leaves per plant, NPB- number of primary branches, PH- plant height (cm), SPAD- soil plant analysis development

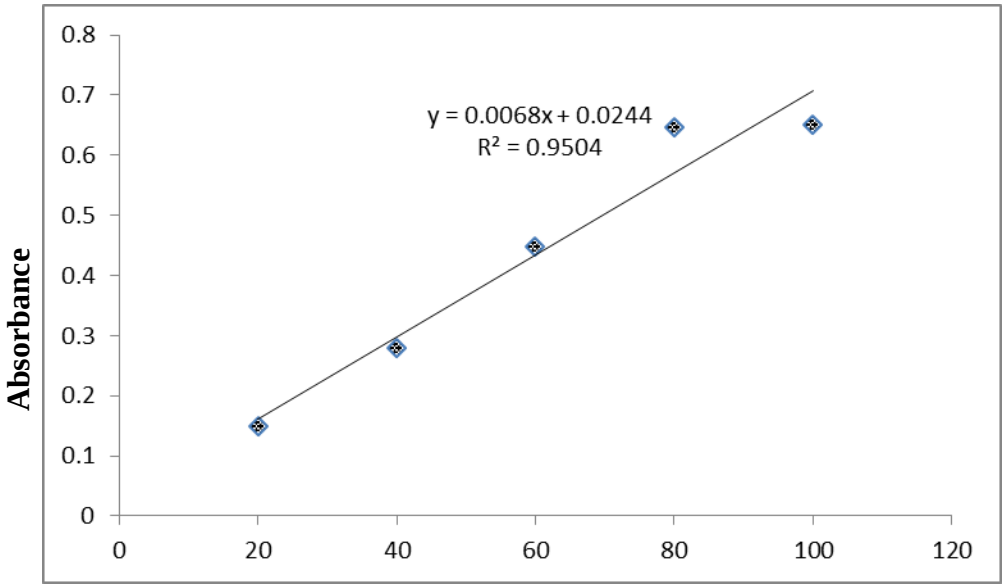
Appendix 3: Vitamin C standard curve



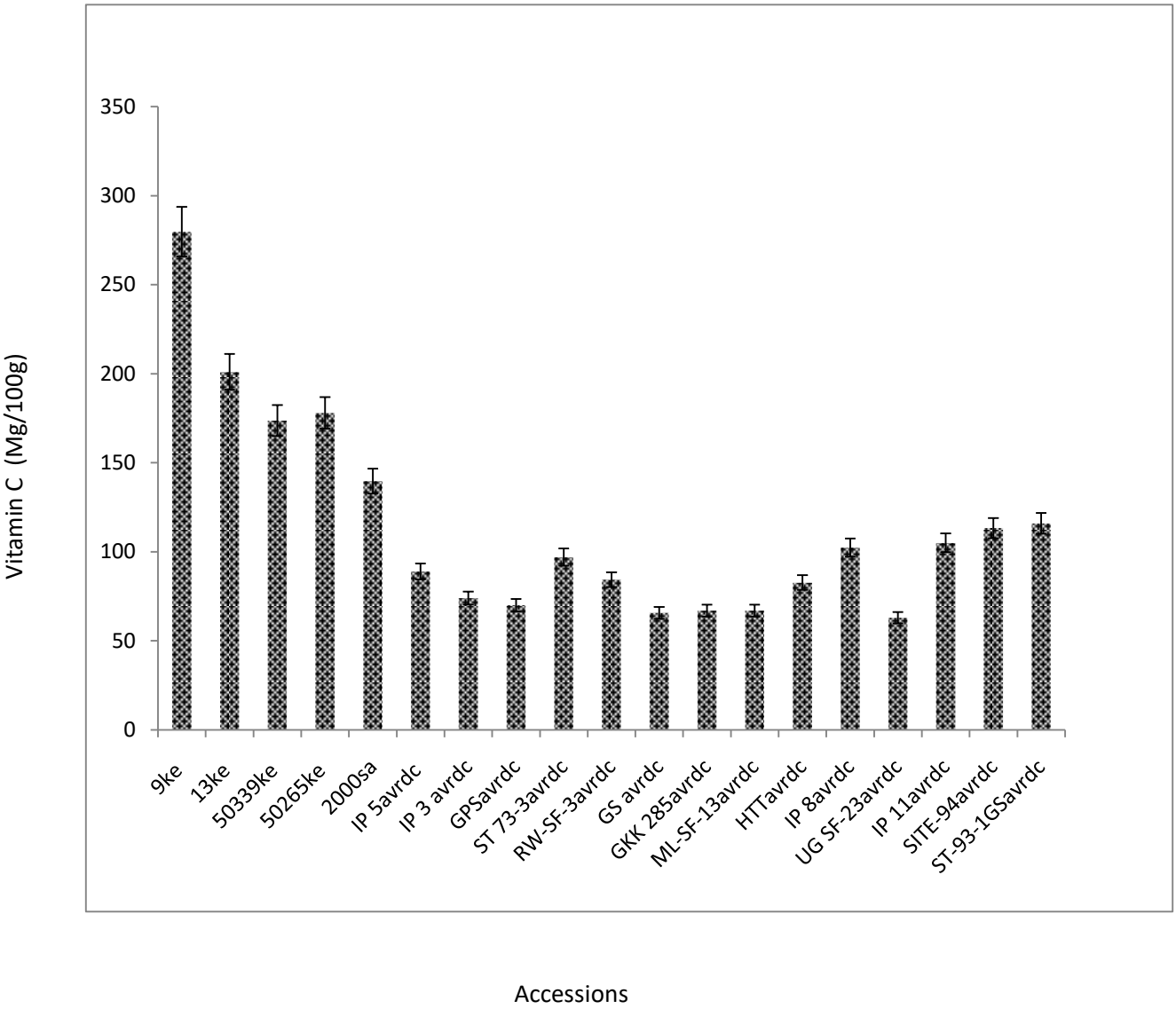
Appendix 4: Beta carotene standard curve



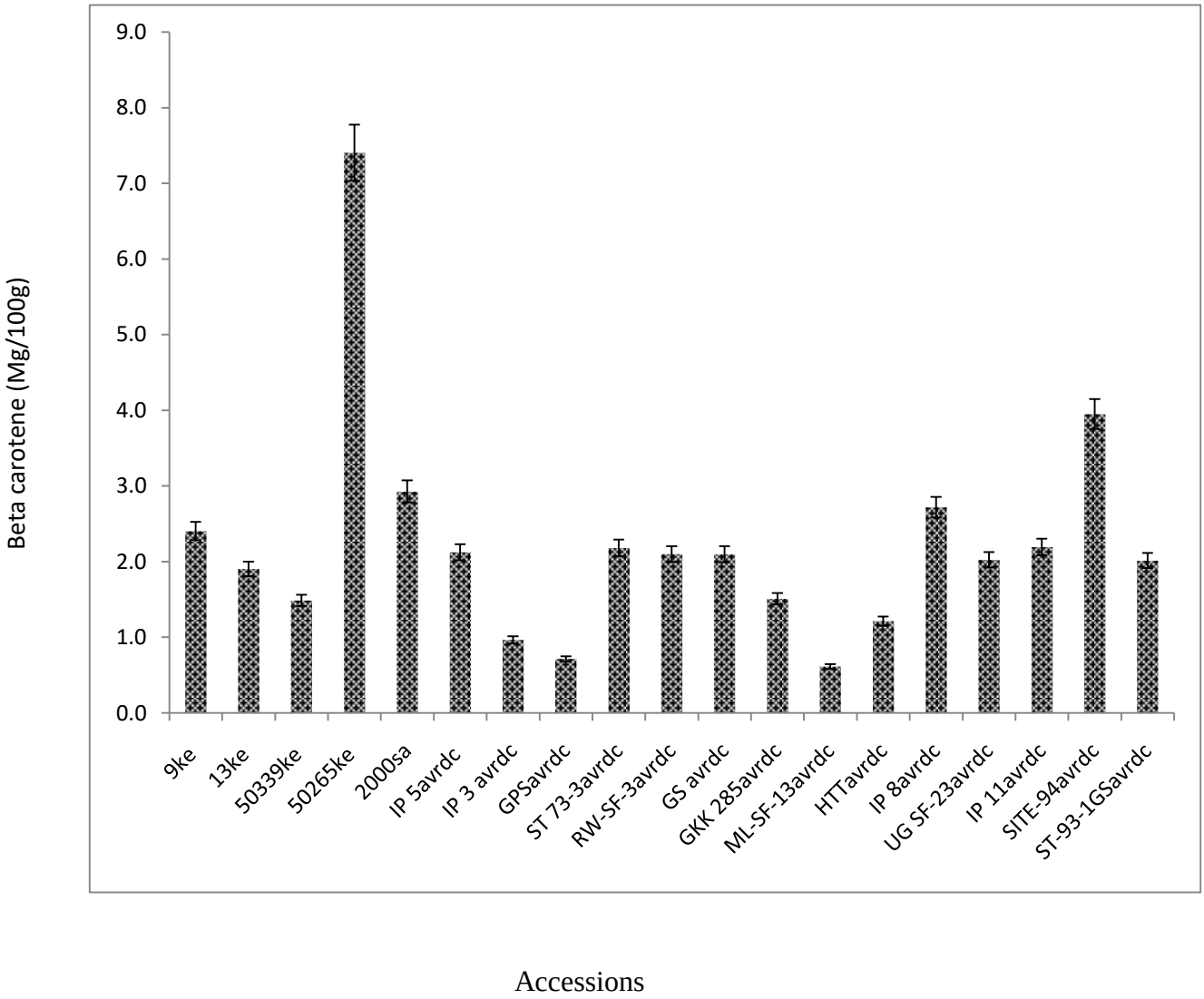
Appendix 5: Standard phenol curve



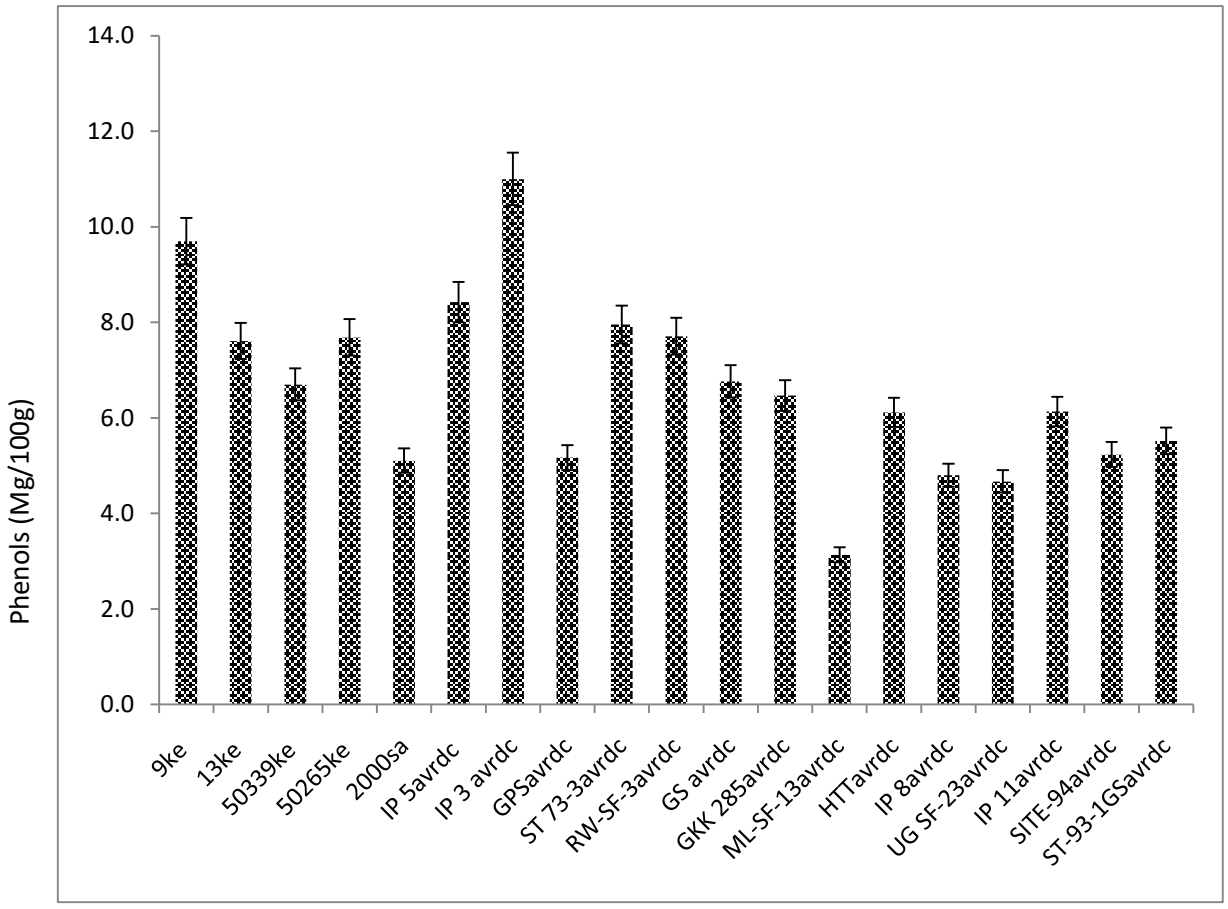
Appendix 6: Variations in vitamin C concentration (mg/100g) among the study accessions



Appendix 7: Variations in beta carotene concentration (mg/100g) among the study accessions.



