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Research Article

Morphological Characterization and Selection of Spider Plant (*Cleome Gynandra*) Accessions from Kenya and South Africa

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Abstract: Characterization of selected spider plant accessions from Kenya and South Africa was performed in order to individuate those with distinct morphological traits for future improvement programs. For this purpose, thirty two accessions of spider plant, 23 sourced from Kenyan genebank and nine sourced from South African genebank, were planted at the University of Nairobi's Kabete field station, in a randomized complete block design with 3 replications. Eleven morphological traits based on modified FAO (1995) spider plant descriptors were used in characterization. Traits evaluated were 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. The scored data were analyzed using DARwin software v6 and Genstat v14. Shannon diversity index (H'), multivariate methods of principal component analysis and hierarchical clustering analyses of unweighted pair group method of arithmetic mean were assessed for all the traits. Estimates of Shannon-Weaver diversity index (H') for the morphological traits were generally high (H'>0.500). The H' index indicated inter-country diversity to be greater than the intra-country diversity. Principal component analysis identified seven important morphological traits (stem colour, stem hairiness, petiole colour, petiole hairiness, leaf hairiness, leaf shape and number of leaflets per leaf) for characterizing spider plant accessions. The hierarchical cluster analysis revealed two major clusters (Cluster I and II) for the 32 accessions grown, 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. The relatively high levels of dissimilarity revealed in this study among the accessions for traits evaluated, especially accessions from the two different countries, indicates high prospects for genetic improvement of the crop through cross breeding by using materials from different geographical origins.

Keywords: Cat's whiskers, indigenous vegetables, Principal Component Analysis (PCA), Unweighted Pair Group Method of Arithmetic Averaging (UPGMA)

INTRODUCTION

Spider plant (Cleome gynandra L.) is among the most important African Leafy Vegetables (ALVs) widely used in Africa (Schippers, 2000). Jansen van Rensburg et al. (2004) noted that indigenous leafy vegetables, which are rich in micronutrients and vitamins, could play an important role in alleviating hunger and malnutrition. Spider plant is a rich source of protein, and its leaves contain high levels of vitamins A and C, calcium, magnesium, iron, antioxidant enzymes (superoxide dismutase, catalase, peroxidases) and nonenzymatic antioxidants (ascorbic acid tocophenols, carotenoids, flavonoids and glutathione) (DAFF, 2010), making it suitable for combating malnutrition and life

style diseases especially in Sub-Saharan Africa (WHO, 2005).

Kenya and South Africa feature a range of agroecologies that represent most parts of the African continent (Nono-Womdim and Openam, 1997). They include both highlands and lowlands. In Southern Africa various ecotypes of spider plants spread from Limpopo region in South Africa to Namibia (DAFF, 2010) while in East Africa, the plants spread from the Coastal region to western regions of Kenya then to Uganda (Chweya, 1997). This signifies high diversity in spider plant populations between Kenya and South Africa. However, most of the collected spider plant accessions by the Kenyan and South African genebanks are undocumented and have not been systematically characterized in terms of their morphological and

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agronomic variability, which would be important for breeding and conservation (K'opondo et al., 2009; Maundu et al., 1999; Chweya and Mnzava, 1997). 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 few traits used in characterization (Masuka and Mazarura, 2012; K'Opondo, 2011). The studies further recommend use of more accessions and characters under field environments to characterize spider plant 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 and evaluation of phenotypic trait expressions.

The objective of this study was to evaluate the phenotypic diversity among Kenyan and South African accessions of spider plant.

MATERIALS AND METHODS

Experimental materials: Thirty two spider plant accessions collected from different eco-regions in Kenya and South Africa were used in the study. The accessions were obtained from National Genebanks of the respective countries hence no exploration work for the source of the planting materials.

Study site: The experiment was conducted at the University of Nairobi's Kabete Field station farm, Kenya (1°15'S, 36°44' E, and 1940 m above sea level). The site is located in Upper Midland Zone three with an annual rainfall of 700-1500 mm and average temperatures of 13-23°C.

