PHENOTYPIC CHARACTERIZATION OF KENYAN AND SOUTH AFRICAN SPIDER PLANT (*Cleome gynandra L.)* ECOTYPES

BY

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DECLARATION

This thesis is my original work and it has not been presented for a degree in any other university.

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DEDICATION

This thesis is dedicated to my father Martin Wasonga and my mother Consolata Wasonga for their financial support, true love and patience during my study years.

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ABBREVIATIONS

ALVs	African leafy vegetables
ANOVA	Analysis of variance
CEC	Cation exchange capacity
DARwin	Dissimilarity analysis representation for windows
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
GBK	Genebank of Kenya
GD	Genetic distance
GDP	Gross domestic product
GS	Genetic similarity
HCDA	Horticultural Crops Development Authority
ITC	International Trade Centre
KARI	Kenya Agricultural Research Institute
MOA	Ministry of Agriculture
MT	Metric tonnes
SPAD	Soil Plant Analysis Development
UPGMA	Unweighted pair-group method using arithmetic averages
USD	United States of America dollar

ABSTRACT

Spider plant (*Cleome gynandra L.*) is an important leafy vegetable that has been used by local African communities as a source of nutrition in their diets for many years. The plant has recently attracted an increasing demand since it is highly nutritive and contains health promoting bioactive compounds important in combating malnutrition and reducing human degenerative diseases. Despite the great value of spider plant, there are limited efforts towards its improvement especially in the area of phenotypic diversity. Spider plant has a rich genetic resource base in Kenya and South Africa and knowledge of its phenotypic diversity in these countries will aid on selection of accessions with desirable traits for breeding and conservation purposes. The aim of this study was to determine the extent of phenotypic variation among selected spider plant accessions from Kenya and South Africa and select those with desirable qualitative and quantitative characters for future improvement. Field and greenhouse experiments were conducted in 2013 and 2014 at the University of Nairobi's Kabete field station, Kenya. A total of 32 spider plant accessions, 23 sourced from Kenyan genebank and nine from South African genebank were used in characterization and evaluation. Both field and greenhouse experiments were laid out as a randomized complete block design with three replications. Eleven qualitative and quantitative traits based on modified FAO (1995) spider plant descriptors were used in characterization. Qualitative characters evaluated included growth habit, flower colour, stem colour, stem hairiness, petiole colour, petiole hairiness, leaf colour, leaf pubescence, leaf shape, leaf blade tip shape, and number of leaflets per leaf. Quantitative characters evaluated were days to 50% flowering, soil plant analysis development values, plant height, stem girth, number of primary branches, leaf length, leaf width, single leaf area, number of leaves per plant, number of pods per plant and seed yield per plant.

The qualitative and quantitative data were analyzed using DARwin software version 5.0 and Genstat version 14. Shannon diversity index (H'), multivariate methods of principal coordinate analysis, principal component analysis and hierarchical clustering analyses of unweighted pair group method of arithmetic averaging were assessed for all the qualitative traits. Analysis of variance was performed at 5% level of significance for the quantitative data and variability calculated using statistical measures of mean, standard deviation and coefficient of variation. Correlation was also performed to estimate quantitative relationships among the traits.

Estimates of Shannon-Weaver diversity index (H') for the qualitative characters assessed in the field and glasshouse were generally high (H'>0.500). The H' index indicated inter-country diversity to be greater than the intra-country diversity. The second plane of principal coordinate analysis separated the two groups of accessions (Kenyan and South African) clearly. Principal component analysis identified seven important qualitative characters for characterizing spider plant accessions. These were stem colour, stem hairiness, petiole colour, petiole hairiness, leaf hairiness, leaf shape and number of leaflets per leaf. The hierarchical cluster analysis revealed two major clusters (Cluster I and II) for the thirty two accessions grown in the field, with clustering of accessions occurring along regional basis. Cluster I consisted of South African accessions only while cluster II had mainly Kenyan accessions and two South African accessions (accession numbers 1959 and 2289). The cluster phenogram grouped the glasshouse grown accessions into three major clusters (Cluster I, II and III). Cluster I had only one accession, GBK045436. Cluster II had two Kenyan accessions, GBK027195 and GBK027212. Cluster III consisted mainly of a mixture of the Kenyan and South African accessions with two sub-clusters (sub-cluster 'a' and 'b'). Sub-cluster 'a' had six South African accessions and two Kenyan accessions. Sub-cluster 'b' had a total of 21 accessions most of which were Kenyan accessions.

The analysis of variance indicated significant differences ($P<0.05$) for most of the accessions grown in the field and glasshouse. Number of leaves per plant was significant ($P<0.05$) and positively correlated with SPAD values % ($r = 0.34$ and 0.03), stem girth % ($r =0.59$ and 0.29), number of pods per plant % ($r = 0.69$ and 0.57) and seed yield per plant % ($r =0.21$ and 0.03) for field and glasshouse grown accessions, respectively. However, number of leaves per plant correlated both positively and negatively with days to flowering % ($r = -0.17$ and 0.12), leaf area ($r = -0.05$ and 0.03), plant height % ($r = 0.52$ and -0.15) and number of branches per plant % ($r = 0.35$ and -0.09), respectively, for field and glasshouse grown accessions. Twelve accessions, namely 1959, 2000, 2279, 2289, GBK027195, GBK027212, GBK031990, GBK031996, GBK032302, GBK040606, GBK043261 and GBK045451 were found to be different from the other accessions for important characters such as late flowering, high SPAD content, large leaf area, high number of primary branches, high number of leaves per plant, high number of pods per plant and high seed yield per plant. These accessions can therefore be used for future spider plant improvement programmes through breeding in view of variety release.

CHAPTER 1: INTRODUCTION

1.1 Background information

Agriculture is the mainstay of Kenya's economy providing the basis of development for other sectors of the economy. The Agricultural sector contributes about 30% of the gross domestic product and accounts for over 75% of the total labour force (MOA, 2010). It is envisaged that the sector will continue to play a leading role in stimulating and supporting the country's economic growth mainly through the vibrant horticulture industry (HCDA, 2008). According to the Horticultural Crops Development Authority of Kenya (HCDA, 2014), vegetables contributed over 40% of the total value of horticultural production between 2011 and 2013. Thirty percent (30%) of the vegetables valued at USD 247 million were exported mainly to the European Union.

Similarly, horticulture plays an important role in South Africa's agriculture sector. From 1980s to 2007 horticultural production increased from 18% to 26% share of total agricultural output, while field crops production declined during the same period (Kirsten *et al.,* 2010). Fresh fruit dominate export sector, whilst vegetable production is largely for the domestic market with less than 3% exported in 2011 (ITC, 2012), although there has been an increased volume of output.

According to Shei (2008), vegetables are a vital constituent of all human diets and traditional vegetable species are highly important. Traditional leafy vegetables (also known as African leafy vegetables or ALVs) are local vegetables whose leaves, young shoots and flowers are consumed (Maundu *et al.,* 1999). Obel-Lawson (2005) reported that hunger and malnutrition threatens millions of people in Sub-Saharan Africa, and an increased consumption of African leafy

vegetables can have a positive effect on nutrition, health and economic wellbeing of both rural and urban populations. Among the factors contributing to preference for exotic vegetables as compared to local ones include non-appreciation of African traditional vegetables (Obel-Lawson, 2005), urbanization and inadequate scientific information on local African vegetable species and their ecotypes (Shei, 2008).

In recent times, ALVs are increasingly playing a central role in horticulture. The percentage contribution of African leafy vegetables such as cowpeas, African nightshades, vegetable amaranths, jute mallow and spider plant to the value of vegetables in the domestic market in Kenya rose from 4.3% in 2011 to 5% in 2013 (HCDA, 2014). The area under these vegetables has also increased over the years from 31,864 Ha in 2011 to over 40,000 Ha in 2013 leading to a production increase from 31,868 MT in 2011 to 178,268 MT in 2013 (HCDA, 2014). The ALVs have several advantages over other exotic vegetables. They have high nutritive value (Chweya and Mnzava, 1997), medicinal value and health benefits (Kokwaro, 1993; Olembo *et al.*, 1995; Opole *et al.*, 1995; Dasgupta and De 2007). These ALVs are also important in conserving a rich diversity of genotypes of importance for future generations and breeding (Chadha, 2003).

Cleome gynandra (L.) is among the most important traditional leafy vegetables widely used in Africa (Schippers, 2000). In English, *Cleome gynandra* is known as spider flower or plant, cats' whiskers, spider wisp, and African cabbage. This tropical plant has different names among the African dialects. Among the different *Cleome* species, *Cleome gynandra* is the most widely used as a leafy vegetable but *Cleome monophylla* and *Cleome hirta*, which are close relatives, are also used occasionally (Vorster *et al.*, 2002).

Spider plant is used as both food and medicine (Venter *et al.,* 2000; Nesamvuni *et al.,* 2001). It was noted by Jansen van Rensburg *et al.,* (2004) that African leafy vegetables, which are rich in micronutrients and vitamins, could play an important role in alleviating hunger and malnutrition. The plant has been evaluated for nutrient content and was shown to have high values especially for calcium, magnesium, iron, zinc, vitamin A, C and E (Ekpong, 2009; Mnzava 1997), making it suitable for combating malnutrition and life style diseases especially in Sub-Saharan Africa (WHO, 2005; FAO, 1993). Plant extracts of spider plants are found to be heat stable and fungitoxic (Pandey *et al.,* 1993) and its essential oils exhibits good repellence against the livestock tick *Rhipicephalus appendiculatus* (Lwande *et al.,* 1999; Chandel *et al*., 1987).

Spider plant is not cultivated as a commercial crop anywhere in the world, but for years it has been a semi-domesticated volunteer crop in home gardens in many parts of sub-Saharan Africa where its leaves are eaten. The species are native to the following regions: Southern Africa, Western Africa, Central Africa, Eastern Africa and South East Asia (DAFF, 2010). The major African countries that produce spider plant are Kenya, Uganda, Tanzania, South Africa, Malawi, Botswana, Cameroon, Namibia, Zimbabwe, Zambia, Swaziland and Ghana (DAFF, 2010). In Kenya, leaf yield production of spider plants increased from 19,428 MT in 2012 to 21,507 MT in 2013. The area under production also increased from 2,256 ha in 2012 to 2,336 ha in 2013 (HCDA, 2014). The increase in production of the plants was due to increase in demand (HCDA, 2014). However, in South Africa, the production levels of the plants are not yet known (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 with key counties producing the plant being: Kisii, Nyamira, Kericho, Migori, and Siaya (HCDA, 2014).

Kenya and South Africa feature a range of agro-ecologies that represent most parts of the African continent (Nono-Womdim and Opena, 1997). They include both highlands and lowlands. In these two sub-regions there are various ecotypes of spider plants. Even though the plant is adapted to these wide ranges of environmental conditions there is lack of 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). A difference in agro-ecological zones, which includes differences in climate and soil types, has an influence on the level of various nutrients in spider plant grown in those zones. According to Chweya and Mnzava (1997), a plant's nutritional value and phenotypic trait expressions may vary with soil fertility, environment, plant type (ecotype), plant age and the production techniques used.

1.2 Problem statement

There are a number of genetically diverse populations among spider plant accessions (Omondi, 1990), but it is not clear to what extent they are genetically different (K'Opondo, 2011; Maundu *et al*., 1999). Spider plant is adapted to a wide range of Agro-ecological conditions in Eastern and Southern Africa (DAFF, 2010). In East Africa, the plant shows from Kenyan Coastal region to western region to Uganda (Chweya, 1997). In Southern Africa, it ranges from Limpopo to Namibia (DAFF, 2010). This signifies high diversity in spider plant populations between Kenya and South Africa. Spider plant germplasm is continuously being collected from farms and forests in all parts of Kenya and South Africa by the respective national genebanks, but most of the collected accessions are undocumented and have not been systematically characterized in terms of their morphological and agronomic variability (Chweya and Mnzava, 1997; K'opondo *et al.,* 2009; Masuka and Mazarura, 2012). Categorizing germplasm accessions into phenotypic similar

and presumably genetically similar groups is most useful when the population structure in a collection is unknown (Marshall *et al.,* 2007). Phenotypic characterization of spider plants will aim at identifying desirable morphological and agronomic traits which can inform future breeding programs for ultimate improvement of the plants.

Previous studies on the diversity of spider plant focusing on morpho-agronomic differences, for example plant height, plant structure and 50% flowering, have evaluated few accessions with only a few traits used in characterization (Masuka and Mazarura, 2012; K'Opondo, 2011). The studies further recommend use of more accessions and more traits under field environments to characterize spider plant diversity. Thus there is need to identify morphological traits which best characterize spider plants for phenotypic diversity. Currently, the identification of these accessions relies on local names, and often, an accession's name may represent several genotypes. Like any other crop species, the first step in spider plant improvement is assessment of local materials, including collection, evaluation of phenotypic trait expressions and morphological characterization of germplasm.

1.3 Justification of the study

In Sub-Saharan Africa, 40% of children under five years in age are chronically undernourished and in poor health partly attributed to micronutrient deficiency. There is an urgent need to address nutritional security essential for human health. Spider plant is an important local vegetable that has been used by local African communities as a source of nutrition in their diets for many years and recently it has attracted an increasing demand among the ALVs. The plant is highly nutritive and contains health promoting bioactive compounds important in combating

malnutrition and reducing human degenerative diseases. Despite the great value of spider plant, not much research has been devoted towards its crop improvement especially in the area of phenotypic diversity. Phenotypic characterization of spider plants will inform on selection of accessions with desirable traits for breeding and conservation purposes. Information on the diversity within and among closely related crop species is essential for their effective use, improvement and management. It is particularly useful in characterizing individual accessions and cultivars, in detecting genetic material with novel genes and thereby rescuing them from erosion, and as a general guide in selecting parents for crossing in breeding programmes. Thus, knowledge of existent diversity in spider plant will allow more informed discussions around crosses in breeding programmes. Characterization of spider plant also promises to increase yield and improve availability of seed leading to more domestication and consumption the crop. Increased production would also lead to production surpluses, which are sold in markets providing reliable and consistent income for the poor farmers.

1.4 Objectives

The main objective of this study was to determine the extent of phenotypic variation among selected spider plant accessions from Kenya and South Africa. The specific objectives were:

1. To evaluate Kenyan and South African spider plant accessions for agronomic and morphological characteristics.

2. To determine the key traits of morphological and agronomic importance that can be used in characterizing spider plants.

1.5 Hypotheses

i. Kenyan and South African spider plant accessions are different in morphological and agronomic traits.

ii. Kenyan and South African accession posses key morphological and agronomic traits that can differentiate them.

CHAPTER 2: LITERATURE REVIEW

2.1 Botany of spider plants

Cleome gynandra (L.) belongs to the family *Capparaceae* of the order *Capparales* (Porter, 1967; Cronquist, 1988). *Capparaceae* is made up mostly of two subfamilies: *Capparoideae*, which are mainly woody and *Cleomoidaea*, which are herbaceous (Porter, 1967). *Cleome gynandra* is wide spread in the tropics as a weed and it is native to Africa, South America, Asia and Middle East (Chweya and Mnzava, 1997; Fletcher, 1999). It is an annual herb commonly used as a vegetable in the tropics (Fox and Young, 1982) and is related to the *Brassicaceae* (Hall *et al.*, 2002), which includes the model plant, *Arabidopsis thaliana*. Spider plant is erect, mainly branched with a long tap root. The height of the plant varies between 0.5 m and 1.5 m, depending on the environment. Leaves are alternate, palmately compound with three to seven leaflets. Stems and leaves are covered with glandular hair. Pigmentation on the stems varies from green to pink and purple. The terminal inflorescences have very distinct small white flowers, but pink and lilac coloured flowers also occur. These plants flower mostly at night after a minimum number of palmetly compound leaves have been formed (Iltis, 1967). The fruit consists of small siliques (Van Wyk and Gericke, 2000). Seeds germinate and develop rapidly and the plants may flower within four to six weeks after planting while fruit development and maturation may take three to four months (Mnzava, 1997).

2.2 Spider plant seed dormancy and plant physiological attributes

Spider plants are both self- and cross pollinated (Mnzava and Chigumira, 2004). Omondi (1990) observed that the species populations indicated uniformity for most characters. Such uniformity could only arise from a predominantly self-pollinating species. It is therefore possible that spider

plants are self-pollinating. However, there is likely to be a high rate of outcrossing, owing to diverse phenotypic variability, and the phenomenon of anthers dehiscing when flowers have been open for a long time and their stigmas exposed (Ayiecho and Omondi, 1992; Mnzava, 1997). Pollinators may include insects (especially honey bees), spiders and the wind. Spider plants have regular meiosis and pollen fertility under ideal conditions (Chweya and Mnzava, 1997). The plants have variable diploid counts of 2n = 18, 20, 30, 32, 34, 36 (Schippers, 2000; Mnzava and Chigumira, 2004), with Schippers (2000) reporting 2n = 20 as being the most common. This makes it possible to produce hybrids among the ecotypes or make interspecific crosses between spider plant and its relatives. According to Omondi (1990), characters targeted for any genetic improvement work are highly influenced by the environment. In spider plant and other plant species, the majority of important agronomic characteristics such as yield, are controlled in a quantitative fashion. Vegetative yield can be improved indirectly, via yield components such as days to flowering, plant height, number of leaves, fresh leaf weight and dry leaf weight. However, morphological characters have low heritability estimates and hence show low expected selection gain due to the genetic uniformity concealing the limited genetic variation present (Ayiecho and Omondi, 1992).

Spider plants have small round or circular black seeds that resemble the shell of a snail and have a rough surface. They have tough brittle seed coats, which are shiny black on the inside and have curved worm-like embryos enveloped in semi-permeable cell membrane (Ochuodho, 2005). The seeds are negatively photoblastic and this plant species has been shown to exhibit poor germination (Borhinger *et al.*, 1999; Chweya and Mnzava, 1997). The seeds may take up to one year, post-harvest, to reach maximum germination (Chweya and Mnzava, 1997). Bohringer, *et*

al. (1999) obtained maximum germination of only 25% at 31^{o}C in darkness six months after harvest. The poor seed germination in spider plant could be due to the hard seed coat, immature embryos or induced secondary dormancy. While Chweya and Mnzava (1997) reported a four to five day *Cleome* germination period, Ochuodho and Modi (2007) concluded that germination of the seeds can be improved by treatment with gibberrelic acid (GA) and also when performed under conditions of darkness or alternating temperatures of 20^{o}C- 30^{o}C.

Spider plants assimilate carbon dioxide through the C_4 photosynthetic pathway (Feodorova *et al.*, 2010). These plants show Kranz-type leaf anatomy with a higher activity of phosphoenolpyruvate carboxylase (Feodorova *et al.,* 2010). They exhibit diaheliotrophic leaf movements which allow them to maximize light-use efficiency throughout the day and avoid the hazard of midday depression of photosynthesis (Rajendru *et al.,* 1996). Because of its tropical origin, Iltis (1967) considered spider plants to be daylength-insensitive.

2.3 Ecological requirements

Spider plants thrive best in the semiarid, sub-humid and humid climates in the tropics. The plants grow well in altitude range of 0-2400 meters above the sea level. They require temperatures of 18°C to 25°C and a high light intensity as they are sensitive to cold. The species grow best when adequately supplied with water especially in areas with short periods of useful rainfall. They do tolerate a degree of water stress (Chweya and Mnzava, 1997), but prolonged water stress hastens flowering and senescence of the plants. Water stress reduces leaf yield and quality. The plants cannot withstand flooding but grow well on a wide range of soils from sandy loams to clay loams with optimum soil pH of 5.5-7.0 (Chweya and Mnzava, 1997).