Appendix 8: Variations in total phenols concentration (mg/100g) among the study accessions.



Appendix 9: A list of morphological data of the assorted accessions used in nutrition analysis

s/no	Acc no	Flower colour	Stem colour	Petiole colour	Stem hairiness	Petiole hairiness	Leaf colour	Leaf hairiness	Growth habit
1	9	white	purple	purple	profuse	profuse	dark green	sparse	Erect
2	13	purple	purple	purple	medium	medium	light green	sparse	Erect
3	50339	purple	purple	purple	medium	medium	dark green	sparse	Erect
4	50265	purple	purple	purple	medium	sparse	dark green	medium	Erect
5	2000	white	green	Green	Glabrous	Glabrous	light green	glabrous	semi erect
6	IP 5	white	purple	Green	Sparse	Glabrous	light green	sparse	Erect
7	IP 3	white	purple	Green	Sparse	Glaburous	light green	Glaburous	Erect
8	GPS	purple	Green	Green	Sparse	Glabrous	light green	glabrous	Erect
9	ST 73-3	White	Purple	Green	Medium	Sparse	light green	medium	Erect
10	RW-SF-3	white	Purple	Green	Medium	Glabrous	light green	Sparse	Erect
11	GS	White	green	Green	medium	sparse	light green	Sparse	Erect
12	GKK 285	Pink	Green	Green	medium	Sparse	light green	Glaburous	Erect
13	ML-SF-13	Pink	Purple	Green	Medium	Sparse	light green	Sparse	Erect
14	HTT	white	Purple	Green	Medium	Sparse	dark green	Sparse	Erect
15	IP 8	White	Purple	purple	Medium	sparse	dark green	Sparse	Erect
16	UG SF-23	Purple	Purple	Purple	Profuse	Medium	light green	Sparse	Erect
17	IP 11	purple	Purple	purple	Medium	Sparse	light green	sparse	Erect
18	SITE 24	purple	Purple	purple	Medium	Sparse	light green	Sparse	Erect
19	ST-93-1GS	Pink	Purple	Green	medium	sparse	dark green	Sparse	Erect

Appendix 10: Number and frequency of motifs in the *Tarenaya hassleriana* genome.