Experimental design and management: Randomized complete block design was used. The experimental field was ploughed and harrowed with a tractor. The field was lined and pegged and divided into blocks replicated three times. Each accession was planted in two rows of 20 seeding holes (ten plants in a row) 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 (September 2013 to March February 2013 and March 2014 to June 2014). Accessions planted were pre-germinated for 72 hours under treatment with 0.2% Gibberellic Acid (GA) purchased from Marty Chemical (KE) supplies. Well decomposed chicken manure was applied to rows and mixed with soil before planting at 60g/line. Top dressing was done with 12 g of calcium ammonium nitrate/ per line (26%N) (80 kg/Nha). The plants were sprayed using an organophosphate insecticide duduthrin (lambdacyhalothrin-250EC), purchased from Twiga Chemicals Industries, at the rate of 65 mL/20 L 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 irrigation was applied two times, at two weeks after planting and two weeks after flower initiation.

Parameters measured: Data were collected from accessions that survived till maturity and harvest. Qualitative variables were scored on individual plant basis, using three randomly selected plants per plot. A list of modified spider plant descriptors from the Food and Agriculture Organization (FAO, 1995) was used as a guide in the selection of parameters and procedures for characterization. Qualitative characters scored included growth habit, flower colour, stem colour, stem hairiness, petiole color, petiole hairiness, leaf colour, leaf pubescence, leaf shape, leaf blade tip shape, and number of leaflets per leaf (Table 1). Dissimilarities were estimated based on Euclidean distance matrix and hierarchical clustering analyses of unweighted pair group method of arithmetic mean was performed in DARwin 5.0 software as described by Perrier and Jacquemud-Collet (2006).The Clusters relationships were displayed as a phenogram and were used to assist in grouping and selecting accessions with promising characters out of which those with high performing traits were selected. 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 phenotypic variation in the spider plants.

Table 1: Character, descriptor and codes used for characterization of qualitative traits in spider plant accessions used in the study

Character	Descriptor and code
Growth habit	Erect (2), semi-erect (4) and prostrate (6)
Flower colour	White (1), purple (2) and pink (3)
Stem colour	Green (1), pink (2), violet (3) and purple (4)
Stem hairiness	Glabrous (1), weak/sparse (3), medium (5) and profuse (7)
Petiole colour	Green (1), pink (2), violet (3) and purple (4),
Petiole hairiness	Glabrous (1), weak/sparse (3), medium (5) and profuse (7)
Leaf colour	Dark green (1), light green (2),
Leaf hairiness	Glabrous (1), weak/sparse (3), medium (5) and profuse (7)
Leaf shape	Linear (1), lanceolate (2), elliptic (3), obovate (4) and ovate (5)
Leaf blade tip shape	Acuminate (1), acute (2), obtuse (3) and cuspidate (4)
No. of leaflets per leaf	Three (1), four (2), five (3), six (4) and seven (5)