2.4 Uses and nutritional importance

Spider plants are a major vegetable. As vegetables, their tender shoots and leaves are boiled and eaten as herb, tasty relish, stew or side dish. However, bitter taste in some ecotypes is derived from polyphenolics, which constitute from 0.5% to 0.9% of the edible leaf (Mathooko and Imungi, 1994). Spider plants are a rich source of protein, and the leaves contain over and above the normal recommended adult daily allowance of vitamins A and C and the minerals calcium, magnesium and iron (DAFF, 2010). Crude Protein content of 3.1-7.7 % has been reported for spider plant (Chweya, 1995). On the other hand, Hassan *et al.* (2007) reported a protein content of 14.30 %-dry weight. Beta-carotene and ascorbic acid levels have been reported to be 6.7-18.9 mg/100 g and 127- 484 mg/100 g, respectively, in leaves (Gomez, 1981; Sreeramulu, 1982; Mathooko and Imungi, 1994). Chweya (1995) reported calcium content of 213-434 mg/100 g in spider plant with the leafy parts of the plant containing relatively higher levels. Spider plant has a magnesium content of 86 mg/100 g (Opole *et al.,* 1995) and iron content of 1-11 mg/100 g (Mathooko and Imungi, 1994; Chweya and Mnzava, 1997).

Spider plant is known to have a variety of ethnomedical uses such as treatment of malaria, piles, rheumatism and anti-tumour activity (Bala *et al.,* 2010). The juice or boiled concoction of the plant is believed to treat scurvy and marasmus (Opole *et al.,* 1995) while regular consumption of sap from pounded leaves eases child birth among women (Kokwaro, 1993). In addition, the methanol extract of spider plant possesses good total antioxidant potential (Muchuweti *et al.,* 2007). These include antioxidant enzymes (superoxide dismutase, catalase, peroxidases) and non-enzymatic antioxidants (ascorbic acid, tocophenols, carotenoids, flavonoids and glutathione).

2.5 Spider plant pests and diseases

Slugs and snails can devour entire *Cleome* seedlings. Other pests that attack spider plant include: pentatomids (*Acrosternum gramineum* and *Agonoselis nubilis*) and their parasitoids, locusts (*Schistocera gregaria*), nematodes (*Meloidogyne* spp.), flea beetles (*Podagrica* spp.), green vegetable bugs (*Nezara* spp.), cabbage sawfly (*Athalia* spp.), cotton jassids (*Empoasca* spp.) and hurricane bugs (*Bagrada* spp.). Applications of insecticides can be used to control the pests. Young seeds are eaten by weaver birds (*Quelea quelea*) and the plant is a host to mildew fungus (*Sphaerotheca fuliginea*) and leaf spots (*Cercospora uramensis)* (Chweya, 1997; Mbugua *et al.*, 2007). Spider plants do not have dense foliage, and are unable to compete with weeds like oxalis (*Oxalis sorrel*), couch grass (*Elymus repens*) and thorn apple (*Datura stramonium*).

2.6 Plant characterization and its importance in crop improvement

In the terminology of genebanks and germplasm management, 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 (Perry and Battencourt, 1997). 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). Perrino and Monti (1991) defined characterization as the scoring of characters that can be easily detected and have high heritability. There are four main subcategories of characters and these are morphological, botanical, agronomic and chemical characters. They can be recorded on plants or their products, for example seed grown only in one environment. It is for this reason that characterization may begin during exploration and collection, and continue in the laboratory before or after multiplication (Perrino and Monti, 1991). From characterization, a number of conclusions can be

drawn. 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).

Characterization data have many applications including identification of materials in a collection or checking their authenticity; distinguishing homonyms or similar names and recognizing the duplicates (UPOV, 2004); identifying or selecting species, clones or cultivars with a desired combination of characteristics (traits); classifying the species, clones, cultivars or varieties; detecting groups with correlated characteristics which may have immediate practical value or may give clues to genetic relationships among accessions; and estimating the variation within a collection (Saad and Idris, 2001). Characterization also avoids the possibility of filling up space in the genebank by keeping material which is essentially the same (Lungu, 1990). An appraisal of the environment in which the crop is grown or is going to be grown is also done during characterization, recognizing the major factors that can limit its performance.

Standard characterization and evaluation of accessions may be routinely carried out using different methods, including traditional practices such as the use of descriptor lists of morphological characters (Huaman *et al.,* 1997; UPOV, 2004). Agronomic evaluation is part of characterization where emphasis is given on performance characteristics and helps in utilization of germplasm (Saad and Idris, 2001). Traits assessed during agronomic evaluation vary according to species. In the case of spider plants, such traits may include days to emergence, days to 50% flowering, yield and yield components, and uniformity of characters (K'Opondo *et al.,* 2009). Characterizing genetic diversity and the degree of association between and within accessions is the first step toward developing crop cultivars.

2.7 Methods of assessing phenotypic variation

Methods that are currently available for analysis of phenotypic diversity in germplasm accessions, breeding lines and segregating populations rely on pedigree, morphological, and agronomic performance (Smith and Smith, 1992; Bar-Hen *et al.*, 1995; Hamrick and Godt, 1997).

2.7.1 Pedigree data

Pedigrees of varieties are defined as a complete documentation of relationships traced back to landraces and wild relatives. If pedigrees of studied material are known it is possible to perform a pedigree analysis. Selection of genetically diverse parents based on pedigree information in order to obtain transgressive segregates 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.

2.7.2 Characterization of crop species using agro-morphological characters

Morphological traits continue to be the first step in the studies of genetic relationships in most breeding programmes (Cox and Murphy, 1990; Van Beuningen and Busch, 1997) because: (1) the existing data bases on the germplasm collection or breeding stocks can often be used for genetic analysis; (2) statistical procedures for morphological trait analysis are readily available; (3) morphological information is essential in understanding the ideotype performance relationships; and (4) explanation of heterosis may be enhanced if morphological measures of distances is included as an independent variable. The assays of qualitative traits do not need any

sophisticated equipment or complex experiments as they are generally simple, rapid and inexpensive to score (Van Beuningen and Busch, 1997). Morphological characterization entails primary and secondary characterization. Primary 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, while secondary characterization deals with more complicated morphological traits of agronomic importance such as pest and disease resistance, fruit set, yield potential and biochemical properties (Ayad *et al*. 1995). Morphological descriptor lists often provides the simplest of formal, standardized, repeatable methods of measuring crop genetic diversity when used in characterization (Watson and Eyzaguirre, 2002).

The use of phenotypic data to identify crop germplasm and breeding materials has shown notable limitations in discrimination/identification power and capacity to accurately predict relatedness and the level of genetic similarity among materials (Roldin-Ruiz *et al.*, 2001). Identification of crop germplasm based on morphological characteristics is often subject to errors caused by changes in the environmental conditions. Morphological appearance requires extensive trials to adequately describe germplasm (Lin and Binns, 1994) and therefore, valid comparisons are only possible for descriptions taken at the same location during the same season (Smith and Smith, 1992). Germplasm identification can also be difficult when the number of collections is large and when the germplasm converge on a few of the most desirable characters (Cooke and Reeves, 1998). Genetic relationship evaluations among germplasm using morphological characteristics are also lenghy and costly (Cooke, 1984). Moreover, during morphological characterization the germplasm have to be vegetatively produced each season and this involves a high risk of exchange and mixing of lots.

Considerable studies have been conducted by researchers on spider plants but few comprehensive studies have reported on phenotypic characterization. Masuka and Mazarura (2012) characterized four spider plant morphs, three from Zimbabwe and one from Kenya using nine morpho-agronomic traits i.e. number of days to seedling emergence, number of days to flowering, number of leaflets/compound leaf, number of pods/plant, stem pigmentation, stem pubescence, petiole length, flower colour, leaf length and fruit length. The study revealed that the morphs differed in most of the traits evaluated and especially on stem pigmentation. The Kenyan morph was smaller with purple stem pigmentation, profusely pubescence and produced a higher number of pods per plant than the Zimbabwean morphs which were green stemmed and glabrous pubescence. This study also showed variation among the four spider plant morphs in plant height, days to flowering, fruit length and number of pods per plant. The study recommended further characterization trials using more accessions, examining more characteristics and also growing the crop in a range of localities especially in the field.

K'Opondo (2011) morphologically characterized four spider plant types collected from western Kenya (Uashin Gishu District and Kakamega District) in a plastic greenhouse at Chepkoilel Campus of Moi University, Eldoret, Kenya. The results of that study revealed that the morphotypes were different for three variables out of the seven scored. These were plant structure, stem pubescence, leaflet shape and leaflet apices. In the case of variable counts, morphotypes differed for three out of the five counted and the variables included days to 50% flowering, stem pubescence and number of leaflets per compound leaf. For variable measurements, morphotypes showed differences for three out of the five measured, and the variables were plant height, petiole length and fruit breadth. The morphotypes were also

clustered into three groups by the phenogram. In characterizing the four morphotypes, it was noted that overlaps in morphological characteristics occur as expected. Despite these overlaps, significant differences were observed in analysis of variance, indicating that apart from stem and petiole colours, other characters of importance also differ. The study recommended further testing of the four morphotypes including more collections but under field trials in view of variety release and for use in breeding programmes.

2.8 Comparisons based on morphological and agronomical markers

Studying the diversity of pre-breeding and breeding germplasm, and determining the uniqueness and distinctness of the phenotypic and genetic constitution of genotypes, is important to protect the plant breeder's intellectual property rights (Franco *et al*., 2001). For conservation, evaluation, and utilization of genetic resources, different types of characters are frequently measured in each genotype: (i) quantitative characters (morpho-agronomic), and (ii) qualitative characters (these are usually multi-state variables) (Franco *et al*., 2001).

When morpho-agronomic data are available for a set of genotypes, hierarchical clustering is performed in which a standard metric distance (such as the squared Euclidean) is computed and a clustering strategy, such as Ward or unweighted pair group method of arithmetic averaging, is applied (Franco *et al*., 2001). Through applying any clustering strategy (such as single or complete linkage, UPGMA, centroid method and Ward method.), genotypes can be clustered into groups that are as homogeneous as possible and heterogeneous among groups. Franco *et al*. (2001) reported that groups formed based on both continuous and categorical classifications had low to medium consensus.

2.9 Correlation of phenotypic marker distance

Relationships between morpho-agronomic data of genotypes may provide useful information in order to determine the most promising entities for future breeding programmes. While phenotypic differences are connected to specific genes or coding regions, molecular markers have the ability to cover the entire genome (coding as well as non-coding regions). Hence, to express accurately the relationships among genotypes, a combination of morphological and molecular information would be necessary for further study (Burstin and Charcosset, 1997). In many cases, the relationships between distances based on morphology are not easy to understand. As a result, a future combination of morphological and molecular analyses may be the most useful to understand all aspects of genetic variation within the species.

2.10 Measures of genetic variation

Two different models of measuring genetic variation and applicable at population level are: (i) "richness" of any population (or sample from it) related to the total number of genotypes present in the population, and (ii) "evenness" or the frequency of different genotypes in the population or samples analyzed (Frankel *et al.* (1995). The evenness of genotype frequencies is accounted by the measures of average observed heterozygosity, expected heterozygosity, and effective number of genotypes. Heterozygosity is the most widespread measure of genetic variation within a population.

Spellerberg (1991) suggested that ‘species diversity’ which is an expression or index of some relation between number of species and number of individuals be retained to refer to ‘species richness’. Several indices of species diversity are used in the large amount of literature on

diversity and ecological monitoring (Niklaus *et al*., 2001). A commonly used index is the 'Shannon's Index' or '*H*' (Spellerberg, 1991), which is sometimes referred to as the 'Shannon–Weaver' Index (Poole, 1974; Niklaus *et al*., 2001) or the 'Shannon–Wiener' Index (Sax, 2002). The Shannon-Weaver diversity index (H') is computed using the phenotypic frequencies to assess the phenotypic diversity for each character for all genotypes studied. The Shannon-Weaver diversity index as described by Perry and McIntosh (1991) is given as:

$$H' = 1 - \sum_{i=1}^{n} pi \ln pi$$

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). By pooling various characters across collection sites, the additive properties of H' are used to evaluate diversity of localities and characters within the population.

2.11 Types of distance measures

Various genetic distance measures have been proposed for analysis of morpho-agronomic data for the purpose of genetic diversity analysis. Genetic distances can be calculated by different statistical measures depending on the data set. Dissimilarity coefficients estimate the distance or the difference of two individuals and the bigger the values, the more diverse the two individuals. In contrast, similarity indices measure the similarity between two individuals and the bigger the

value the more related the two individuals are (Kosman and Leonard, 2005). Kaufman and Rousseeuw (1990) reported that Euclidean distance, and square Euclidean distance are the most commonly used measures for morphological data to estimate genetic distance (GD) between individuals, whereas Gower's distance (Gower, 1971) can be used to measure genetic distance between individuals on the basis of different types of characters, such as qualitative and quantitative.

2.12 Multivariate analysis

The pattern of genetic relationship among accessions can be conveniently shown by multivariate analysis procedures. Accessions are characterized using morphological data from the growing plants. Data is then analysed using multivariate techniques, such as cluster analysis, principal component analysis (PCA), principal coordinate analysis (PCoA), and multidimensional scaling (MDS) (Cruz and Carneiro, 2006).

2.12.1 Cluster analysis

Clustering is a useful tool for studying relationships among closely related cultivars or accessions by grouping units according to similarity for certain characteristics or response patterns (Hair *et al*., 1995). It involves a stepwise procedure of calculating similarities and dissimilarities between observations and grouping together those that are most similar in a hierarchy (Mutsaers *et al.,* 1997). Basically, there are two types of clustering methods: (i) distance-based methods, in which a pair-wise distance matrix is used as an input for study by a specific clustering algorithm (Johnson and Wichern, 1992), and (ii) model-based methods, in which analysis from each cluster is assumed to be 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 clustering methods are classified into two groups: hierarchical and nonhierarchical. Hierarchical clustering methods are more frequently used in analysis of genetic diversity in crop species. Initially, each observation is a "cluster" by itself. Then, in a first step the two most similar clusters (observations) are grouped together to form a new cluster. Merging cluster together step-by-step is done in this way until all observations are grouped together into one final cluster. Amongst different agglomerative hierarchical methods, UPGMA (Sneath and Sokal, 1973; Panchen, 1992) is the most commonly used, followed by Ward's minimum variance method (Ward, 1963). The nonhierarchical methods referred to as K- means clustering measures do not occupy the structure of dendrograms and are based on chronological threshold (Everitt, 1980).

Extensive characterization studies have been conducted on other ALVs but none has been reported on spider plant (Maundu *et al.*, 1999; Chweya and Mnzava, 1997). Nkouannessi (2005) characterized 20 accessions of cowpea genotypes from Kenya, Cameroon and South Africa using 15 qualitative and 12 quantitative traits. Results showed a considerable variation among the accessions studied. The clustering pattern separated the accessions into three distinct groups according to the geographical region of collection. In addition, the results identified some important characters such as the high number of seeds per pod, pod length, seed weight and number of pods per plant as important for future cowpea improvement programmes.

Ondieki *et al.* (2011) studied variations in growth and yield characteristics of three black night shade species (*Solanum. villosum*, *S. scabrum* and *S. americanum*) grown in high altitude areas in Kenya. *Solanum americanum* exhibited good growth characteristics such as higher number of branches and larger stems than *Solanum. villosum* and *S. scabrum*. *Solanum americanum* also had the highest yields.

Mwase *et al.* (2014) characterized 37 accessions of *Amaranthus* from Central Malawi using 26 agronomic and morphologic traits. Axillary inflorescence were absent in 59.3% of the accessions and present in 40.7%. About 74% of the accession depicted erect growth habit while 18.5 % of the accessions had prostrate growth habit and 7.4% had semi-erect growth habit. In addition, a cluster phenogram generated from qualitative and quantitative traits summarized the 37 accessions in two major clusters according to their area of collection. Growth habit was the major trait that separated the two clusters with cluster I (30 accessions) having erect plants and cluster II (seven accessions) having prostrate plants.

2.12.2 Principal component analysis

Principal component analysis (PCA) as defined by Wiley (1981), is a technique of statistical decrease to describe relations among two or more characters and to split the total difference of the novel characters into a partial number of uncorrelated new variables. The decrease is created by linear conversion of the original variables into a new set of uncorrelated variables known as principal components (PCs). 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).

Wiley (1981) reported that 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.

2.12.3 Principal coordinate analysis

Principal coordinate analysis **(**PCoA) is an ordination technique that begins with a matrix of similarities or dissimilarities between a set of individuals and aims to create a low-dimensional graphical plot of the statistics in a way that distances between points in the plot are close to novel dissimilarities. Rohlf (1972) recognized that the treatment of missing information is more reasonable in PCoA than that in PCA.

2.12.4 Multidimensional scaling

Multidimensional scaling (MDS) is a procedure that represents a set of individuals or genotypes *(n)* in a few dimensions *(m)* using a similarity/distance matrix between them (Johnson 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). The closeness between original similarities-distances and inter-individual proximities in the map can be tested by different methods. The most commonly used test is a numerical measure of closeness called

"stress", which shows the percentage of the variance of the disparities not accounted for by the MDS

Rohlf (1972) reported that the actual arrangement of individuals consequential from PCA, PCoA, and MDS are typically related. On the contrary, results based on MDS contrast with PCA and PCoA since (i) differences among close individuals are, in common, reflected better by MDS, and (ii) the smaller or greater distances among individuals are not essentially represented by MDS to the equivalent scale. MDS is preferable over PCA and PCoA when the number of individuals is large (Rohlf, 1972). Simply, if there are no missing data or many more individuals than characters, PCA should be employed.

CHAPTER 3: MATERIALS AND METHODS

3.1 Plant materials

Thirty two (32) spider plant accessions comprising local landraces and wild types were used in this study. Twenty three (23) of the accessions were sourced from the genebank of Kenyan while nine (9) accessions were sourced from South African genebank. Figure 3.1 and 3.2 show the spider plant collection areas conducted by the Kenyan and South African genebanks. The respective genebanks have coded the accessions based on collection eco-regions as shown in Table 3.1. The 23 Kenyan accessions were all the collections available in genebank of Kenya while the 9 South African accessions were selected from the available 15 collections, by the South African genebank due to high seed quantity and provided for this study. Apart from the codes, no characteristics were given to the accessions by the genebanks.

3.2 Study site

Field and glasshouse experiments were conducted at the University of Nairobi's Kabete Field station, Kenya. The study site is situated on latitude of 1° 15''S, longitude 36°44' E and an altitude of 1940 m above sea level. The agro-ecological zone of the area is Upper Midland Zone three (Jaetzold and Schmidt, 1983). It has a bimodal distribution of rainfall with long rains from early March to late May and short rains from October to December (Appendix 2). Mean annual maximum and minimum temperatures are 23°C and 13°C, respectively (Siderus, 1976). The average annual rainfall is about 1,000 mm with a range of between 700 mm $year^{-1}$ and 1,500 mm $year^{-1}$ (Mburu, 1996). Kabete soils are classified as humic nitisols according to the FAO – UNESCO System (FAO, 1990). They are deep well-drained, dark reddish brown and friable clays when moist.