Motif	total	Motif	total	motif	total	motif	total
AT/AT	26862	TTAGGG/CCCTAA	3	GCAGTG/CACTGC	1	GCTGGA/T	1
TA/TA	19702	TCGGT/ACCGA	3	TATTAG/CTAATA	1	TCAA/TTG	1
GA/TC	7355	ATATT/AATAT	3	TGCAGG/CCTGCA	1	CTCACC/G	1
CT/AG	7312	TATAA/TTATA	3	GACCC/GGGTC	1	AGAAC/GT	1
GT/AC	3360	TCATGG/CCATGA	3	GAAGATG/CATCTTC	1	TGAAAA/T	1
TG/CA	2601	AAAAAT/ATTTTT	3	TGCGTC/GACGCA	1	CCACGT/A	1
ATT/AAT	1317	CCGAC/GTCGG	3	AGAGA/TCTCT	1	CAGCTA/T	1
TAT/ATA	1015	ATGAT/ATCAT	3	TTTGACGAG/CTCGTCAAA	1	CAGTGTG/	1
TTA/TAA	1012	CTAAACC/GGTTTAG	3	ACGT/ACGT	1	CCCACGG/	1
TTC/GAA	551	AAATAA/TTATTT	3	CCTT/AAGG	1	CTCCT/AG	1
CAT/ATG	527	TAAACCC/GGGTTTA	3	TAAC/GTTA	1	AACGCA/T	1
TCT/AGA	487	TTTTTTTAA/TTAAAAAAA	3	CTTTCC/GGAAAG	1	GACCATG	1
GAT/ATC	441	AACCCT/AGGGTT	3	TCTCGG/CCGAGA	1	GGGAGAT	1
CTT/AAG	438	CATC/GATG	3	GAACCA/TGGTTC	1	total	total
CATA/TATG	417	TCTT/AAGA	3	TCATGT/ACATGA	1	252	76280
TGA/TCA	413	AACCG/CGGTT	3	TTCAAGA/TCTTGAA	1		
ATGT/ACAT	337	CCAT/ATGG	3	CGGTCG/CGACCG	1		
GTAT/ATAC	286	CATGGTCA/TGACCATG	2	GCAC/GTGC	1		
TGTA/TACA	274	TATTA/TAATA	2	AAAAAAATT/AATTTTTTT	1		
TGT/ACA	117	TAAAA/TTTTA	2	TTTTAT/ATAAAA	1		
GAG/CTC	102	TCCA/TGGA	2	TCTTCA/TGAAGA	1		
GTT/AAC	99	AAAC/GTTT	2	TGGGGCT/AGCCCCA	1		
GGA/TCC	89	TCAAACTCG/CGAGTTTGA	2	CTCCAC/GTGGAG	1		
TTG/CAA	89	ATGGTCAC/GTGACCAT	2	AGAATCA/TGATTCT	1		
AGG/CCT	58	AACA/TGTT	2	GTGACCC/GGGTCAC	1		
CAG/CTG	56	CGTCAAACT/AGTTTGACG	2	GTGCGT/ACGCAC	1		
AAAT/ATTT	56	TGATTGA/TCAATCA	2	AGATGG/CCATCT	1		
CCG/CGG	53	TTCTGG/CCAGAA	2	ACCATC/GATGGT	1		
GCA/TGC	35	TAAAAAA/TTTTTTA	2	TTCGG/CCGAA	1		
AATA/TATT	33	TCCATG/CATGGA	2	ACCCGG/CCGGGT	1		
GCT/AGC	32	ATATA/TATAT	2	AAACCA/TGGTTT	1		
CCA/TGG	31	ACACAT/ATGTGT	2	TACATATT/AATATGTA	1		
GCG/CGC	29	GGAT/ATCC	2	GGAGATG/CATCTCC	1		
GC/GC	28	ATTAA/TTAAT	2	TGTGACCT/AGGTCACA	1		
CAC/GTG	28	TTTG/CAAA	2	ACGCAG/CTGCGT	1		
TAAA/TTTA	26	GAAG/CTTC	2	ACACGCC/GGCGTGT	1		
TCG/CGA	26	AATC/GATT	2	TTTTTATT/AATAAAAA	1		
CG/CG	23	AAAGAA/TTCTTT	2	AAGCCC/GGGCTT	1		