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

Table 2: 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}	Cleome gynandra	Mpumalanga	Loopspruit	SH
2	1988 ^{Za}	Cleome gynandra	Mpumalanga	Silverhills	SH
3	2000 ^{Za}	Cleome gynandra	Mpumalanga	Gemsbokspruit	SH
4	2232 ^{Za}	Cleome gynandra	Northern province	Arthurstone	SA
5	2241 ^{Za}	Cleome gynandra	Northern province	Arthurstone	SA
6	2249 ^{Za}	Cleome gynandra	Northern province	Arthurstone	SA
7	2279 ^{Za}	Cleome gynandra	Northern province	Arthurstone	SA
8	2289 ^{Za}	Cleome gynandra	Mpumalanga	Rooikoppen	SH
9	2299 ^{Za}	Cleome gynandra	Mpumalanga	Loding	SH
10	GBK-027131 ^{Ke}	Cleome gynandra	Rift valley	Elgeyo Marakwet	LH 3
11	GBK-027195 Ke	Cleome gynandra	Rift valley	Keiyo Marakwet	LM 1
12	GBK-027212 Ke	Cleome gynandra	Rift valley	Keiyo Marakwet	LH 2
13	GBK-028554 Ke	Cleome gynandra	Nyanza	Siaya	LM 1
14	GBK-031990 Ke	Cleome gynandra	Western	Busia	LH 2
15	GBK-031992 Ke	Cleome gynandra	Western	Busia	LM 3
16	GBK-031993 Ke	Cleome gynandra	Western	Busia	LM 1
17	GBK-031996 Ke	Cleome gynandra	Western	Busia	LM 1
18	GBK-031997 Ke	Cleome gynandra	Western	Busia	LM 3
19	GBK-032134 Ke	Cleome gynandra	Eastern	Makueni	UM 4
20	GBK-032253 Ke	Cleome gynandra	Rift valley	West Pokot	UM 5
21	GBK-032302 Ke	Cleome gynandra	Western	Mbale	LM 1
22	GBK-040606 Ke	Cleome gynandra	Rift valley	Elgeyo Marakwet	LH 3
23	GBK-043261 Ke	Cleome gynandra	Rift valley	Nandi	LH 1
24	GBK-043760 Ke	Cleome gynandra	Rift valley	Koibatek	LH 2
25	GBK-045408 Ke	Cleome gynandra	Western	Vihiga	LM 1
26	GBK-045426 Ke	Cleome gynandra	Western	Vihiga	LM 1
27	GBK-045436 Ke	Cleome gynandra	Nyanza	Kisumu	LM 3
28	GBK-045446 Ke	Cleome gynandra	Nyanza	Kisii	LH 1
29	GBK-045451 Ke	Cleome gynandra	Central	Kiambu	UH 1
30	GBK-045456 Ke	Cleome gynandra	Central	Kiambu	UH 1
31	GBK-045494 Ke	Cleome gynandra	Coast	Kilifi	L 2
32	GBK-045497 Ke	Cleome gynandra	Coast	Kilifi	L 2

Refers to identifier code used to identify an accession in the collection at genebank in Kenya and South Africa; Ke Refers to Kenyan accession; Ze refers to South African accession; 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

RESULTS AND DISCUSSION

The existence of morphological types among the spider plant accessions (Table 2) was evident as there were variations in forms and features of the different character traits measured and evaluated.

Growth habit: It was observed that 84.4% of the accessions studied produced an erect growth habit with 15.6% showing semi-erect growth habit (Fig. 1). Accessions with semi-erect growth habit were 1988, 2000, GBK-028554, GBK-045436 and GBK-045494.

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 two of these 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 large 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

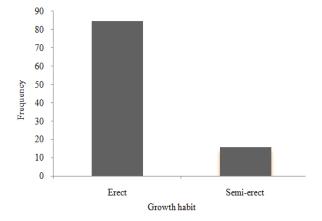


Fig. 1: Frequency distrilbution of growth habit of spider plant accessions

farmers in mixed cropping while the erect types are good for intercrop adaptability (Masuka and Mazarura, 2012). According to K'opondo (2011) the erect spider plant accessions have high reproductive efficiency while the semi-erect ones have higher total pod yield. This attribute was also observed in this study.

Flower colour and stem characteristics: The observed flower colours of the accessions used in this study are presented in Fig. 2. About 46.8% of the

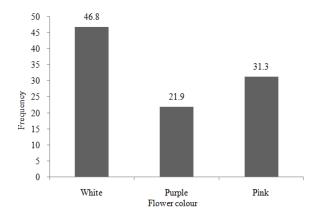


Fig. 2: Frequency distribution of flower colour of spider plant accessions

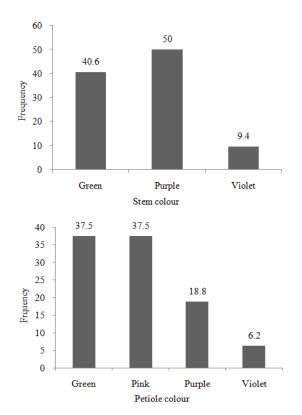


Fig. 3: Frequency distribution of stem colour and petiole colour of spider plant accessions

accessions produced white flowers, 31.3% produced pink flowers and 21.9% produced purple flowers.