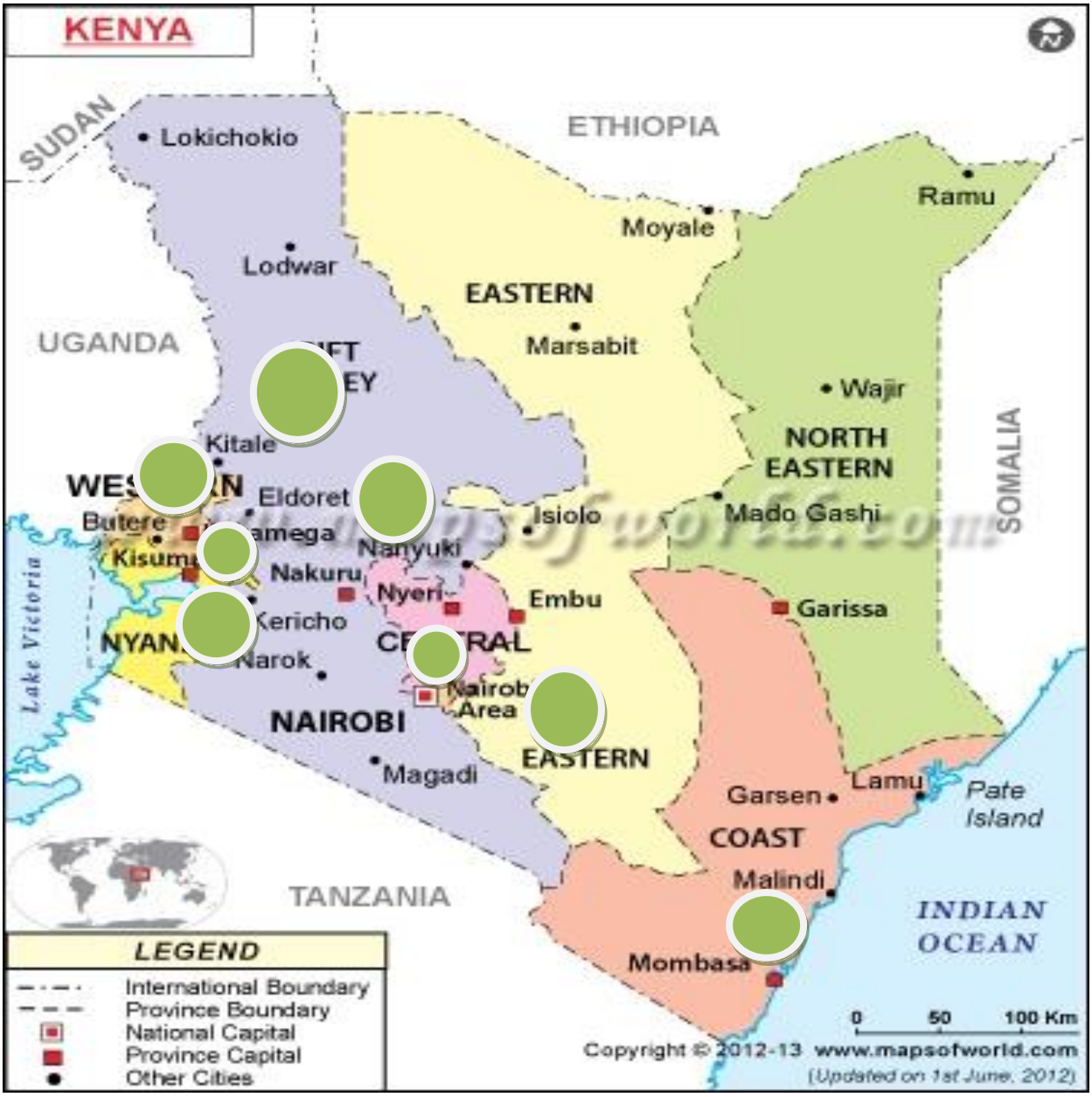


Figure 3.1: Map of Kenya showing the collection sites for the accessions evaluated in this study

- Regions where collections were done. Small circles indicate less than five collections in the area; large circles indicate more than five collections in the area.

Figure 3.2: Map of South Africa showing the collection sites for the accessions evaluated in this study

- Regions where collections were done. Small circles indicate less than five collections in the area; large circles indicate more than five collections in the area.

Table 3.1: List of Kenyan and South African spider plant accessions evaluated in the study

S/no.	[I]Accession no.	Species name	Region	County/area	AEZ
1	1959 [Za]	*C. gynandra*	Mpumalanga	Loopspruit	SH
2	1988 [Za]	*C. gynandra*	Mpumalanga	Silverhills	SH
3	2000 [Za]	*C. gynandra*	Mpumalanga	Gemsbokspruit	SH
4	2232 [Za]	*C. gynandra*	Northern province	Arthurstone	SA
5	2241 [Za]	*C. gynandra*	Northern province	Arthurstone	SA
6	2249 [Za]	*C. gynandra*	Northern province	Arthurstone	SA
7	2279 [Za]	*C. gynandra*	Northern province	Arthurstone	SA
8	2289 [Za]	*C. gynandra*	Mpumalanga	Rooikoppen	SH
9	2299 [Za]	*C. gynandra*	Mpumalanga	Loding	SH
10	GBK-027131 [Ke]	*C. gynandra*	Rift valley	Elgeyo Marakwet	LH 3
11	GBK-027195 [Ke]	*C. gynandra*	Rift valley	Elgeyo Marakwet	LM 1
12	GBK-027212 [Ke]	*C. gynandra*	Rift valley	Elgeyo Marakwet	LH 2
13	GBK-028554 [Ke]	*C. gynandra*	Nyanza	Siaya	LM 1
14	GBK-031990 [Ke]	*C. gynandra*	Western	Busia	LH 2
15	GBK-031992 [Ke]	*C. gynandra*	Western	Busia	LM 3
16	GBK-031993 [Ke]	*C. gynandra*	Western	Busia	LM 1
17	GBK-031996 [Ke]	*C. gynandra*	Western	Busia	LM 1
18	GBK-031997 [Ke]	*C. gynandra*	Western	Busia	LM 3
19	GBK-032134 [Ke]	*C. gynandra*	Eastern	Makueni	UM 4
20	GBK-032253 [Ke]	*C. gynandra*	Rift valley	West Pokot	UM 5
21	GBK-032302 [Ke]	*C. gynandra*	Western	Mbale	LM 1
22	GBK-040606 [Ke]	*C. gynandra*	Rift valley	Elgeyo Marakwet	LH 3
23	GBK-043261 [Ke]	*C. gynandra*	Rift valley	Nandi	LH 1
24	GBK-043760 [Ke]	*C. gynandra*	Rift valley	Koibatek	LH 2
25	GBK-045408 [Ke]	*C. gynandra*	Western	Vihiga	LM 1
26	GBK-045426 [Ke]	*C. gynandra*	Western	Vihiga	LM 1
27	GBK-045436 [Ke]	*C. gynandra*	Nyanza	Kisumu	LM 3
28	GBK-045446 [Ke]	*C. gynandra*	Nyanza	Kisii	LH 1
29	GBK-045451 [Ke]	*C. gynandra*	Central	Kiambu	UH 1
30	GBK-045456 [Ke]	*C. gynandra*	Central	Kiambu	UH 1
31	GBK-045494 [Ke]	*C. gynandra*	Coast	Kilifi	L 2
32	GBK-045497 [Ke]	*C. gynandra*	Coast	Kilifi	L 2

[I] Refers to identifier code used to identify an accession in the collection at genebank in Kenya and South Africa.
[Ke] Refers to Kenyan accession; [Za] refers to South African accession.

C- stands for *cleome*

AEZ- agroecological zone; LH- lower highland; LM- lower middle land; UM- upper middle land; UH- upper highland; L- lowland; SH- sub-humid; SA- semi-arid.

3.3 Soil analyses

Before the start of the experiments, the top 0- 15 cm of field soil in all the plots was sampled and bulked for testing. Soil testing and analysis was done at University of Nairobi's Soil Science Laboratories. 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 10N NaOH 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 0.025N H_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.

3.4 Experimental design and crop husbandry

3.4.1 Field experiments

Evaluations were performed on 32 spider plant accessions using a randomized complete block design with three replications. The experimental field was ploughed and harrowed with a tractor. Each accession was planted from seeds by hand in two 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. The experiments were carried out in two seasons (October 2013 to January 2014 and March 2014 to May 2014). Accessions planted were pre-germinated for 72 hours under treatment with 0.2 % gibberellic acid (SinoHarvest, Shenzen, China). Well decomposed cow manure and Di-ammonium phosphate (DAP) fertilizer (18:46:0) at the rate of 10.5 g/line was applied to rows and mixed with soil before planting. Top dressing was done with 17 g of calcium ammonium nitrate (26%N) per line. The plants were sprayed using an organophosphate insecticide lambdacyhalothrin-250EC (Twiga Chemical Industries, Nairobi, Kenya) at the rate of 65 ml/ 20 litre of water to kill cutworms and aphids after emergence and before flowering to prevent insect damage. The plants were kept weed free throughout the experimental period by hand weeding. The experiment was conducted under rainfed conditions. However, supplemental overhead irrigation was applied two times, at two weeks after planting and two weeks after flower initiation.

3.4.2 Glasshouse experiments

Thirty two (32) accessions of spider plant were evaluated in pots in a glasshouse using a randomized complete block design with three replications. The glasshouse experiments were carried out two times (September 2013 to December 2014 and March 2014 to May 2014). 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. The 32 pots per replication were each filled with 5 kg of air-dried soil mixture each. Di-ammonium phosphate (DAP) fertilizer (18:46:0) was applied at 3.15 g/pot just before sowing. Pre-germinated seeds treated with 0.2 per cent gibberellic acid (SinoHarvest, Shenzen, China), were then sparingly sown 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. Thirty days after seedling emergence, top dressing with 5.1 g of calcium ammonium nitrate (26%N) per pot was applied. The plants were watered at least two times each week and sprayed with lambdacyhalothrin-250EC (Twiga Chemical Industries, Nairobi, Kenya) to control insect pests.

3.5 Data collection

3.5.1 Qualitative traits

Three plants of each accession were randomly selected in each plot in the field and tagged. The three plants in each pot in the glasshouse were also tagged. These were done just before flowering for the determination of morphological data/ qualitative traits. Seeds used in both field and glasshouse experiments were mixtures collected from specific eco-regions by genebank from where the accessions were obtained in two batches before the planting period. Eleven qualitative traits: growth habit, flower colour, stem colour, stem hairiness, petiole colour, petiole hairiness, leaf colour, leaf pubescence, leaf shape, leaf blade tip shape, and number of leaflets per leaf were characterized based on the list of modified spider plant descriptors (FAO, 1995) as shown in

Table 3.1. All observations for each character were made on the same day for all accessions after 50% flowering to avoid differences in the developmental stages of growth.

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)
9	Leaf shape	Linear (1), lanceolate (2), elliptic (3), obovate (4) and ovate (5)
10	Leaf blade tip shape	Acuminate (1), acute (2), obtuse (3) and cuspidate (4)
11	No. of leaflets per leaf	Three (1), four (2), five (3), six (4) and seven (5)

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.

3.5.2 Quantitative traits

Quantitative data was collected in the field and glasshouse for eleven agronomical characters which included: days to 50% flowering, SPAD values, plant height, stem girth, number of primary branches, leaf length, leaf width, single leaf area, number of leaves per plant, number of pods per plant and seed yield per plant. All measurements and counts of a given trait were done on the same day for the field and glasshouse grown accessions to avoid bias.

3.5.2.1 Growth components

Days to flowering was recorded as the number of days from planting to when 50% of the plants in each plot/pot had flowered. The leaf chlorophyll content was taken at flower initiation stage on a fully expanded young leaf from three plants in each stand and averaged. This value was taken using a non-destructive, hand-held chlorophyll meter Soil Plant Analysis Development (SPAD-502, Minolta Camera Co., Ltd., Japan). SPAD-502 determines the relative amount of chlorophyll present in the leaf by measuring the transmittance of the leaf in two wave bands (600 to700 nm and 400 to 500 nm). Chlorophyll has absorbance peaks in the blue (400-500 nm) and red (600-700 nm) regions, with no transmittance in the near-infrared region. SPAD-502 measures the absorbance of the leaf in the red and near-infrared regions. Using these two transmittances, the meter calculates a numerical SPAD value, ranging from 0 to 80 which is usually proportional to the amount of chlorophyll present in the leaf (Jarvis, 2008). Plant height was measured in centimeters from the base of the plant to the tip of the main stem using a meter rule by selecting three plants at random from the inner rows of a plot, and their vertical heights measured after 50% flowering. Stem girth (cm) was determined by measuring the circumference of the middle portions of stems of three tagged plants at flowering. 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. Three basal leaves in each of the three tagged plants per plot were randomly selected at flowering and leaf measurements recorded. Leaf length was measured in centimeters from the pulvinus to the tip of the leaf while leaf width (cm) was measured at the widest part of the basal leaves. The single leaf area (cm^2) was calculated using leaf length and leaf width measurements following the formulae of Rivera *et al.,* (2007) as follows: $SLA = 0.763L + 0.34W$, where *SLA* is single leaf area, *L* is leaf length and *W* is leaf width.

3.5.2.2 Yield and yield components

The number of leaves per plant was counted from three tagged plants in each plot at flowering and the mean calculated. Mature pods from three tagged plants in each plot/pot were counted and the number of pods per plant calculated. Seed yield per plant was determined by taking the dry weight of seeds harvested from tagged plants and calculating the mean.

3.6 Data analysis

3.6.1 Qualitative traits

Phenotypic frequency distributions of the characters were calculated for all accessions based on the Shannon-Weaver diversity index (H') as described by Perry and McIntosh (1991). Dissimilarities were estimated based on Euclidean distance matrix and hierarchical clustering analyses of unweighted pair group method of arithmetic averaging performed in DARwin 5.0 software as described by Perrier and Jacquemoud-Collet (2006). The clusters and relationships were displayed as a phenogram. Multivariate-principal component analysis (PCA) was conducted between variance-covariance matrix using Genstat software programme, version 14 (Payne *et al.,* 2011) to identify the most significant descriptors in capturing the morphological variation in the germplasm. Coded data from the eleven morphological traits were used to generate biplot analysis in DARwin 5.0 software.

3.6.2 Quantitative traits

Analysis of variance (ANOVA) for the quantitative data was performed using Genstat version 14 (Payne *et al.,* 2011) at 5% level of significance. For treatment effects that were significant, mean separation was done by Fisher's protected least significant difference (LSD) test using Genstat

version 14 at P = 0.05. Variability within each quantitative trait was calculated using statistical measures of mean, standard deviation and coefficient of variation. A correlation analysis was performed in Genstat to estimate quantitative relationships among the traits and also to determine key agronomic traits of importance in breeding work.

CHAPTER 4: RESULTS

4.1 Qualitative characteristics

The spider plant accessions showed variations in forms and features of the different character traits measured and evaluated. However, there were no differences in qualitative characteristics between the two seasons. Figures 4.1 - 4.3 show selected accessions of visible variations. All pictures were captured the same day to avoid growth differences.

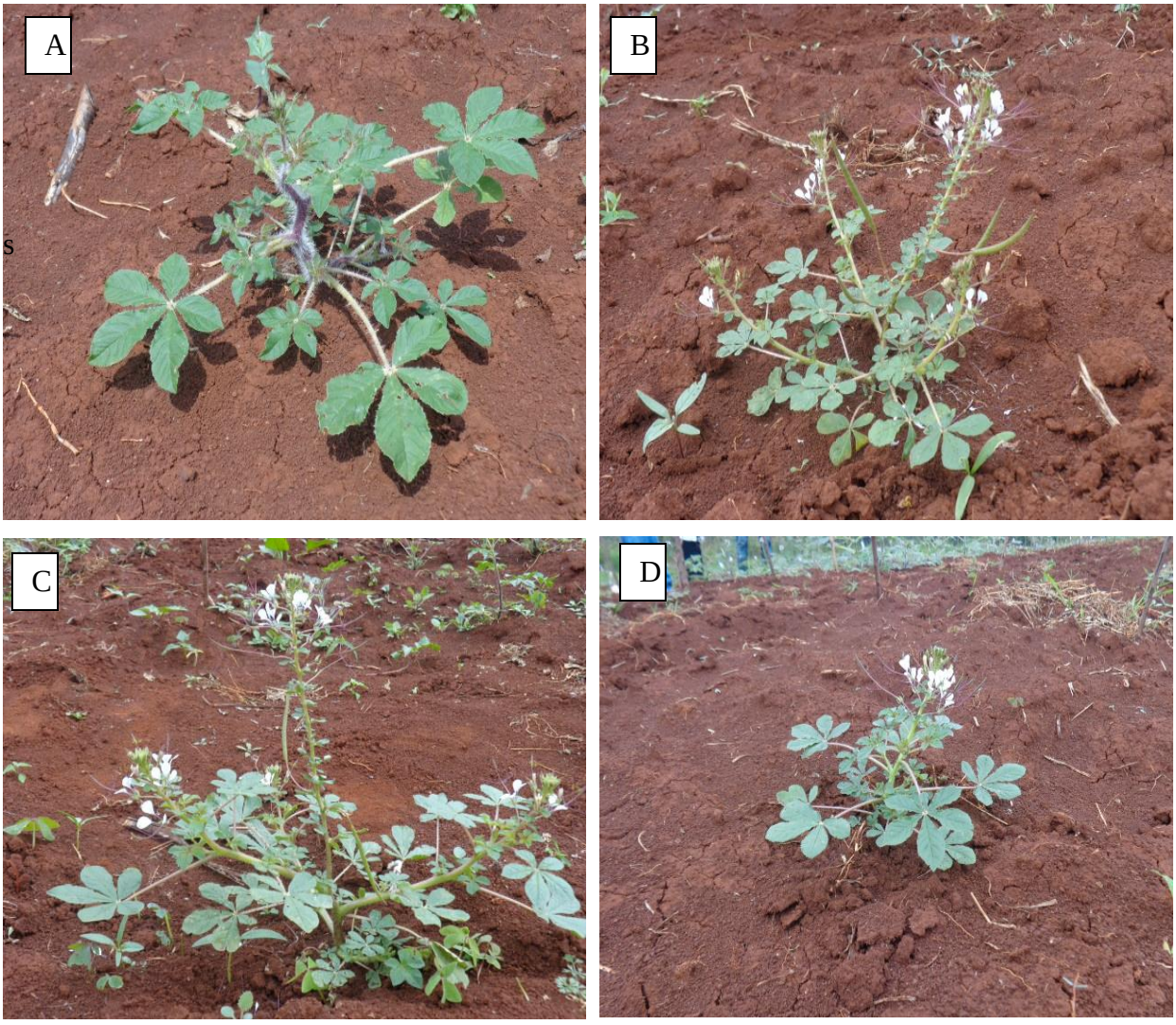


Figure 4.1: (A) Accession 1959: pink flower, purple stem and pink petiole; (B) Accession 2000: white flower, green stem and green petiole; (C) Accession 2279: white flower, green stem, and green petiole; (D) Accession 2289: white flower, green stem and pink petiole.