GGC/GCC	21	AAAAT/ATTTT	2	GCGTTT/AAACGC	1		
GGT/ACC	18	ACTCGTCAA/TTGACGAGT	2	ACCATGTG/CACATGGT	1		
ACT/AGT	17	CGTA/TACG	2	CCTC/GAGG	1		
GAC/GTC	16	TCTGCG/CGCAGA	2	ACCCTA/TAGGGT	1		
ATAA/TTAT	15	GTTTGACGA/TCGTCAAAC	2	GAATGC/GCATTC	1		
TTAA/TTAA	12	TCGGGA/TCCCGA	2	TTTGAA/TTCAAA	1		
CTAT/ATAG	11	CAAT/ATTG	2	ACCCTC/GAGGGT	1		
GTTAGG/CCT	11	TTCT/AGAA	2	TTTGGT/ACCAAA	1		
ATACAT/ATG	10	TTCA/TGAA	2	TCACCA/TGGTGA	1		
GATA/TATC	10	TCACATGG/CCATGTGA	2	CTCCTT/AAGGAG	1		
TGTATA/TAT	10	ATATAA/TTATAT	2	ACGCAA/TTGCGT	1		
TAC/GTA	9	TGCA/TGCA	2	GGGTTTC/GAAACCC	1		
TATATG/CAT	9	TAATTTT/AAAATTA	2	GCTGGG/CCCAGC	1		
CTTT/AAAG	8	CGGG/CCCG	1	TCAT/ATGA	1		
ATTA/TAAT	8	AAATT/AATTT	1	CCCCTG/CAGGGG	1		
TAG/CTA	8	GTCTGC/GCAGAC	1	ACCAT/ATGGT	1		
AATT/AATT	8	TCTCCCA/TGGGAGA	1	CAAACTCGT/ACGAGTTTG	1		
AGAT/ATCT	7	AAATTAA/TTAATTT	1	TTTTAA/TTAAAA	1		
ACG/CGT	7	GAATC/GATTC	1	TGAACA/TGTTCA	1		
ATATAC/GTA	7	GATAGGA/TCCTATC	1	GCCACA/TGTGGC	1		
GAAAA/TTTT	6	CGCTCT/AGAGCG	1	GTATGT/ACATAC	1		
CATGTGAC/G	6	TCATCAT/ATGATGA	1	TTTATT/AATAAA	1		
CCATCA/TGA	6	ATTC/GAAT	1	ATCACCC/GGGTGAT	1		
TCATCAA/TT	6	GAGGCG/CGCCTC	1	CCGTTA/TAACGG	1		
TCTA/TAGA	6	TCTCCA/TGGAGA	1	AAACCCC/GGGGTTT	1		
CATG/CATG	6	GGCGTC/GACGCC	1	GGGA/TCCC	1		
TATGTA/TAC	6	ATGTATGC/GCATACAT	1	AAAAATA/TATTTTT	1		
CCCATCT/AG	6	GTTCCA/TGGAAC	1	GGAGAG/CTCTCC	1		
ATAAT/ATTA	5	CGTACC/GGTACG	1	AAAAG/CTTTT	1		
ATATGT/ACA	5	CATGCA/TGCATG	1	TCGGG/CCCGA	1		
ATGC/GCAT	5	TATAAA/TTTATA	1	TGATCG/CGATCA	1		
AGGGTTT/AA	5	AAAAAC/GTTTTT	1	AAAAACT/AGTTTTT	1		
CTAACC/GGT	4	GGAGCC/GGCTCC	1	GTTTTTG/CAAAAAC	1		
TTTAGGG/CC	4	AAATAAA/TTTATTT	1	CATTTTC/GAAAATG	1		
TGTATG/CAT	4	TTTTAAT/ATTAAAA	1	AAATCT/AGATTT	1		
GTCAAACTC/	4	TCGCCA/TGGCGA	1	CGATGA/TCATCG	1		
TGATGAT/AT	4	CACACTT/AAGTGTG	1	GTTTGACGT/ACGTCAAAC	1		
TACACA/TGT	3	AGGGAG/CTCCCT	1	GGTCACAA/TTGTGACC	1		
TATTTT/AAA	3	CTGTT/AACAG	1	AAAAAG/CTTTTT	1		
GAAA/TTTC	3	CACATA/TATGTG	1	AAACCC/GGGTTT	1		
GTTTAGG/CC	3	GGTCACAT/ATGTGACC	1	CGGGT/ACCCG	1		
		CTCG/CGAG	1	AAATAT/ATATTT	1		