Masuka and Mazarura (2012) made a similar observation on 4 accessions they studied. Three (3) accessions had white flowers while 1 accession had purple flower. The results of the present study showed all three flower colours documented by FAO (1995). In contrast, studies done by AVRDC (2009), reported additional flower colours such as mauve-pink, lilacpink and violet. This may be attributed to varying environmental factors such as temperature, nutrients and stress where the evaluations were conducted.

Stem colour of the accessions was mainly purple (50%) and green (40.6%) while only 9.4% of the stems were violet in colour (Fig. 3). Proportion of accessions with white flowers and green stems were 73.4% while those with white flowers and purple stems were 13.3%. White flowers and violet stems accounted for 13.3% of the accessions. Accessions with white flowers had mostly green stems and green petiole (66.7%) while purple flowered accessions with purple stems and purple petiole were 22.2%. Pink flowered accessions were noted to have 10% violet stems and 90% purple stems. The proportion of accessions characterized with pink petioles was 37.5%, green petioles 37.5%, purple petiole pigmentation 18.8%, and violet petioles 6.2% (Fig. 3).

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 play key role in pollination and subsequent fruit production (Dasgupta and De. 2007). These plant pigments have antiinflamatory, antitumor, antioxidant, and antihepatotoxic properties in human (Opole et al., 1995). The accumulation of these anthocyanins in plant tissues are environmentally controlled by factors such as temperature, nutrients, and heat 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).

Stem hairiness for the study accessions was mainly profuse 56.2%, while medium and glabrous accounted for 21.9% each (Fig. 4). 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%) as shown in Fig. 4.

The study revealed 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. This observation was in conformity with the findings of Makgakga (2011), who reported that purple stemmed plants are usually more glandular 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

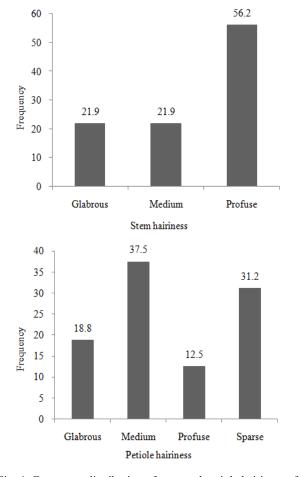
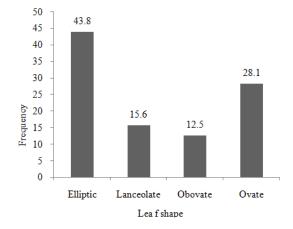


Fig. 4: Frequency distribution of stem and petiole hairiness of spider plant accessions

several advantages from these hairs. They interfere with the feeding by herbivores due to stiffness and irritability to the palate. 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, open habitats. This explains why most of the study accessions in the field had profuse hairs on leaves, stems and petioles. The more profuse Kenyan accessions are thus better adapted with regards to this character compared to the glabrous South African accessions. However, the glabrous accessions are preferred for consumption due to their higher ease of palatability.

Leaf characteristics: The basal leaf shape for the study accessions varied from elliptic (43.8%) to ovate (28.1%) to lanceolate (15.6%) and obovate (12.5%) as shown in Fig. 5. Leaf blade tip shapes for the study accessions ranged from obtuse (43.8%), acute (28.1%), acuminate (15.6%), and cuspidate (12.5%). while leaf colour was either dark green (50%) or light green (50%).



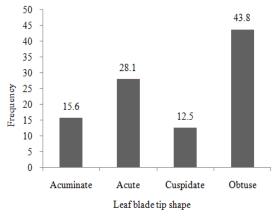


Fig. 5: Frequency distribution of leaf shape and of leaf blade tip shape of spider plant accessions

The proportion of leaf hairiness of the study accessions ranged from sparse (50%), glabrous (28.1%), medium (18.8%), and profuse (3.1%) (Fig. 6). The number of leaflets per leaf were mostly five (81.3%) but three (3.1%), six (6.3%) and seven (9.4%) leaflets per leaf were also noted in other accessions (Fig. 6).