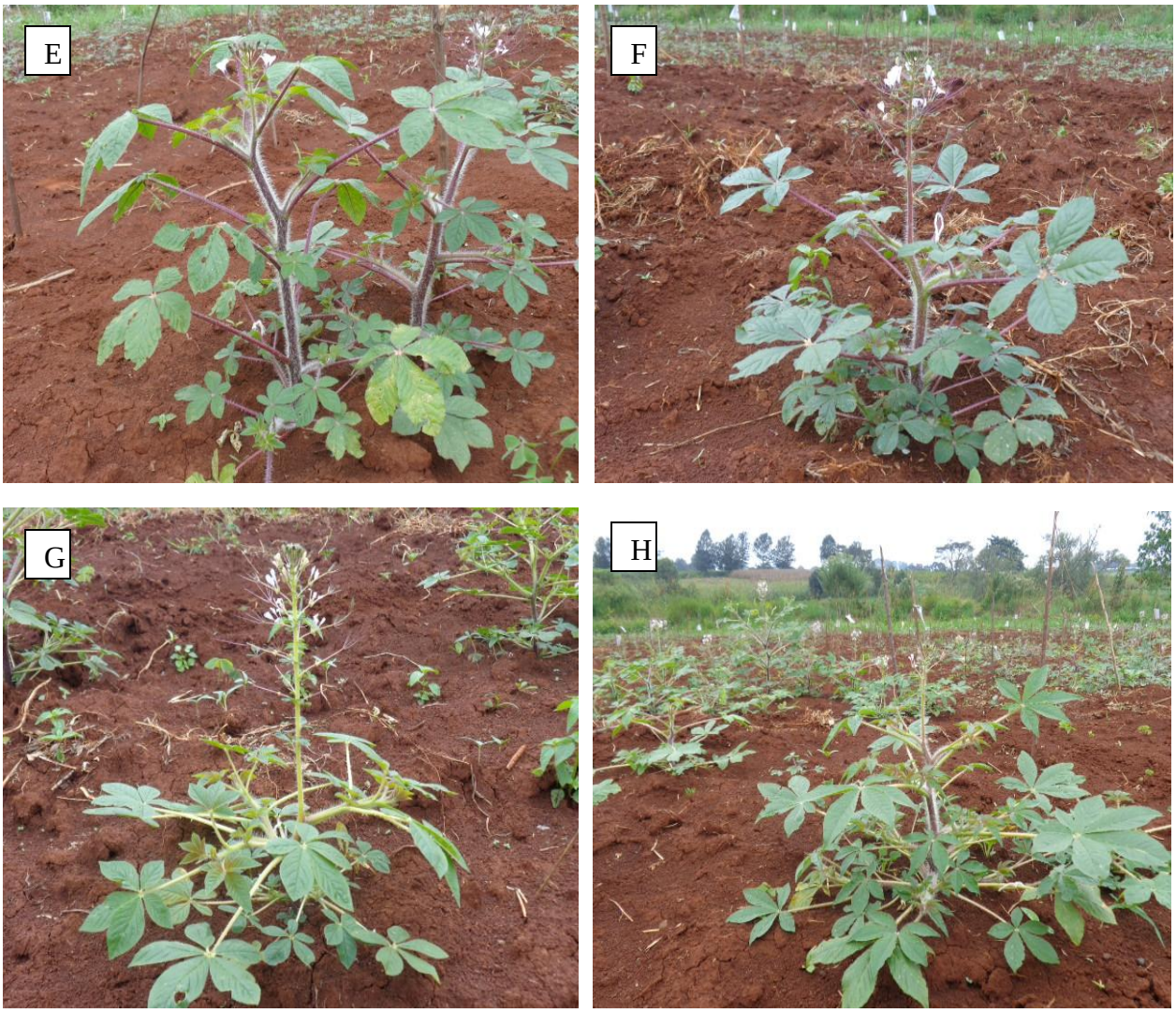


Figure 4.2: (E) Accession GBK-027195: white flower, purple stem and purple petiole; (F) Accession GBK-027212: pink flower, purple stem and pink petiole; (G) Accession GBK-031990: purple flower, green stem and green petiole; (H) Accession GBK-031996: purple flower, purple stem and green petiole.

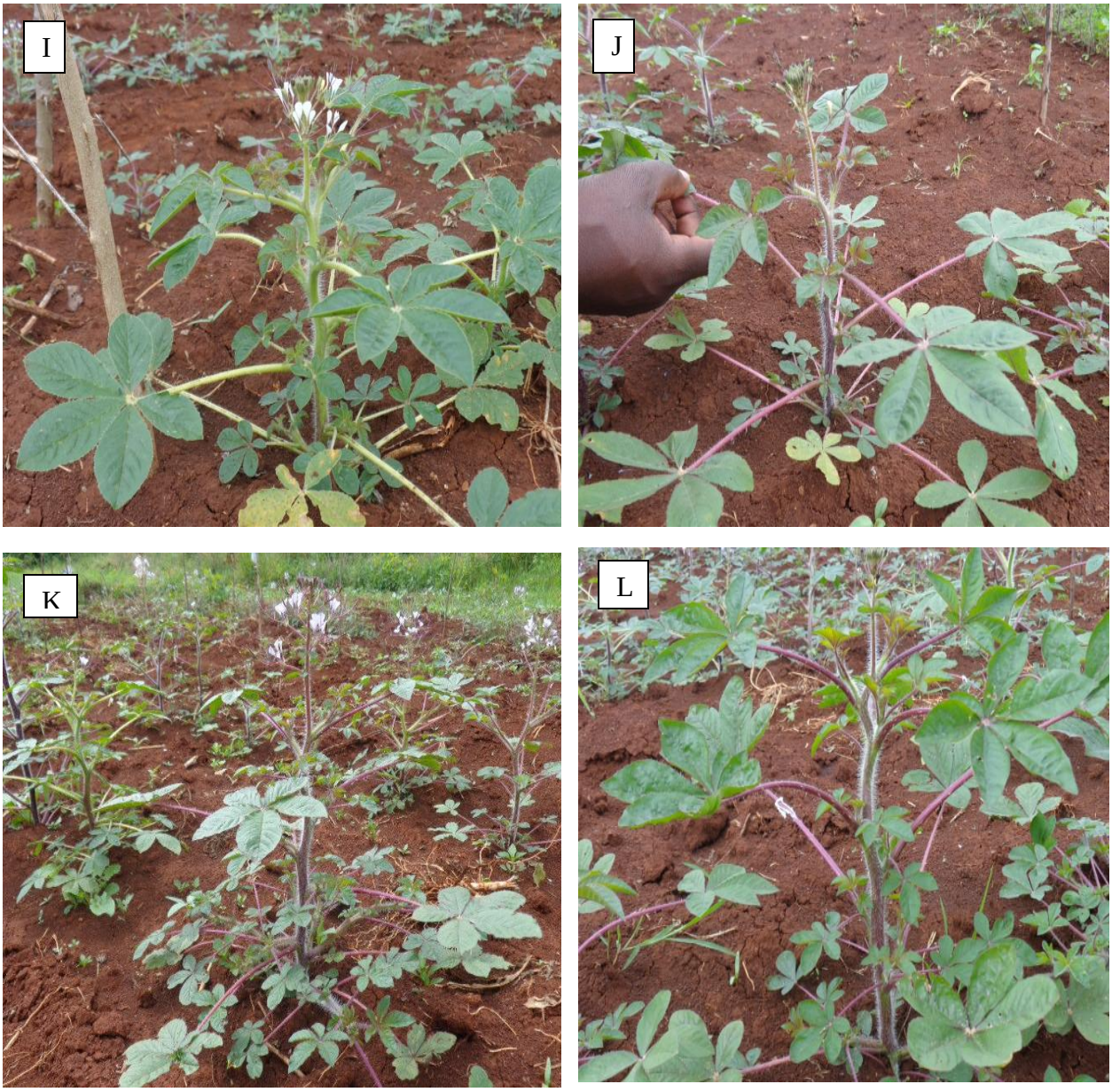


Figure 4.3: (I) Accession GBK-032302: purple flower, green stem and green petiole; (J) Accession GBK-040606: purple flower, purple stem and pink petiole; (K) Accession GBK-043261: pink flower, purple stem and purple petiole; (L) Accession GBK-045451: pink flower, purple stem and purple petiole.

4.1.1 Growth habit

It was observed that 81.2% of the accessions studied in the field and in the glasshouse produced an erect growth habit with 18.8% showing semi-erect growth habit (Table 4.1a and 4.1b). Accessions with semi-erect growth habit were 1988, 2000, GBK-028554, GBK-045436 and GBK-045494.

4.1.2 Flower colour and stem characteristics

About 47% of the accessions grown in the field produced white flowers, 31.3% produced pink flowers and 21.9% produced purple colour. On the other hand, about 43.8% of the accessions grown in the glasshouse produced white flowers, 21.8% produced pink flowers and 34.4% had purple colour.

Stem colour of the study accessions grown in the field and in the glasshouse were mainly purple (50%) and green (40.6%) while only 9.4% of the stems were violet in colour (Table 4.1a and 4.1b). South African accessions contributed largely to the green stem pigmentation observed in both experiments. Proportion of accessions with white flowers and green stems were 73.4% and 71.4%, for field and glasshouse grown accessions, respectively, while those with white flowers and purple stems were 13.3% and 21.4%, respectively, white flowers and violet stems accounted for 13.3% and 7.2%, respectively. Accessions with white flowers had mostly green stems and green petiole (66.7% and 44.4% for field and glasshouse grown accessions, respectively) while purple flowered accessions with purple stems and purple petiole comprised 22.2% of the field grown accessions. Pink flowered accessions were noted to have 10% violet stems and 90% purple stems for field grown accessions and 14.3% green stems, and 85.7% purple stems for

Table 4.1a: Morphological descriptors of 32 spider plant accessions grown in the field

Accession No.	Growth habit	Flower color	Stem color	Stem hairiness	Petiole color	Petiole hairiness	Leaf color	Leaf hairiness	Leaf shape	Leaf blade tip shape	No. leaflets/ Leaf
1959	Erect	Pink	Purple	Profuse	Pink	Medium	Light green	Medium	Elliptic	Acuminate	5
1988	Semi-erect	White	Green	Glabrous	Green	Glabrous	Light green	Glabrous	Obovate	Obtuse	5
2000	Semi-erect	White	Green	Glabrous	Green	Glabrous	Light green	Glabrous	Elliptic	Cuspidate	5
2232	Erect	White	Green	Glabrous	Green	Glabrous	Dark green	Glabrous	Obovate	Cuspidate	5
2241	Erect	White	Green	Glabrous	Pink	Glabrous	Light green	Glabrous	Ovate	Cuspidate	7
2249	Erect	White	Green	Glabrous	Pink	Glabrous	Light green	Glabrous	Obovate	Obtuse	5
2279	Semi-erect	White	Green	Glabrous	Green	Glabrous	Light green	Glabrous	Elliptic	Obtuse	3
2289	Erect	White	Green	Medium	Pink	Sparse	Dark green	Sparse	Lanceolate	Acute	5
2299	Erect	White	Green	Glabrous	Green	Sparse	Light green	Glabrous	Elliptic	Obtuse	5
GBK-027131	Erect	White	Green	Profuse	Green	Medium	Dark green	Medium	Elliptic	Acute	5
GBK-027195	Erect	White	Purple	Profuse	Purple	Profuse	Dark green	Profuse	Ovate	Acute	5
GBK-027212	Erect	Pink	Purple	Profuse	Pink	Sparse	Dark green	Sparse	Elliptic	Acute	5
GBK-028554	Semi-erect	Pink	Violet	Medium	Violet	Sparse	Light green	Sparse	Elliptic	Obtuse	5
GBK-031990	Erect	Purple	Green	Medium	Green	Sparse	Light green	Glabrous	Ovate	Obtuse	5
GBK-031992	Erect	Pink	Purple	Profuse	Purple	Medium	Dark green	Medium	Lanceolate	Acuminate	5
GBK-031993	Erect	White	Purple	Medium	Pink	Profuse	Light green	Medium	Ovate	Obtuse	5
GBK-031996	Erect	Purple	Purple	Profuse	Green	Medium	Light green	Sparse	Elliptic	Acuminate	6
GBK-031997	Erect	Purple	Purple	Profuse	Pink	Medium	Dark green	Sparse	Elliptic	Acute	5
GBK-032134	Erect	Pink	Purple	Profuse	Purple	Medium	Dark green	Sparse	Elliptic	Acute	5
GBK-032253	Erect	White	Violet	Profuse	Pink	Profuse	Light green	Medium	Lanceolate	Acute	5
GBK-032302	Erect	Purple	Green	Profuse	Green	Medium	Dark green	Sparse	Ovate	Obtuse	7
GBK-040606	Erect	Purple	Purple	Profuse	Pink	Medium	Dark green	Sparse	Lanceolate	Acuminate	5
GBK-043261	Erect	Pink	Purple	Profuse	Purple	Medium	Dark green	Sparse	Ovate	Obtuse	5
GBK-043760	Erect	White	Green	Medium	Pink	Sparse	Light green	Sparse	Elliptic	Obtuse	5
GBK-045408	Erect	Purple	Purple	Profuse	Pink	Profuse	Dark green	Sparse	Ovate	Acute	5
GBK-045426	Erect	Pink	Purple	Medium	Green	Sparse	Dark green	Sparse	Ovate	Obtuse	5
GBK-045436	Semi-erect	Purple	Purple	Profuse	Purple	Medium	Dark green	Sparse	Elliptic	Obtuse	5
GBK-045446	Erect	White	Green	Profuse	Pink	Medium	Dark green	Sparse	Ovate	Cuspidate	6
GBK-045451	Erect	Pink	Purple	Profuse	Purple	Profuse	Dark green	Medium	Lanceolate	Acuminate	5
GBK-045456	Erect	Pink	Purple	Profuse	Green	Sparse	Light green	Sparse	Elliptic	Obtuse	5
GBK-045494	Semi-erect	White	Violet	Medium	Green	Sparse	Light green	Glabrous	Obovate	Obtuse	5
GBK-045497	Erect	Pink	Purple	Profuse	Green	Medium	Dark green	Sparse	Elliptic	Acute	7

Table 4.1b: Morphological descriptors of 32 spider plant accessions grown in the glasshouse

Accession No.	Growth habit	Flower color	Stem color	Stem hairiness	Petiole color	Petiole hairiness	Leaf color	Leaf hairiness	Leaf shape	Leaf blade tip shape	No. leaflets/ leaf
1959	Erect	Pink	Purple	Profuse	Pink	Medium	Light green	Sparse	Elliptic	Acuminate	5
1988	Semi-erect	White	Green	Sparse	Green	Glabrous	Light green	Glabrous	Obovate	Obtuse	5
2000	Semi-erect	White	Green	Glabrous	Green	Glabrous	Light green	Glabrous	Elliptic	Cuspidate	5
2232	Erect	White	Green	Sparse	Green	Sparse	Dark green	Sparse	Obovate	Cuspidate	5
2241	Erect	White	Green	Glabrous	Pink	Glabrous	Light green	Glabrous	Ovate	Cuspidate	7
2249	Erect	White	Green	Glabrous	Pink	Glabrous	Light green	Glabrous	Obovate	Obtuse	7
2279	Semi-erect	White	Green	Glabrous	Green	Glabrous	Light green	Glabrous	Elliptic	Obtuse	3
2289	Erect	White	Green	Medium	Pink	Sparse	Dark green	Sparse	Lanceolate	Acute	5
2299	Erect	White	Green	Glabrous	Green	Glabrous	Light green	Glabrous	Elliptic	Obtuse	5
GBK-027131	Erect	White	Green	Profuse	Green	Medium	Light green	Medium	Elliptic	Acute	5
GBK-027195	Erect	Pink	Purple	Profuse	Purple	Profuse	Dark green	Profuse	Ovate	Acute	7
GBK-027212	Erect	White	Purple	Profuse	Pink	Medium	Dark green	Profuse	Elliptic	Acute	7
GBK-028554	Semi-erect	Purple	Violet	Medium	Violet	Sparse	Dark green	Glabrous	Elliptic	Obtuse	5
GBK-031990	Erect	Purple	Green	Profuse	Green	Sparse	Light green	Sparse	Ovate	Obtuse	5
GBK-031992	Erect	Purple	Purple	Profuse	Purple	Sparse	Dark green	Sparse	Lanceolate	Acuminate	5
GBK-031993	Erect	Purple	Purple	Profuse	Pink	Sparse	Dark green	Sparse	Ovate	Obtuse	7
GBK-031996	Erect	Purple	Purple	Profuse	Green	Sparse	Dark green	Medium	Elliptic	Acuminate	3
GBK-031997	Erect	Purple	Purple	Profuse	Pink	Medium	Dark green	Medium	Elliptic	Acute	5
GBK-032134	Erect	Purple	Purple	Profuse	Purple	Medium	Dark green	Profuse	Elliptic	Acute	6
GBK-032253	Erect	Purple	Violet	Profuse	Pink	Medium	Light green	Medium	Lanceolate	Acute	7
GBK-032302	Erect	Purple	Green	Profuse	Green	Medium	Light green	Medium	Ovate	Obtuse	7
GBK-040606	Erect	Pink	Purple	Medium	Pink	Medium	Dark green	Glabrous	Lanceolate	Acuminate	5
GBK-043261	Erect	Pink	Purple	Medium	Purple	Sparse	Light green	Sparse	Ovate	Obtuse	5
GBK-043760	Erect	White	Green	Profuse	Pink	Sparse	Light green	Sparse	Elliptic	Obtuse	5
GBK-045408	Erect	Pink	Purple	Profuse	Pink	Sparse	Light green	Medium	Ovate	Acute	5
GBK-045426	Erect	Purple	Purple	Profuse	Green	Profuse	Dark green	Sparse	Ovate	Obtuse	5
GBK-045436	Semi-erect	Purple	Purple	Medium	Purple	Sparse	Dark green	Glabrous	Elliptic	Obtuse	5
GBK-045446	Erect	Pink	Green	Glabrous	Pink	Sparse	Dark green	Medium	Ovate	Cuspidate	5
GBK-045451	Erect	White	Purple	Profuse	Purple	Sparse	Light green	Sparse	Lanceolate	Acuminate	5
GBK-045456	Erect	Pink	Purple	Medium	Green	Sparse	Dark green	Sparse	Elliptic	Obtuse	5
GBK-045494	Semi-erect	White	Violet	Glabrous	Green	Glabrous	Light green	Glabrous	Obovate	Obtuse	5
GBK-045497	Erect	White	Purple	Glabrous	Green	Glabrous	Light green	Glabrous	Elliptic	Acute	5

glasshouse grown accessions. The proportion of the studied accessions in the field and glasshouse with pink petioles was 37.5%, green petioles 37.5%, purple petiole 18.8%, and violet petioles 6.2%.

Stem hairiness for the study accessions in the field was mainly profuse at 56.2%, 21.9% were medium as well as 21.9% being glabrous. In the glasshouse, 50% of accessions had profuse stems, 18.7% were medium, 6.3% sparse and 25% glabrous. Glabrous accessions were mainly South African accessions. Petiole hairiness varied from medium (37.5%), to sparse (31.2%), glabrous (18.8%) and profuse (12.5%) in the field while for the accessions grown in the glasshouse petiole hairiness was mostly sparse (40.6%), medium (25%), glabrous (25%) and profuse (9.4%).

4.1.3 Leaf characteristics

The shapes of the basal leaves for the study accessions in both the field and glasshouse were elliptic (43.8%), ovate (28.1%), lanceolate (15.6%) or obovate (12.5%). Blade tip shapes for the study accessions in the field and glasshouse were obtuse (43.8%), acute (28.1%), acuminate (15.6%), or cuspidate (12.5%). Leaf colour was either dark green (50%) or light green (50%) for field grown accessions but more light green (53.1%) in the glasshouse study accessions.

Proportion of leaf hairiness of the study accessions grown in the field were either sparse (50%), glabrous (28.1%), medium (18.8%), or profuse (3.1%). In the glasshouse, the proportion of leaf hairiness were either glabrous (34.4%), sparse (34.4%), medium (21.8%) or profuse (9.4%). Number of leaflets per leaf were mostly five (81.3%, 68.8%), but three (3.1%, 6.3%), six (6.3%,

3.1%) and seven (9.4%, 21.9%) leaflets per leaf were also noted in field and glasshouse grown accessions, respectively.

4.2 Diversity index

Estimates of Shannon-Weaver (H') for the qualitative characters evaluated in the study accessions were generally high for both field and glasshouse experiments (Table 4.2). The indices ranged from 0.935 (stem hairiness) to 0.999 (number of leaflets per leaf) with an average of 0.970 for study accessions grown in the field. In the glasshouse, H' ranged from 0.872 (leaf hairiness) to 0.999 (growth habit) with an average of 0.968 (Table 4.2). All traits showed high (H'>0.500) levels of polymorphism in both experiments.

Table 4.2: Standard Shannon Weaver diversity index (H') for qualitative characters in 32 spider plant accessions grown in the field and in the glasshouse

Qualitative trait	Shannon-Weaver index (H')	
	Field	Glasshouse
Growth habit	0.998	0.999
Flower colour	0.977	0.983
Stem colour	0.970	0.965
Stem hairiness	0.935	0.920
Petiole colour	0.974	0.979
Petiole hairiness	0.936	0.911
Leaf colour	0.993	0.995
Leaf hairiness	0.902	0.872
Leaf shape	0.997	0.997
Leaf blade tip shape	0.990	0.994
No. leaflets per leaf	0.999	0.997
Average diversity index	0.970	0.968

4.3 Principal coordinate analysis

Figure 4.4 and 4.5 show the genetic relationships based on first (axis 1 and 2) and second (axis 1 and 3) planes of the PCoA, respectively, for field grown accessions. Generally, separation between the two groups of accessions (Kenyan and South African) was high with the second plane separating the two groups clearly. The accessions grown in the glasshouse were also clearly separated by the second plane (axis 1 and 3) (Fig 4.7) but less with the first plane (axis 1 and 2) (Fig 4.6). Overall, there was a clear separation of the field grown accessions from the glasshouse grown accessions. This could be due to character expression being more vivid in the field than in the glasshouse.

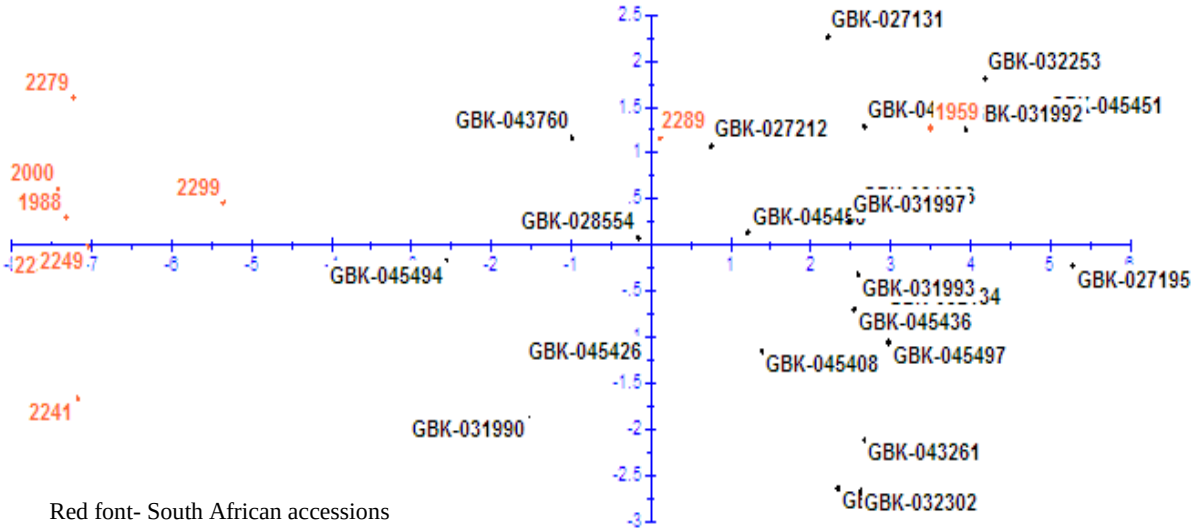
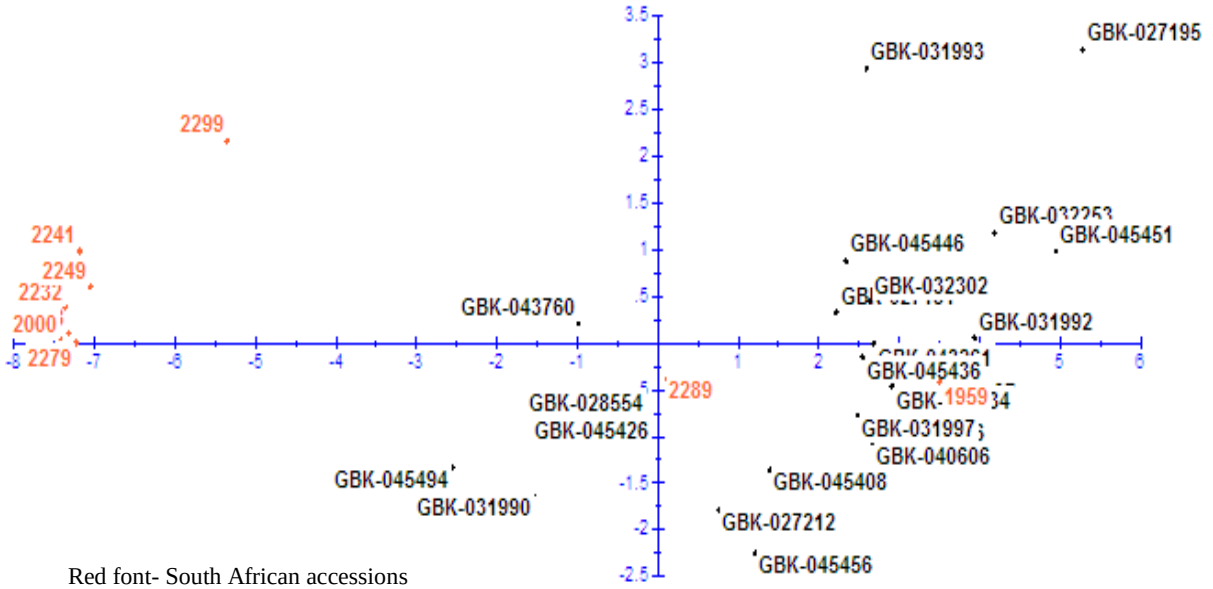


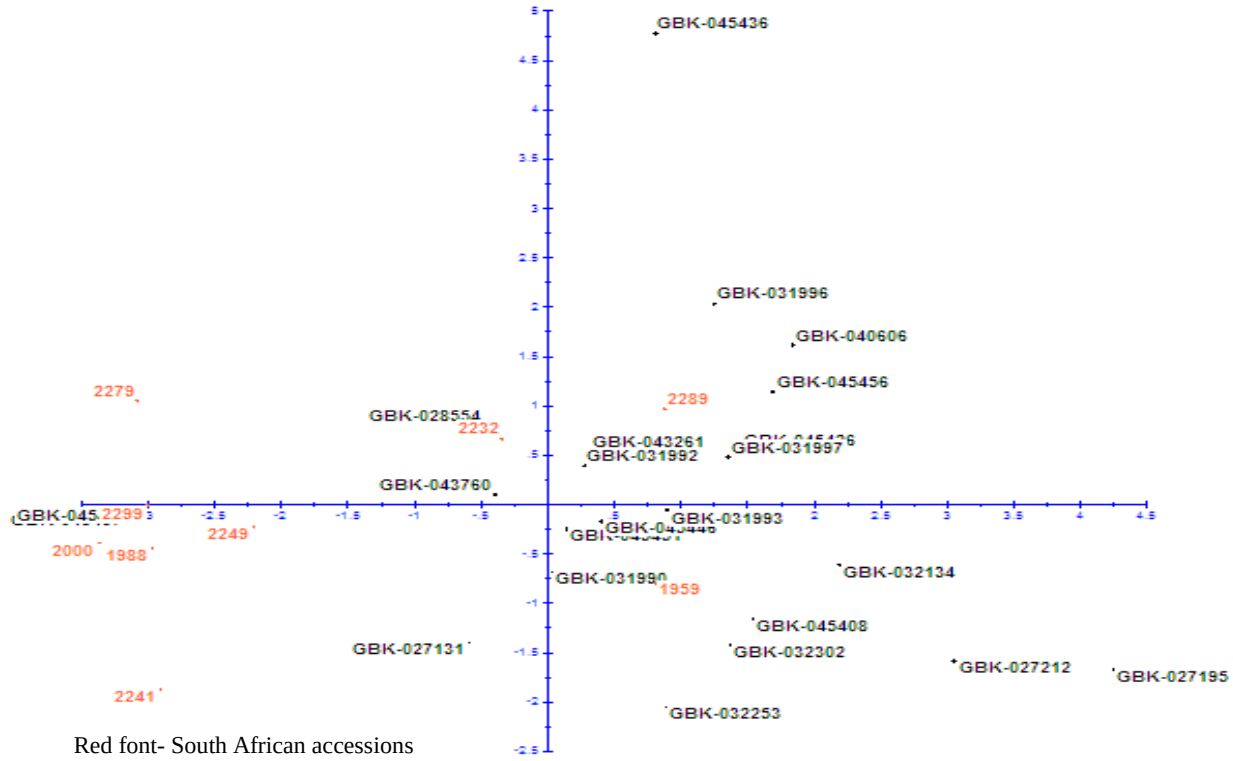
Figure 4.4: Biplot analysis of axis 1 and 2 of principal coordinate analysis of 32 spider plant accessions grown in the field based on dissimilarity of the qualitative characters



Red font- South African accessions

Normal font- Kenyan accessions

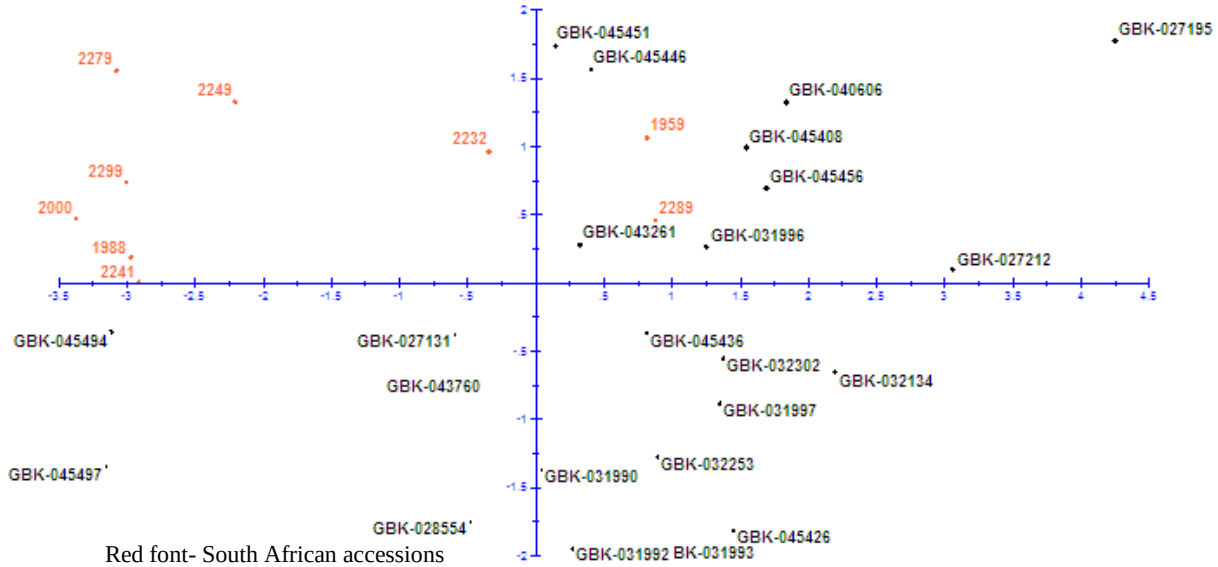
Figure 4.5: Biplot analysis of axis 1 and 3 of principal coordinate analysis of 32 spider plant accessions grown in the field based on dissimilarity of the qualitative characters



Red font- South African accessions

Normal font- Kenyan accessions

Figure 4.6: Biplot analysis of axis 1 and 2 of principal coordinate analysis of 32 spider plant accessions grown in the glasshouse based on dissimilarity of the qualitative characters



Red font- South African accessions

Normal font- Kenyan accessions

Figure 4.7: Biplot analysis of axis 1 and 3 of principal coordinate analysis of 32 spider plant accessions grown in the glasshouse based on dissimilarity of the qualitative characters

The three main axes (axis 1, 2 and 3) explained 70.9, 6.8 and 6.2% of the total variation respectively for the accessions grown in the field giving a cumulative total variation of 84% (Table 4.3). Axes 1, 2, 3, 4 and 5 contributed 16.6, 1.61, 1.46, 1.04 and 0.73 of the eingenvalues respectively, for the field grown accessions (Table 4.3). For the accessions grown in the glasshouse, the three main axes explained 36.6, 16.2 and 12% of the total variations, respectively giving a cumulative total of 64.85% of the Variance (Table 4.3). The five axes (1, 2, 3, 4, and 5) contributed 3.91, 1.73, 1.28, 1.11, and 0.72 of the eingenvalues respectively, for glasshouse grown accessions as shown in Table 4.3.

Table 4.3: Eigenvalues and total variation of five principal components for 32 spider plant accessions grown in the field and in the glasshouse

Axis	Eigenvalue		% of variance		Cumulative % of variance	
	Field	Glasshouse	Field	Glasshouse	Field	Glasshouse
1	16.62	3.90	70.92	36.64	70.92	36.64
2	1.61	1.73	6.86	16.22	77.78	52.86
3	1.46	1.28	6.22	11.99	84.00	64.85
4	1.04	1.11	4.44	10.43	88.44	75.28
5	0.73	0.72	3.09	6.73	91.53	82.01

4.4 Cluster analysis

The phenogram generated using eleven morphological descriptors (growth habit, flower colour, stem colour, stem hairiness, petiole colour, petiole hairiness, leaf colour, leaf pubescence, leaf shape, leaf blade tip shape, and number of leaflets per leaf) based on Euclidean Distance Coefficient and UPGMA clustering method clearly showed the phenetic relationship among the accessions. Cluster analysis revealed two major clusters (Cluster I and II) for study accessions grown in the field (Figure 4.8). Cluster I had seven accessions while cluster II had 27 accessions. Cluster I had South African accessions while cluster II had Kenyan accessions mainly and two South African accessions (2289 and 1959). Within country variation was observed for both the Kenyan and South African accessions with accessions collected from the same region closely related. Figure 4.8 cluster I shows a close relationship between South African accessions 2249 and 2232 which were collected from Nothern province. Likewise, accessions 1988 and 2000 which were collected from Mpumalanga region had similar clades suggesting a close resemblance in their genetic traits. However, the South African accessions collected from different regions expressed dissimilarity in variations as was the case with accessions 2299, 2241 and 2279 in cluster I figure 4.8. Accession 2299 which was collected from Mpumalanga region

had a longer genetic distance from the other two accessions, 2241 and 2279 which were collected from Northern Province. Within country variation was also observed in the Kenyan accessions (Figure 4.5). In sub-cluster 'c' of three accessions originating from one node, two accessions (GBK045426 and GBK05408) from the western region were more closely related to each other than to accession GBK045456 which was collected from central Kenya. Similarly, accession GBK031997 and GBK031996 in sub-cluster 'd' which were collected from western region, were more closely related to each other than to accession GBK040606 which was collected from Rift valley region. However, in cluster II sub-cluster 'b', accessions GBK031993 and GBK027195 which were collected from Western and Rift valley regions, respectively, were closely related despite being collected from the two different regions.

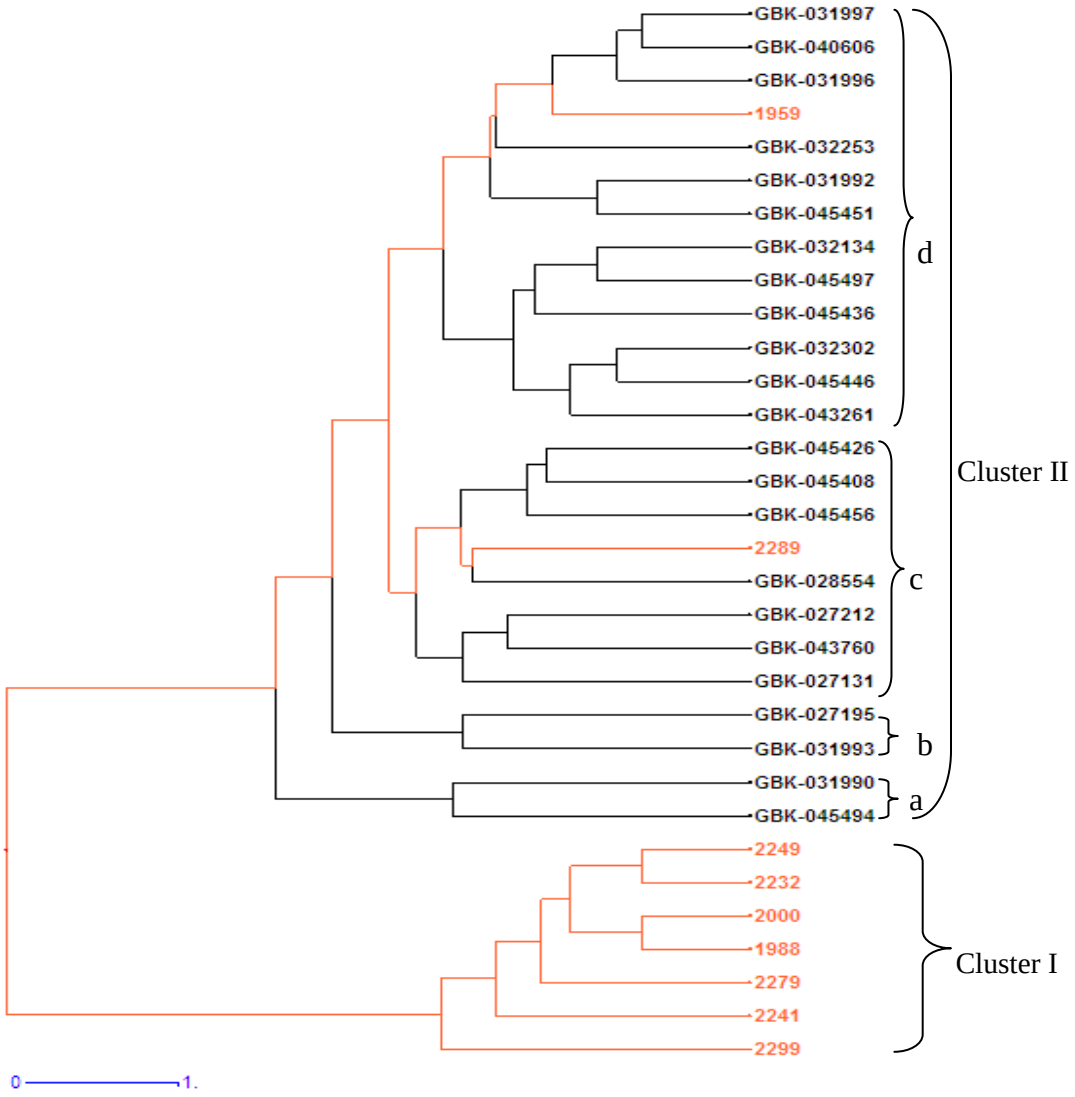


Figure 4.8: Unweighted pair-group method using arithmetic averages cluster analysis phenogram showing the relationships among the 32 spider plant accessions grown in the field

For the glasshouse grown accessions, cluster phenogram exposed three major clusters (Cluster I, II and III) (Figure 4.9). Cluster I revealed a simplifolious clade having Kenyan accession GBK045436. Cluster II had a bifolious clade with two Kenyan accessions, GBK027195 and GBK027212. Cluster III consisted mainly of a mixture of the Kenyan and South African accessions but with two sub-clusters (sub-cluster 'a' and 'b'). Sub-cluster 'a' contained most

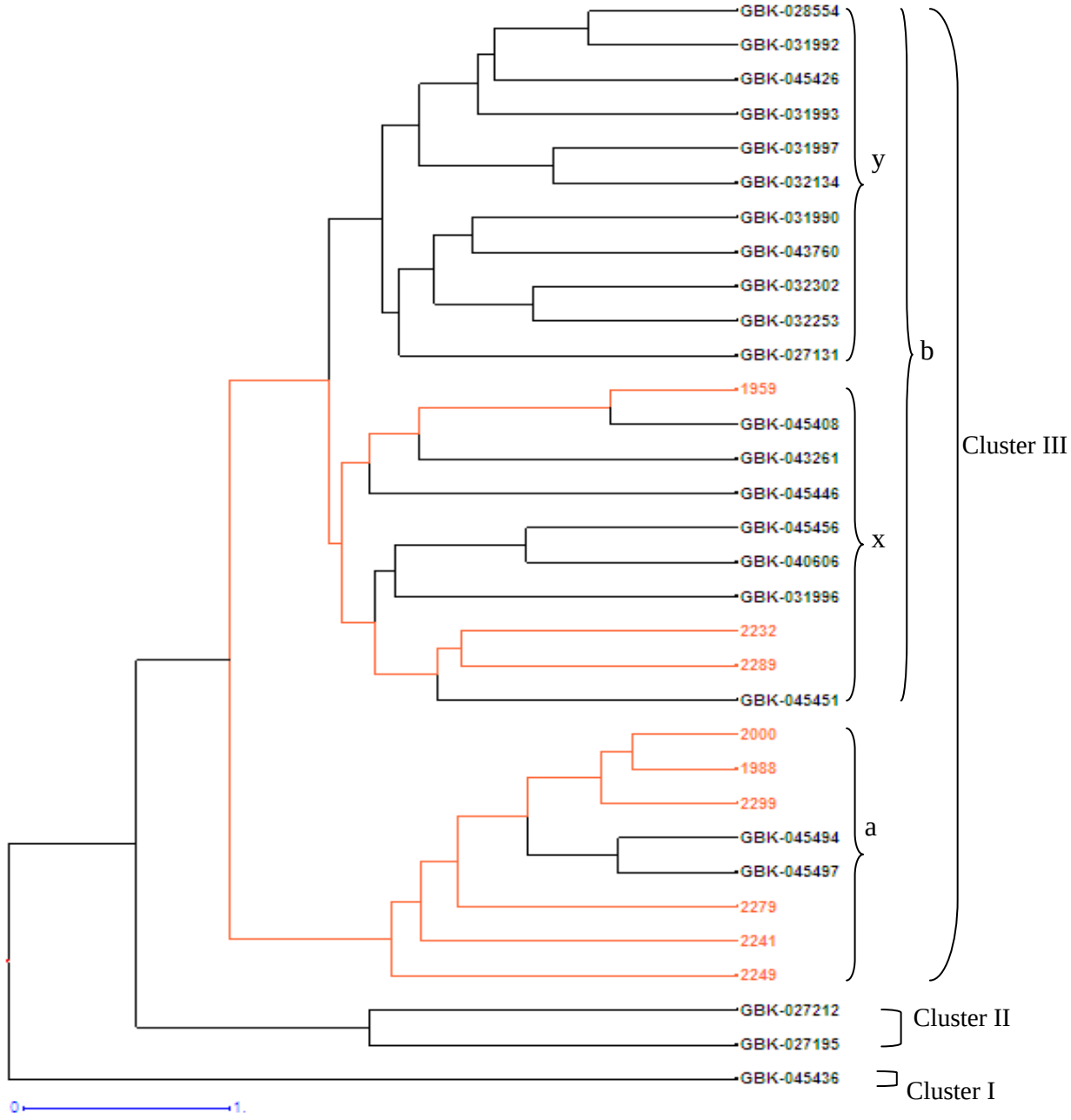


Figure 4.9: Unweighted pair-group method using arithmetic averages cluster analysis phenogram showing the relationships among the 32 spider plant accessions grown in the glasshouse

South African accessions (6) with only two Kenyan accessions (GBK-045497 and GBK-045494). This shows that the two Kenyan accessions were more closely related to the South

African accessions than to other Kenyan accessions. Sub-cluster 'b' had the most accessions (21). This sub-cluster was dived into two primary sub-groups (x and y) with three South African accessions (2289, 2232 and 1959) in sub-group 'x'. Sub-group 'y' had only Kenyan accessions.

4.5 Principal component analysis

The percentage variation explained by the first six principal components (PC) and the vector loadings for each character and PC are presented in Tables 4.4a and 4.4b. The first six PCs explained 94.2% of the variation among the 32 field grown accessions and 96.1% among the glasshouse grown accessions studied.

Table 4.4a: Eigenvalues[a], eigenvectors[b] and percentage of variation explained by the first six principal components for 32 spider plant accessions grown in the field

Qualitative character	Principal component					
	1	2	3	4	5	6
Variation explained (%)	70.92	6.86	6.22	4.44	3.09	2.65
Eigenvalue	17.16	1.66	1.51	1.08	0.75	0.64
Growth habit	-0.056	-0.003	-0.081	0.089	-0.328	-0.282
Flower color	0.110	0.072	-0.360	0.322	0.176	0.350
Stem color	0.279	0.319	-0.092	0.154	**0.756**	-0.234
Stem hairiness	[c]**0.653**	0.118	**-0.565**	-0.198	-0.357	-0.011
Petiole color	0.160	0.307	0.337	**0.691**	-0.251	0.207
Petiole hairiness	**0.500**	-0.039	0.464	-0.016	-0.053	**-0.539**
Leaf color	-0.075	-0.069	0.032	-0.091	0.072	-0.180
Leaf hairiness	0.419	-0.326	0.403	-0.247	0.087	**0.572**
Leaf shape	-0.045	**0.667**	0.171	-0.485	0.048	0.205
Leaf blade tip shape	-0.145	0.356	0.117	-0.171	-0.193	-0.058
No leaflets per leaf	0.031	0.317	0.045	0.115	-0.211	0.074

[a]Eigenvalues indicate the amount of variance explained by each principal component
[b]Eigenvectors are the weights in a linear transformation when computing principal components
[c]Values in bold indicate the most relevant descriptors that contributed most to the particular component.

Table 4.4b: Eigenvalues[a], eigenvectors[b] and percentage of variation explained by the first six principal components for 32 spider plant accessions grown in the glasshouse

Qualitative character	Principal component					
	1	2	3	4	5	6
Variation explained (%)	65.14	11.39	6.37	5.44	4.53	3.23
Eigen value	16.86	2.95	1.65	1.41	1.18	0.84
Growth habit	-0.003	-0.062	-0.071	-0.009	0.085	-0.033
Flower color	0.098	0.028	-0.221	-0.001	0.225	0.321
Stem color	0.135	**-0.570**	0.341	**-0.567**	0.340	0.203
Stem hairiness	[c]**0.675**	-0.465	-0.035	0.235	-0.321	-0.369
Petiole color	0.095	0.237	0.025	-0.010	0.274	-0.251
Petiole hairiness	**0.499**	0.142	-0.281	0.310	0.312	0.489
Leaf color	-0.050	0.031	0.060	0.153	-0.194	-0.088
Leaf hairiness	0.493	**0.573**	0.370	-0.411	-0.214	0.070
Leaf shape	-0.057	-0.147	**0.554**	**0.500**	0.061	0.341
Leaf blade tip shape	-0.090	0.039	0.369	0.130	-0.416	0.286
No leaflets per leaf	0.054	0.164	0.407	0.260	**0.540**	-0.454

[a]Eigenvalues indicate the amount of variance explained by each principal component
[b]Eigenvectors are the weights in a linear transformation when computing principal components
[c]Values in bold indicate the most relevant descriptors that contributed most to the particular component.

Stem hairiness and petiole hairiness were the main traits that contributed positively to PC1 for the accessions grown in the field (Table 4.4a). It was also observed that growth habit, leaf colour, leaf shape and leaf blade tip shape had negative loadings to this component at -0.056, -0.075, -0.045 and -0.145 respectively. For the glasshouse grown accessions, stem colour, stem hairiness, petiole hairiness and leaf hairiness were the most important characters contributing to the first principal component (Table 4.4b). Leaf shape was the most important character that contributed to the second principal component in field grown accessions while for the glasshouse grown accessions the second principal component was highly contributed by stem colour. Nearly all the characters that made significant contributions to a particular principal component were important contributors to another principal component.

4.6 Quantitative characteristics

Results for the quantitative characters were not different between the two seasons. Spider plant accessions grown in the field showed significant variation ($P<0.05$) for days to 50% flowering (Appendix 3), chlorophyll level (Appendix 4), plant height (Appendix 5), stem girth (Appendix 6), number of primary branches (Appendix 7), number of pods per plant (Appendix 12) and seed yield per plant (Appendix 13) but not for leaf length (Appendix 8), leaf width (Appendix 9), single leaf area (Appendix 10) and number of leaves per plant (Appendix 11). In contrast, all spider plant accessions grown in the glasshouse showed significant variation ($P<0.05$) in all the quantitative traits measured.

4.6.1 Days to 50% flowering

The range and mean for the number of days to 50% flowering of the accessions studied are presented in Tables 4.5a and 4.5b. Two field study accessions were first to flower within a range of 33 to 34 days with a mean of approximately 34 days. Accession number 2000 flowered first in 33 days while accession number 1988 took 34 days to flower after emergence (Table 4.5a). Twenty accessions registered the longest days to flowering within a range of 45 to 51 days. Among the twenty accessions that flowered late, accession number 2297 recorded the longest time of flowering according to this study in 51 days. The coefficient of variation for the number of days to 50% flowering was 7.4%. In the glasshouse, accession number GBK045436 days flowered the earliest at 35 days while accession number 2299 flowered the latest at 56 days after emergence (Table 4.6b). The mean days to flowering in the glasshouse were 43 days and the coefficient of variation was 8%. It was observed that accessions which were grown in the field flowered earlier than those grown in the glasshouse (Table 4.6a and 4.6b).

Table 4.5a: Quantitative trait means of 32 spider plant accessions from Kenya and South Africa grown in the field

S/no.	Accession no.	DTF	SPAD	PH	SG	NPB	LL	LW	SLA	NLPP	NPP	SYPP
1	1959	46.0	52.5	33.7	3.0	6.7	6.0	12.6	8.9	87.0	37.5	0.8
2	1988	34.0	50.4	45.0	3.2	5.3	6.0	12.3	8.7	48.3	29.7	0.9
3	2000	33.3	50.2	31.0	1.8	4.0	6.8	13.3	9.7	18.0	13.7	1.0
4	2232	40.7	21.1	26.0	1.9	4.3	5.1	10.4	7.4	54.3	11.3	0.8
5	2241	46.3	44.6	41.0	2.2	8.0	7.6	15.1	10.9	38.0	7.0	0.7
6	2249	40.0	44.2	23.5	1.2	4.3	6.7	13.3	9.6	14.7	5.7	1.1
7	2279	51.0	34.9	29.0	2.2	6.0	6.2	12.8	9.1	21.0	8.3	0.9
8	2289	45.3	43.7	41.0	3.1	6.7	7.5	14.0	10.5	28.3	4.3	1.7
9	2299	40.0	44.0	29.7	2.7	7.0	5.2	9.9	7.3	72.7	20.3	0.9
10	GBK-027131	46.0	52.0	45.7	3.7	5.1	5.9	10.8	8.2	60.2	36.3	3.2
11	GBK-027195	44.0	55.2	60.8	4.2	8.6	5.3	9.4	7.2	59.8	43.6	2.8
12	GBK-027212	44.7	52.3	45.0	3.3	7.2	9.3	17.7	13.1	41.6	35.9	4.6
13	GBK-028554	45.3	52.3	46.8	2.9	6.9	6.5	12.1	9.1	54.3	40.7	4.2
14	GBK-031990	44.7	53.1	49.0	3.5	7.4	7.7	14.2	10.7	73.0	52.9	4.4
15	GBK-031992	44.7	54.5	56.6	3.6	7.4	8.9	17.6	12.8	46.8	31.0	4.2
16	GBK-031993	46.0	55.3	40.1	3.6	6.2	6.7	13.0	9.5	60.2	23.4	4.2
17	GBK-031996	48.0	53.1	47.3	3.6	7.3	6.4	12.0	9.0	55.8	34.1	5.2
18	GBK-031997	46.0	55.1	50.4	4.2	7.2	5.2	10.2	7.4	54.9	23.2	3.6
19	GBK-032134	44.7	51.5	51.8	3.2	7.4	7.9	16.2	11.5	54.7	38.3	4.7
20	GBK-032253	46.7	51.9	41.1	3.0	6.4	8.5	17.2	12.4	50.1	25.6	4.7
21	GBK-032302	45.3	53.2	53.2	2.9	7.1	7.0	12.9	9.7	46.1	28.2	3.7
22	GBK-040606	44.0	56.8	57.8	4.0	7.7	6.2	12.9	9.1	70.8	28.2	2.6
23	GBK-043261	45.3	54.8	41.3	2.9	6.6	6.1	13.3	9.2	52.3	21.9	5.3
24	GBK-043760	45.3	51.9	43.0	3.2	5.9	5.9	12.0	8.6	51.2	20.6	2.9
25	GBK-045408	43.3	52.8	48.7	3.1	5.9	6.4	10.3	8.4	53.3	38.0	3.0
26	GBK-045426	42.7	54.0	47.9	3.5	7.3	7.7	16.1	11.3	45.9	32.9	4.5
27	GBK-045436	42.0	55.4	46.3	3.8	6.9	8.3	14.1	11.1	66.9	39.8	4.7
28	GBK-045446	44.7	53.0	50.0	3.3	7.4	4.9	9.4	7.0	57.7	37.3	5.1
29	GBK-045451	44.7	48.6	48.9	4.4	8.2	7.3	15.4	10.8	63.3	33.0	1.7
30	GBK-045456	41.3	52.5	52.8	3.1	7.6	7.7	15.5	11.1	52.4	45.9	2.7
31	GBK-045494	42.0	48.9	27.6	1.7	4.8	7.6	13.4	10.4	38.8	33.7	0.7
32	GBK-045497	44.7	50.2	43.4	3.1	6.6	8.0	16.2	11.6	70.4	33.3	3.6
	Mean	43.8	50.1	43.6	3.1	6.6	6.8	13.3	9.7	52.0	28.6	3.0
	Fpr	<.001	<.001	0.003	<.001	<.001	0.611	0.373	0.508	0.015	0.006	<.001
	Lsd $_{(p<0.05)}$	5.3	7.8	17.9	1.2	1.5	NS	NS	NS	NS	24.0	1.9
	Cv%	7.4	9.5	25.2	23.9	13.6	31.0	29.4	29.8	38.8	51.5	18.3

Fpr – F probability, LSD- Least significant difference, ns- not significant, DTF- days to 50% flowering, SPAD- chlorophyll content, PH- plant height (cm), SG- stem girth (cm), NPB- number of primary branches, LL- single leaf length, LW- leaf width, SLA- single leaf area (cm^2), NLPP- number of leaves per plant, NPP- number of pods per plant, SYPP- seed yield per plant.

Table 4.5b: Quantitative trait means of 32 spider plant accessions from Kenya and South Africa grown in the glasshouse

S/no.	Accession no.	DTF	SPAD	PH	SG	NPB	LL	LW	SLA	NLPP	NPP	SYPP
1	1959	55.0	39.9	16.3	2.6	6.0	3.6	6.3	9.4	63.3	30.7	0.9
2	1988	48.7	40.2	23.7	2.2	7.3	4.1	7.9	11.3	22.7	8.3	1.1
3	2000	51.3	36.6	22.0	2.4	7.0	3.4	6.5	9.4	21.0	7.3	1.3
4	2232	43.7	40.1	44.0	2.8	8.0	6.7	10.2	15.6	43.3	20.7	1.0
5	2241	46.3	44.6	36.0	2.2	8.0	8.6	14.5	21.4	38.0	14.3	0.7
6	2249	47.0	41.6	37.3	2.2	7.0	8.9	14.0	21.1	31.3	15.7	1.4
7	2279	51.0	34.9	24.0	2.2	6.0	4.3	7.6	11.3	17.0	7.0	1.0
8	2289	45.3	43.7	36.0	3.1	6.7	8.3	15.6	22.3	32.3	6.7	1.2
9	2299	56.3	11.4	7.0	1.1	4.0	1.7	4.2	5.8	10.3	5.0	0.8
10	GBK-027131	41.0	51.4	101.2	2.6	10.4	7.7	15.0	21.3	30.7	13.9	2.1
11	GBK-027195	43.7	48.8	80.8	2.7	11.2	7.3	12.8	18.7	21.3	13.5	1.8
12	GBK-027212	38.3	60.3	90.8	2.4	10.3	7.8	14.7	21.0	20.0	13.8	3.1
13	GBK-028554	41.0	54.3	82.3	2.1	10.3	6.5	12.6	18.0	16.3	12.2	2.8
14	GBK-031990	41.7	51.2	95.2	2.8	10.3	6.9	13.8	19.4	21.0	13.0	2.9
15	GBK-031992	40.3	54.1	86.8	2.6	9.2	7.4	13.8	19.8	18.0	13.0	2.8
16	GBK-031993	39.7	56.5	89.9	2.5	9.0	6.9	13.8	19.4	28.7	12.4	2.8
17	GBK-031996	40.3	52.5	81.8	2.8	10.0	7.2	12.8	18.7	26.0	13.1	3.5
18	GBK-031997	45.0	53.8	98.8	2.5	11.1	6.8	13.7	19.2	24.7	13.0	2.4
19	GBK-032134	37.7	53.2	79.2	2.5	10.7	6.9	13.6	19.2	22.3	12.9	3.1
20	GBK-032253	40.3	53.0	80.9	2.3	10.4	6.8	13.3	18.8	18.0	12.7	3.1
21	GBK-032302	42.3	58.0	102.3	2.6	12.0	6.9	14.2	19.8	24.7	13.5	2.5
22	GBK-040606	45.0	51.8	101.3	2.4	11.6	6.7	12.9	18.4	32.7	12.9	1.8
23	GBK-043261	41.0	57.1	96.7	2.2	11.4	5.9	11.1	15.9	17.0	11.8	3.5
24	GBK-043760	41.0	51.4	76.7	2.5	8.6	6.8	12.1	17.6	28.0	12.0	1.9
25	GBK-045408	45.0	46.4	87.3	2.7	9.9	6.1	10.9	15.8	22.7	11.6	2.0
26	GBK-045426	39.7	55.4	82.4	2.4	10.8	8.4	15.0	21.7	24.7	14.7	3.0
27	GBK-045436	35.0	56.7	69.0	2.0	8.8	7.3	14.2	20.1	31.7	12.6	3.1
28	GBK-045446	39.0	52.9	88.4	2.6	10.3	6.9	12.3	17.9	33.3	12.8	3.4
29	GBK-045451	43.0	46.4	70.2	2.5	9.3	6.2	10.5	15.6	26.0	11.5	1.1
30	GBK-045456	41.7	51.4	75.4	2.5	10.9	6.5	12.7	18.0	29.3	12.5	1.8
31	GBK-045494	39.0	41.2	43.9	1.7	7.2	4.4	10.9	14.6	21.0	8.6	0.5
32	GBK-045497	40.3	55.7	87.0	2.4	9.6	6.3	13.0	18.1	30.0	11.8	2.4
	Mean	43.3	48.3	68.6	2.4	9.2	6.4	12.1	17.3	26.5	12.7	2.1
	Fpr	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Lsd $_{(p<0.05)}$	5.7	7.5	19.5	0.7	1.8	1.7	3.0	4.1	14.0	4.8	1.3
	Cv%	8.0	9.5	17.4	16.6	12.3	16.6	15.2	14.6	32.3	23.3	19.4

Fpr – F probability, LSD- Least significant difference, ns- not significant, DTF- days to 50% flowering, SPAD- chlorophyll content, PH- plant height (cm), SG- stem girth (cm), NPB- number of primary branches, LL- single leaf length, LW- leaf width, SLA- single leaf area (cm^2), NLPP- number of leaves per plant, NPP- number of pods per plant, SYPP- seed yield per plant.

4.6.2 SPAD value

The SPAD values measured across the 32 spider plant accessions in the field ranged from 21.1 to 56.8 (Table 4.6a). Accession number GBK-040606 recorded the highest value at 56.8 while accession number 2232 had the lowest value at 21.1 with a mean of 50.1. In the glasshouse, accession number 2299 recorded the lowest SPAD value at 11.4 while accession number GBK-027212 recorded the highest value at 60.3 (Table 4.6b) with a mean of 48.3. It was revealed that accessions grown in the field had higher SPAD values than accessions grown in the glasshouse. In both experiments, the SPAD values of South African accessions were lower than the SPAD values of the Kenyan accessions.

4.6.3 Plant height

Significant ($P<0.05$) differences were observed among the study accessions in the field and glasshouse for plant heights (Tables 4.6a and 4.6b). Among the study accessions in the field, accession number GBK-027195 was the tallest with a height of 60.8 cm while accession number 2249 was the shortest with a height of 37.3 cm. On the other hand, accession number GBK032302 was the tallest in the glasshouse with a height of 102.3 cm and the shortest was accession number 2299 with a height of 7 cm. Generally for this character, glasshouse grown accessions were relatively tall with a mean of 68.6 cm compared to the field grown accessions which had a mean of 43.6 cm. Also, Kenyan accessions were taller than the South African accessions.

4.6.4 Stem girth

Stem girth is an important trait that determines lodging in plants. Significant variation ($P<0.05$) was observed among the accessions and also between the field and glasshouse experiments with

respect to this character (Appendix 6 and 17). Stem girths varied from 1.2 cm to 4.4 cm in accession number 2249 and number GBK045451, respectively, for the field grown accessions (Table 4.6a). The mean girth of the accessions was 3.1 cm with 4 accessions noted to be of wide girth. These were accession numbers GBK027195, GBK031997, GBK040606 and GBK045451 which had girths of 4.2 cm 4.2 cm, 4.0 cm and 4.4 cm, respectively. Stem girths among glasshouse grown accessions ranged from 1.1 cm for accession number 2299 to 3.1 cm for accession number 2289 with a mean of 2.4 cm. The mean stem girth in the field grown accessions was 3.1 cm higher than the mean observed in glasshouse which was 2.4 cm. The Kenyan accessions had thicker stem girths with a mean of 3.4 cm and 2.4 cm in field and glasshouse, respectively, compared to the South African accessions which had slender stem girths averaging 2.4 cm and 2.3 cm for the field and glasshouse experiments, respectively.

4.6.5 Number of primary branches

A significant ($P<0.05$) variation in the number of primary branches among the field and glasshouse grown accessions was observed (Appendix 7 and 18). In the field, the mean number of primary branches ranged from 4.0 branches for accession 2000 to 8.6 branches for accession number GBK-027195 with a mean of 6.6 branches (Table 4.6a). On the other hand, the glasshouse grown accessions had 4 branches (accession number 2299) to 12.0 branches (accession number GBK032302) per plant with an average of 9.2 branches (Table 4.6b). The average number of branches observed among the Kenyan accessions for the field grown and glasshouse grown accessions was 7 branches and 10 branches, respectively. This was higher than 6 branches and 7 branches recorded for the South African accessions in the field and glasshouse grown accessions, respectively.

Table 4.6a: Quantitative trait measurements of 32 field grown spider plant accessions from Kenya and South Africa with their minimum and maximum values

VARIATE	Minimum	Mean	Maximum	SED	P value
Days to 50% flowering	33.0	43.8	51.0	2.6	<.001**
SPAD value	21.1	50.1	56.8	3.9	<.001**
Plant height (cm)	23.5	43.6	60.8	9.0	0.003*
Stem girth (cm)	1.2	3.1	4.4	0.6	<.001**
No. of primary branches	4.0	6.6	8.6	0.7	<.001**
Leaf length (cm)	4.9	6.8	9.3	1.7	NS
Leaf width (cm)	9.4	13.3	17.7	3.2	NS
Single leaf area (cm^2)	7.0	9.7	13.1	2.4	NS
No. of leaves per plant	14.7	52.0	87.0	16.5	0.015*
No. of pods per plant	4.3	28.6	52.9	12.0	0.006*
Seed yield per plant (g)	0.7	3.0	5.3	0.9	<.001**

** = highly significant, * = Significant, NS= Not significant. SED = Standard error of difference. P value = significance level test. Data are means of three replications of three plants each for the 32 spider plant accessions.

Table 4.6b: Quantitative trait measurements of 32 glasshouse grown spider plant accessions from Kenya and South Africa with their minimum and maximum values

VARIATE	Minimum	Mean	Maximum	SED	P value
Days to 50% flowering	35.0	43.3	56.0	2.8	<.001**
SPAD value	11.4	48.3	60.3	3.8	<.001**
Plant height (cm)	7.0	68.6	102.3	9.8	<.001**
Stem girth (cm)	1.1	2.41	3.1	0.3	<.001**
No. of primary branches	4.0	9.2	12.0	0.9	<.001**
Leaf length (cm)	1.7	6.4	8.9	0.9	<.001**
Leaf width (cm)	4.2	12.1	15.6	1.5	<.001**
Single leaf area (cm^2)	5.8	17.3	22.3	2.1	<.001**
No. of leaves per plant	10.0	26.5	63.0	7.0	<.001**
No. of pods per plant	5.0	12.7	30.7	2.4	<.001**
Seed yield per plant (g)	0.5	2.1	3.5	0.6	<.001**

** = highly significant * = Significant, NS= Not significant. SED = Standard error of difference. P value = significance level test. Data are means of three replications of three plants each for the 32 spider plant accessions.

4.6.6 Leaf length

There were no significance differences among the field study accessions for the leaf lengths (Table 4.6 a). However, significant differences were observed for the glasshouse grown accessions (Table 4.6b) which ranged from 1.7 cm for accession 2299 to 8.9 cm for accession 2249 with a mean of 6.4 cm. (Tables 4.5b and 4.6b).

4.6.7 Leaf width

Leaf widths showed no significance differences among the field grown accessions (Table 4.6a). In the glasshouse grown accessions, significant variations were recorded with leaf widths ranging from 4.2 cm to 15.6 cm for accession numbers 2299 and 2289, respectively, with a mean of 12.1 cm (Table 4.6b).

4.6.8 Leaf area

In the field experiments, there was no significant difference among the accessions for leaf area (Table 4.6a). Leaf area among the accessions ranged from 7 cm^2 in accession GBK045446 to 13.1 cm^2 in accession GBK027212 with a mean of 9.7 cm^2 (Table 4.5b). In the glasshouse experiments, there were highly significant ($P<0.01$) differences among the accessions in leaf area (Table 4.5b). Accession 2289 had the highest leaf area of 22.3 cm^2 while accession 2299 had the lowest leaf area of 5.8 cm^2 (Table 4.6b). Generally, accessions grown in the glasshouse recorded a higher leaf area with a mean of 17.3 cm^2 than accessions grown in the field which had a mean of 9.7 cm^2 (Tables 4.6a and 4.6b). In the field experiments, the Kenyan accessions had a higher leaf area of 10 cm^2 than the South African accessions which had an average of 9.1 cm^2.

4.6.9 Number of leaves per plant

Significant ($P<0.05$) variation was detected in number of leaves per plant among the Kenyan and South African accessions. In the field grown accessions, the number of leaves per plant ranged from as low as 15 to as high as 87 for accessions 2249 and 1959, respectively, with a mean of 52 (Table 4.6a). The average number of leaves per plant for the Kenyan accessions was a high of 56 compared to a low of 42 for the South Africa accessions. As for the glasshouse grown accessions, the number of leaves per plant ranged from 10 to 63 for accessions 2299 and 1959, respectively, with a mean of 26 (Table 4.6b). Kenyan accessions grown in the glasshouse had a lower number of leaves with a mean of 25 leaves than the South African accessions with a mean of 31 leaves per plant. Two accessions in the field, accession 2000 and 2249, and two accessions in the glasshouse accession 2279 and 2299, had less than 20 leaves per plant.

4.6.10 Number of pods per plant

Generally, the Kenyan accessions had more pods per plant than the South African accessions in the field and glasshouse grown accessions (Tables 4.5a and 4.5b). The number of pods per plant were significantly ($P<0.05$) different from each other ranging from a low of 4 pods to a high of 53 pods per plant for accessions 2289 and GBK031990, respectively, in the field (Table 4.6a). In the glasshouse, the number of pods per plant was low with an average of 13 pods per plant compared to the average number of pods in the field of 29 pods per plant. The lowest pod number per plant was observed in accession 2299 with 5 pods per plant while the highest number of pods per plant was seen in accession 1959 with 31 pods per plant. It was also observed that field grown accessions had higher pod counts per plant than the glasshouse grown accessions.

4.6.11 Seed yield per plant

There was a significant ($P<0.05$) variation in seed yield per plant among the accessions in the field and glasshouse grown accessions (Appendix 13 and 14). Seed yield per plant in the field ranged from 0.7g for accession 2241 to 5.3g for accession GBK043261 with a mean yield of 3.0g (Table 4.6 a). Three accessions were fairly distinguishable in terms of high seed yield; accessions GBK-043261, GBK-031996, GBK-045446 with yield of 5.3g, 5.2g and 5.1g, respectively (Table 4.5a). Seed yield per plant for the glasshouse grown accessions varied from 0.5g for accession GBK045494 to 3.5g for two accessions GBK031996 and GBK043261 (Table 4.5b). The mean seed yield per plant was 2.1g (Table 4.6b). Field experiments had high seed yields per plant than the glasshouse experiments (Table 4.6a and 4.6b). Generally, Kenyan accessions produced more seeds per plant (3.7g and 2.5g) than the South African accessions (1.0g and 1.0g) for the field and glasshouse grown accessions, respectively.

4.7. Correlation among the traits

Results from Table 4.7a of field grown accessions showed significant negative (-0.17) correlations between days to 50% flowering and number of leaves per plant. However, for the glasshouse grown accessions (Table 4.7b), correlations between days to 50% flowering and the number of leaves per plant was positive (0.12) but not significant.

Table 4.7a: Correlation table for the quantitative traits in combined seasons recorded for accessions grown in the field

Traits	DTF	SLA	LL	LW	NLPP	NPP	NPB	PH	SYPP	SPAD	SG
Days to 50% flowering	-										
Single Leaf area (cm^2)	-0.02	-									
Leaf length (cm)	-0.03	0.99*	-								
Leaf width (cm)	0.00	0.98*	0.94*	-							
No of leaves per plant	-0.17	-0.04	-0.03	-0.05	-						
No of pods per plant	-0.21	0.10	0.12	0.07	0.69*	-					
No of primary Branches	0.33	0.20	0.18	0.21	0.35	0.26	-				
Plant height (cm)	-0.22	0.08	0.08	0.08	0.52*	0.64*	0.51*	-			
Seed yield per plant (g)	0.16	0.11	0.11	0.11	0.21	0.36	0.33	0.42	-		
SPAD value	-0.13	0.07	0.08	0.05	0.34	0.46	0.38	0.61*	0.52*	-	
Stem girth (cm)	-0.01	-0.03	-0.02	-0.04	0.59*	0.54*	0.48	0.71*	0.39	0.54*	-

*Correlation is significant at the P>0.05 level (1-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, NPP- number of pods per plant, PH- plant height (cm), SPAD- soil plant analysis development, SG- stem girth (cm) SYPP- seed yield per plant (g).

Table 4.7b: Correlation table for the quantitative traits in combined seasons recorded for accessions grown in the glasshouse

Traits	DTF	SLA	LL	LW	NPB	NLPP	NPP	PH	SYPP	SPAD	SG
Days to 50% flowering	-										
Single Leaf area (cm^2)	-0.65	-									
Leaf length (cm)	-0.58	0.95*	-								
Leaf width (cm)	-0.66	0.99*	0.90*	-							
No of primary Branches	-0.51	0.52*	0.48	0.53*	-						
No of leaves per plant	0.12	0.03	0.10	0.00	-0.09	-					
No of pods per plant	-0.06	0.13	0.21	0.10	0.18	0.57*	-				
Plant height (cm)	-0.58	0.58*	0.51*	0.59*	0.80*	-0.15	0.07	-			
Seed yield per plant (g)	-0.54	0.45	0.42	0.46	0.60*	-0.16	0.06	0.58*	-		
SPAD value	-0.78	0.70*	0.65*	0.70*	0.72*	0.03	0.24	0.74*	0.65*	-	
Stem girth (cm)	-0.06	0.41	0.45	0.37	0.35	0.29	0.28	0.34	0.22	0.32	-

*Correlation is significant at the P>0.05 level (1-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, NPP- number of pods per plant, PH- plant height (cm), SPAD- soil plant analysis development, SG- stem girth (cm) SYPP- seed yield per plant (g).

Correlations between leaf chlorophyll (SPAD) and the number of leaves per plant for both the field and glasshouse grown accessions showed positive (0.34 and 0.03, respectively) correlation values (Table 4.7a and 4.7b). A strong positive (0.52) correlation was also observed between plant height and the number of leaves per plant for the field grown accessions. In the glasshouse grown accessions, a significant negative (-0.15) correlation between the plant height and leaf count (Table 4.7b).

In the field and glasshouse grown accessions, correlation results between stem girths and number of leaves per plant was positive. This shows that accessions with wider stem girths had many leaves per plant (Tables 4.7a and 4.7b). A strong positive (0.35) correlation was observed between the number of branches per plant and the number of leaves per plant (Table 4.7a) in the field. Contrary to this finding, glasshouse grown accessions recorded a slightly negative (-0.09) correlation between the number of primary branches and the leaf count per plant.

Correlations between single leaf area and number of leaves per plant in the field (Table 4.7a) recorded a significant negative (-0.05) value. Accessions with high area per leaf were observed to have few leaves per plant. However, correlations in the glasshouse recorded a slight positive (0.03) relationship between the leaf area and number of leaves per plant (Table 4.7b).

Results from Tables 4.7a and 4.7b for field and glasshouse grown accessions, showed a strong positive (0. 69 and 0.57 respectively) correlations between the number of leaves per plant and the number of pods per plant.

Correlations between seed yield per plant and the number of leaves per plant in the field showed a positive (0.21) value (Table 4.7 a). Similarly, a positive (0.03) correlation value was observed for the glasshouse grown accessions.

CHAPTER 5: DISCUSSION

All the 32 spider plant accessions planted emerged within the range of those reported by Ekpong (2009). These accessions took between three and five days to emerge from the soil media after a pre-germination period of three days. Kenyan accessions readily germinated but it was noticed that the South African accessions had an extended germination period probably because seeds were stored in the genebank for a long time (more than 5 years) since collection and their viability was reduced. Physiologically mature seeds are either dormant or nondormant and would germinate once the dormancy is broken or optimum conditions are provided (Ochuodho and Modi, 2007).

The Food and Agriculture Organization FAO (1995) descriptor describes three main growth habits in spider plants. These are erect, semi-erect and prostrate. However, in this study only erect and semi-erect growth habits were found as was also observed by Chigumira (2004). This may be attributed to the fact that the FAO report was based on assessment of larger number of spider plant accessions collected from many parts of the world as compared to this study where the assessment was based on only a small number of spider plant accessions from Kenya and South Africa. Growth habit is very important in the cropping system of spider plant and an influential character in harvesting of the plant (Mnzava, 1997). The semi-erect types could be used by peasant farmers in mixed cropping while the erect types are good for intercrop adaptability (Masuka and Mazarura, 2012).

The study revealed that most accessions had white flowers both in the field (15 white, 10 pink, 7 purple) and in the glasshouse (14 white, 7 pink, 11 purple). Masuka and Mazarura (2012) made a

similar observation on 4 accessions they studied. Three (3) accessions had white flowers while one (1) accession had purple flower. The results of the present study showed all three flower colours documented by FAO (1995). In contrast, studies by AVRDC (2009), reported additional flower colours such as mauve-pink, lilac-pink and violet. This may be attributed to varying environmental factors such as temperature, nutrients and stress where the evaluations were conducted (Chweya and Mnzava, 1997).

The polymorphism in colour observed in flowers, stems and petioles which ranged from violet, pink and purple is due to the accumulation of anthocyanins in the plant tissues. Anthocyanins are glycosides and acylglycosides of anthocyanids and belong to the general class of flavanoids (Dasgupta and De, 2007). These plant pigments are responsible for a variety of red, blue, and purple colours in fruits and vegetables. These pigments which occur in flowers are very useful to plants and have been reported to be a key component of pollination and subsequent fruit production (Dasgupta and De, 2007). These plant pigments have anti-inflamatory, antitumor, antioxidant, and antihepatotoxic properties in human (Opole *et al.,* 1995), hence providing the vital health promoting bioactive compounds when spider plant is consumed. The accumulation of these anthocyanins in plant tissues are environmentally controlled by factors such as temperature, nutrients, and stress. Hence ability by the spider plant ecotypes to grow under diverse environmental conditions is enhanced in those ecotypes that have anthocyanin accumulation on both stems and petioles rather on either stem or petiole only, or even no accumulation on both the plant parts (K'opondo, 2011).

The study showed that green stem accessions were mostly glabrous in stems and petioles compared to the purple stem accessions which had profuse pubescence on stems and petioles (Tables 4.1a and 4.1b). This observation was in conformity with the findings of Makgakga, (2011), who reported that purple stemmed plants are usually more hairy than green stemmed plants. The Kenyan purple stem accessions were significantly different from most of the South African accessions except for South African accessions 1959, 2289 and 2232. According to Imbamba (1976), leaf, stem and petiole hairs are mostly pronounced in plants growing in the field. Plants derive several advantages from these hairs. They interfere with the feeding by herbivores due to stiffness and irritability to the palate (Subhash, 2010). In windy locations, hairs break up the flow of air across the plant surface, reducing transpiration. Dense coatings of hairs reflect sunlight, protecting the more delicate tissues underneath in hot, dry and open habitats (Subhash, 2010; Rajendru *et al*., 1996). This explains why most of the study accessions in the field had more profuse hairs on leaves, stems and petioles than accessions grown in the glasshouse. The more profuse Kenyan accessions are thus better adapted with regards to this character than to the glabrous South African accessions.

The qualitative characters showed high levels of diversity indices with more than three phenotypic classes for both field and glasshouse grown accessions. Growth habit, flower colour and leaf shape showed high diversity indices of more than 0.500 i.e. 0.998, 0.977, and 0.997, respectively, for field grown accessions and 0.999, 0.983 and 0.997, respectively, for glasshouse grown accessions. These results concurred with those of Ayana and Bekele (1998) and Persson *et al*. (2006) who observed that characters having three or four phenotypic classes, generally have higher diversity indices than characters with less than three classes. Another reason for the

high levels of diversity of spider plant accessions in the current study might be due to the fact that these accessions were found in relatively complex and heterogeneous ecologies (farm, forest and wild) and the non-uniform climatic conditions (Chweya, 1997; Chayamarit, 1993).

Cluster analysis substantiated the existence of diversity among the 32 spider plant accessions for the morphological traits studied. The clustering pattern shows that accessions from Kenya were genetically distant from each other and from the South African accessions. The clustering patterns based on UPGMA and principal coordinate analysis were similar and revealed clear cut groupings based on collection regions (i.e. Kenyan and South African accessions) for the field experiment. This indicates a difference in the genetic makeup of the two accession groupings (cluster I and II). Compared to accessions grown in the field, the clustering analysis of glasshouse grown accessions failed to completely distinguish the accessions into clear cut groups but showed them as four groups with South African accessions in between Kenyan accessions. Findings of this study also revealed that qualitative traits which included flower colour, stem hairiness, petiole hairiness, leaf hairiness and leaf colour scored differently in the field and in glasshouse study accessions. Several authors have concluded that additive gene action is responsible for much of the genetic variation of qualitative traits (Lal *et al*., 1976; Mak and Yap, 1980; Zaveri *et al*., 1980). Other reports, however, indicate that action by non-additive genes and interactions between genotype and environment are important in some instances for the variations (Singh and Rachie, 1985). This may explain the observed differences in the field and in the glasshouse. The findings of this study demonstrate that morphological traits may not be reliable when characterizing spider plants under different environments.

Most accessions used in this study for the field and glasshouse experiments were grouped according to their geographic origin. For example, cluster I grouping of the field grown accessions had South African accessions from Mpumalanga clustered together and those from Northern Province also clustered together. Cluster II of field grown accessions had Kenyan accessions mainly grouped together such as sub-cluster 'c' with western accessions grouped closely. Findings of this study also revealed the existence of little variation among accessions from the same collection areas for most characters. This close resemblance indicates the possibility that the accessions in each collection region may have come from similar genetic background. This could also be attributed to possibility of seed trade among the farmers from the regions that share a border. A close relationship has also been detected among spider plant genotypes following the evaluation of the variability in seed proteins among them (K'opondo *et al*., 2009). The close relationship reported among spider plant accessions may also be due to it being a self-pollinated crop (Omondi, 1990). Furthermore, a big genetic distance was observed among the accessions from the two major geographical regions (Kenya and South Africa), presenting a great possibility for the development of suitable varieties for the various agro-ecological zones of Africa by making use of the available potential of the germplasm.

Multivariate principal component analysis has been previously used to identify the most important traits for characterizing genotypes and accessions of different species including pigeonpea (Upadhyaya *et al.,* 2007), sweetpotato (Yada *et al*., 2010) and wheat (Al Khanjari *et al.,* 2008). In the present study, PCA identified six traits (stem colour, stem hairiness, petiole colour, petiole hairiness, leaf hairiness and leaf shape) for the field grown accessions and five characters (stem colour, stem hairiness, petiole hairiness, leaf shape and number of leaflets per

leaf) for the glasshouse grown accessions. Future collections and characterization to broaden the Kenyan and South African spider plant resource base could focus on the characters identified above.

Green stemmed plants flowered earlier than purple stemmed accessions. Most South African accessions were green stemmed and flowered earlier than mostly purple stemmed Kenyan accessions. This is the case with the findings of Masuka and Mazurura (2012) who found that green stemmed Zimbabwean morphs flowered earlier than the purple stemmed Kenyan morphs. The purple stemmed Kenyan accessions also produced higher number of pods per plant than the green stemmed South African accessions. Late flowering enables a genotype to have a longer vegetative phase during growth period (Omondi, 1990). The relatively high coefficient of variations observed are an indication that the accessions studied have relatively high variability in number of days to flowering and thus have the potential to flower, pod and mature late. Indications are that the late flowering accessions identified in the present study would be very useful to adopt because of their ability to maintain green leaves for long. From the correlation results between days to flowering and number of leaves per plant, it implies that the longer the days to flowering the higher the number of leaves. The late flowering accessions identified in 2013 and 2014 can be used as good sources for selection and breeding for extended harvesting of spider plant. Water stress can however trigger flowering even at the seedling stages, therefore distorting the count of number of days to flowering (Chayamarit, 1993).

The number of pods in a plant is a trait that indicates the quantity of seeds. Accessions GBK031990 and 1959 produced more pods per plant than the other accessions in the field and

glasshouse, respectively. This confirmed the findings of K'Opondo (2011) who found that purple stemmed plants generally produced more fruits than green stemmed plants. The strong positive correlations between number of leaves per plant and the number of pods per plant suggests that an increase in leaf count number may increase photosynthetic area leading to increased translocation of photosynthates into pod sinks.

The purple stemmed plants were generally taller than the green stemmed plants. On the other hand, it is highly conceivable that accessions stem colour cannot be globally related to stem height (Mnzava, 1997). The range of plant heights of the field grown accessions coincided with those recorded by Makgakga (2011) in South Africa who observed a variation ranging from 25-60 cm. It is worth noting that plant height is a key trait in reflecting drought escape, biomass apportioning and yield. Tall accessions in field and glasshouse had wider stem girths and were generally easy to harvest. The positive correlation between stem girths and the number of leaves per plant can be attributed to the wider stemmed plants translocating more nutrients from the soil to the leaves and photosynthates to roots and other plant parts.

Shorter statured vegetable plants are often preferred since they translocate materials faster, escape drought easily (Chigumira, 2004). The number of leaves per plant which is the most vegetative part in spider plant is associated with biomass yield and productivity of the plant. Accessions with large single leaf areas had few leaves per plant but with high SPAD value. These accessions were tall plants and had few branches with majority being Kenyan accessions. However, the fact that accessions grown in the glasshouse had large leaves with many leaf counts could be attributed to the uniform soil nutrients in the potting bags used as opposed to the

field where there is high heterogeneous nutrient distribution and availability for uptake by the plants. Kenyan accessions had higher SPAD values than South African accessions. The SPAD value is proportional to the amount of chlorophyll concentration present in the sampled leaf (Jarvis, 2008). Higher SPAD values indicate greater absolute chlorophyll and N concentration in the leaf materials. This suggests that Kenyan accessions had more leaf N than the South African accessions.

The number of primary branches is a measure of crop's resilience under water limited conditions and reflects its vegetative productivity (Nkouannessi, 2005). This was observed in most of the short statured South African accessions which had small leaf areas with many leaves per plant. The small leaves observed in the South African accessions may explain the low seed yield per plant. In the same way, accessions with many branches had many leaf counts. However, in the glasshouse the negative correlation result observed could be due to plants exposure to water loss by the warm glasshouse temperatures (27 ^{o}C) and hence the plants developed few leaves to minimize this loss.

In general, most agronomic traits showed variation, and especially for important characters such as days to flowering, SPAD value, plant height, stem girth, number of leaves per plant, number of pods per plant and seed yield per plant. The variations shown among the Kenyan and South Africa spider plant accessions studied could partly be attributed to different evolutionary pathways of development among the accessions. It is suggested that while genes interact with other genes, the way they are expressed is influenced by their environment (Phillips, 2006). The variations could also be due to the selection pressure being effected by farmers especially in

Kenya for those characters they consider to be of importance to them, as they continue putting spider plant under domestication through cultivation (K'Opondo, 2011); whereas in South Africa, domestication is still largely confined to research stations (DAFF, 2010).

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

A range of observations were made in the current analyses of phenotypic diversity of spider plant using both their qualitative and quantitative traits. Relatively high level of dissimilarity was observed among the accessions for most of the traits evaluated, especially accessions from the two different countries. This indicates potential for genetic improvement of the crop through selection and cross breeding. However, a comparatively high level of similarity was revealed between accessions from the same region for most of the characters studied. The use of materials from different geographical origins in any cross breeding programme aiming to develop suitable varieties with specific characters is therefore strongly recommended. This would avoid the use of material with a similar genetic background, as well as avoiding spending time, money and other resources on materials not having the best chance to produce the best result. For example, the use of accession GBK027212 (from Kenya) in a breeding programme aiming to improve accession 1959 (from South Africa) for leaf size and the yield of seeds per plant would have a better chance of success than the use of accession 2289 from the same region. This study also revealed that some qualitative and quantitative traits discriminated more efficiently between the accessions than others. The qualitative traits were stem colour, and stem hairiness, petiole colour, petiole hairiness, leaf hairiness, leaf shape and number of leaflets per leaf. Quantitative traits on the other hand were days to flowering, SPAD value, single leaf area, number of primary branches, number of leaves per plant, number of pods per plant and seed yield per plant. The identified agro-morphological characters had a high discrimination capacity and could be suitable in undertaking genetic diversity studies based on morphological traits.

This study identified 12 accessions namely 1959, 2000, 2279, 2289, GBK027195, GBK027212, GBK031990, GBK031996, GBK032302, GBK040606, GBK043261 and GBK045451 that were different from the other accessions for important traits. They were superior in flowering, SPAD content, leaf area, number of primary branches, number of leaves per plant, number of pods per plant and seed yield per plant. They can therefore be used for future spider plant improvement programmes through cross breeding. Accordingly, future spider plant collections should take into account all levels of variation from the important characters identified. It is concluded that the 12 selected accessions may be useful for further breeding programmes in view of variety release.

6.2 Recommendations

The study recommends that farmers should adopt and increase production of spider plant ecotypes found to have good morpho-agronomic traits rather than rely only on the commercially available Kenyan Kitale spider plant ecotype. These ecotypes are from Loopspruit (1959), Gemsbokspruit (2000), Arthurstone (2279) and Roikoppen (2289) areas of South Africa and from Elgeyo Marakwet (GBK027195; GBK027212; GBK040606), Busia (GBK031990; GBK031996), Mbale (GBK032302), Nandi (GBK043261), and Kiambu (GBK045451) counties in Kenya. However, further characterization trials should be conducted by growing the plants in multiples sites under different environments to ascertain the differences observed in the field and glasshouse. This study also recommends that molecular markers such as simple sequence Repeats (SSRs) be used to supplement this work by identifying the polymorphism that is not due to environmental conditions. In contrast to morphological traits, molecular markers can reveal abundant difference among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation and management, and untouched by environmental influence.

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APPENDICES

Appendix 1: Chemical characteristics of sampled field soil

Chemical property	Value
pH	5.30
Organic carbon (%)	3.00
Total nitrogen (%)	0.28
Phosphorous (ppm)	17.20
Sodium (cmol/kg)	0.30
Potassium (cmolL/kg)	1.56
Magnesium (cmol/kg)	2.40
Calcium (cmol/kg)	4.66
CEC (cmol/kg)	17.50

Appendix 2: Weather conditions at Kabete field station between September 2013 and May 2014 cropping season

	Temperature (^{o}C)		Relative humudity (%)	Rainfall (mm)
Month	Mean max	Mean min	Mean	Total
September	24.5	12.2	64.6	25.9
October	25.6	13.3	54.3	76.0
November	23.6	14.5	71.8	128.4
December	22.9	14.1	74.3	163.2
January	25.1	13.4	61.4	30.2
February	25.1	14.3	66.9	146.5
March	24.3	14.2	64.8	154.7
April	23.0	14.2	71.4	81.7
May	23.5	14.8	68.3	72.8

Source: Kenya Meteorological Department, Kabete Agro-met Station (June 2014).

Appendix 3: Glasshouse conditions at Kabete field station between September 2013 and May 2014 cropping season

Month	Mean temperature (^{o}C)	Mean relative humidity (%)
September	28.5	55.1
October	29.6	46.3
November	24.4	64.6
December	25.1	63.0
January	27.9	57.6
February	29.2	53.9
March	28.2	54.6
April	25.1	62.8
May	26.4	60.1

Source: Kenya Meteorological Department, Kabete Agro-met Station (June 2014).

Appendix 4: Analysis of variance (ANOVA) table for the days to flowering for the field grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	189.58	94.79	9.05	
Accession	31	1136.7	36.67	3.5	<.001**
Residual	62	649.08	10.47		
Total	95	1975.3			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 5: Analysis of variance (ANOVA) table for the SPAD value for the field grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	331.47	165.74	7.3	
Accession	31	4513.9	145.61	6.41	<.001**
Residual	62	1407.4	22.7		
Total	95	6252.7			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 6: Analysis of variance (ANOVA) table for the plant height for the field grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	1354.2	677.1	5.62	
Accession	31	8425.4	271.8	2.25	0.003*
Residual	62	7472.7	120.5		
Total	95	17252			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 7: Analysis of variance (ANOVA) table for the stem girth for the field grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	8.2181	4.1091	7.54	
Accession	31	52.323	1.6878	3.1	<.001**
Residual	62	33.775	0.5448		
Total	95	94.316			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 8: Analysis of variance (ANOVA) table for the number of branches for the field grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.9797	0.4899	0.6	
Accession	31	123.56	3.986	4.92	<.001**
Residual	62	50.261	0.8107		
Total	95	174.81			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 9: Analysis of variance (ANOVA) table for the leaf length for the field grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	14.375	7.187	1.61	
Accession	31	125.16	4.037	0.9	0.611ns
Residual	62	276.62	4.462		
Total	95	416.15			

***** = significant, ****** = highly significant, ns = not significant, F pr = F probability value

Appendix 10: Analysis of variance (ANOVA) table for the leaf width for the field grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	31.83	15.91	1.04	
Accession	31	518.83	16.74	1.09	0.373ns
Residual	62	948.22	15.29		
Total	95	1498.9			

***** = significant, ****** = highly significant, ns = not significant, F pr = F probability value

Appendix 11: Analysis of variance (ANOVA) table for the leaf area for the field grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	22.933	11.467	1.37	
Accession	31	255.65	8.247	0.98	0.508ns
Residual	62	520.08	8.388		
Total	95	798.66			

***** = significant, ****** = highly significant, ns = not significant, F pr = F probability value

Appendix 12: Analysis of variance (ANOVA) table for the number of leaves per plant for the field grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	3446.1	1723.1	4.23	
Accession	31	24223	781.4	1.92	0.015*
Residual	62	25268	407.5		
Total	95	52937			

***** = significant, ****** = highly significant, ns = not significant, F pr = F probability value

Appendix 13: Analysis of variance (ANOVA) table for the number of pods per plant for the field grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	2087.2	1043.6	4.81	
Accession	31	14141	456.2	2.1	0.006*
Residual	62	13446	216.9		
Total	95	29674			

***** = significant, ****** = highly significant, ns = not significant, F pr = F probability value

Appendix 14: Analysis of variance (ANOVA) table for seed yield per plant for the field grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	18.948	9.474	7.13	
Accession	31	238.4	7.69	5.78	<.001**
Residual	62	82.433	1.33		
Total	95	339.78			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 15: Analysis of variance (ANOVA) table for the days to flowering for the glasshouse grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	190.08	95.04	7.84	
Accession	31	2240.24	72.27	5.96	<.001**
Residual	62	751.92	12.13		
Total	95	3182.24			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 16: Analysis of variance (ANOVA) table for the SPAD value for the glasshouse grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	159.11	79.56	3.74	
Accession	31	8422.29	271.69	12.78	<.001**
Residual	62	1318.32	21.26		
Total	95	9899.72			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 17: Analysis of variance (ANOVA) table for the plant height for the glasshouse grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	307	153.5	1.07	
Accession	31	78445.7	2530.5	17.67	<.001**
Residual	62	8879	143.2		
Total	95	87631.8			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 18: Analysis of variance (ANOVA) table for the stem girth for the glasshouse grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.4366	0.2183	1.36	
Accession	31	13.0705	0.4216	2.63	<.001**
Residual	62	9.9419	0.1604		
Total	95	23.4491			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 19: Analysis of variance (ANOVA) table for the number of branches for the glasshouse grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	2.414	1.207	0.96	
Accession	31	352.583	11.374	9.01	<.001**
Residual	62	78.253	1.262		
Total	95	433.25			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 20: Analysis of variance (ANOVA) table for the leaf length for the glasshouse grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	1.124	0.562	0.49	
Accession	31	242.513	7.823	6.86	<.001**
Residual	62	70.723	1.141		
Total	95	314.36			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 21: Analysis of variance (ANOVA) table for the leaf width for the glasshouse grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	6.505	3.252	0.96	
Accession	31	741.587	23.922	7.09	<.001**
Residual	62	209.094	3.372		
Total	95	957.185			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 22: Analysis of variance (ANOVA) table for the leaf area for the glasshouse grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	10.394	5.197	0.81	
Accession	31	1486.812	47.962	7.45	<.001**
Residual	62	399.001	6.436		
Total	95	1896.207			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 23: Analysis of variance (ANOVA) table for the number of leaves per plant for the glasshouse grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	234.65	117.32	1.61	
Accession	31	8677.29	279.91	3.83	<.001**
Residual	62	4532.02	73.1		
Total	95	13443.96			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 24: Analysis of variance (ANOVA) table for the number of pods per plant for the glasshouse grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	51.023	25.512	2.92	
Accession	31	1812.081	58.454	6.68	<.001**
Residual	62	542.153	8.744		
Total	95	2405.257			

***** = significant, ****** = highly significant, F pr = F probability value

Appendix 25: Analysis of variance (ANOVA) table for seed yield per plant for the glasshouse grown accessions during the seasons of 2013 and 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	10.5101	5.2551	8.55	
Accession	31	82.0592	2.6471	4.31	<.001**
Residual	62	38.1024	0.6146		
Total	95	130.6717			

***** = significant, ****** = highly significant, F pr = F probability value