Cluster analysis: The phenogram generated using eleven morphological descriptors based on Euclidean Distance Coefficient and Unweighted Pair Group Method of Arithmetic mean (UPGMA) clustering method (Fig. 7) clearly showed the phenetic relationship among the accessions. The phenogram separated the 32 accessions into two major clusters (Cluster I and II) by grouping accessions sharing close phenotypic similarities into distinct clusters. Cluster I had seven accessions while cluster II had 27 accessions. Cluster I had South African accessions while cluster II mainly had Kenyan accessions and two South African accessions (2289 and 1959). Within country variation was observed for both the Kenvan and South African accessions with accessions collected from the same region closely related. Cluster I shows a close relationship between South African accessions 2249 and 2232 which were collected from Northern Province. Likewise, accessions 1988 and 2000 which

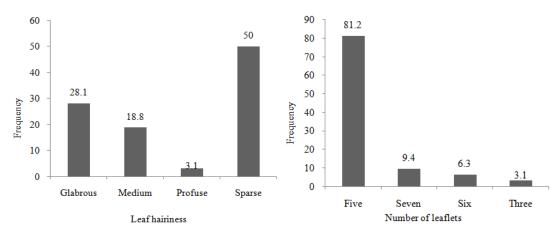


Fig. 6: Frequency distribution of leaf hairiness and of number of leaflets/leaf of spider plant accessions

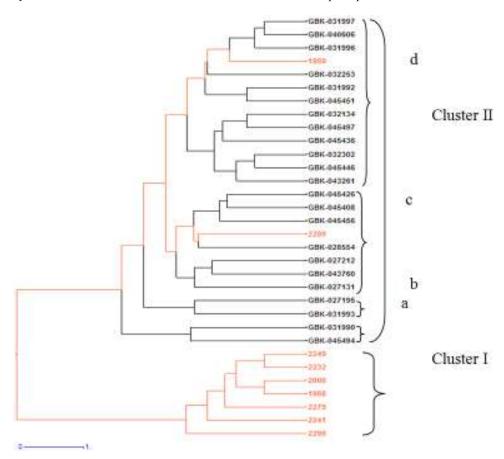


Fig. 7: UPGMA cluster analysis phenogram showing the relationship among the 32 spider plant accessions grown in the field

were collected from Mpumalanga region had similar clades suggesting a close resemblance in their qualitative 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 Fig. 7. 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. In sub-cluster 'c' of three accessions originating from one node, two accessions GBK045426, GBK05408 from western region had a closer connection 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 than to accession GBK040606 which was collected from Rift valley region. However, in cluster II

Table 3: Eigenvalues^a, eigenvectors^b and percentage of variation explained by the first six principal components for the 32 spider plant accessions

Principal component

	Fineipal component						
Qualitative character	1	2	3	4	5	6	
Variation explained (%)	70.92	6.860	6.220	4.440	3.090	2.650	
Eigenvalue	17.16	1.660	1.510	1.080	0.750	0.640	
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	°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	

^aEigenvalues indicate the amount of variance explained by each principal component; ^bEigenvectors are the weights in a linear transformation when computing principal components; ^cValues in bold indicate the most relevant descriptors that contributed most to the particular component

sub-cluster 'b', accessions GBK031993 and GBK027195 which were from Western and Rift valley regions respectively expressed a close connection despite being collected from the two different regions. This could be attributed to the possibility of seed trade among the farmers from the two regions which border one another.

The clustering pattern shows that accessions from Kenya were genetically more distant from each other and from the South African accessions. This indicates a difference in the genetic makeup of the two accession groupings (K'opondo, 2011). The findings also reveal that most accessions used in this study were grouped according to their geographic origin. The similarity observed between the collection regions and genetic diversity of the accessions explained a slim variability among the accessions from the same collection areas for most of the characters. This close resemblance indicates the possibility that the accessions in each collection region may have come from similar genetic backgrounds. High levels of similarity have been detected among spider plant genotypes based on analysis of seed proteins (K'opondo et al., 2009). The high level of relationship reported among spider plant accessions may also be due to it being a self-pollinated crop (Omondi, 1990). Furthermore, 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 agroecological zones of Africa with different agro-climatic conditions.

The clustering pattern of accessions obtained in phenogram was further investigated through Principal Component Analysis (PCA) to determine the relationship between plant traits and accessions. It indicated set of traits that caused clustering of accessions into specific groups and that most effectively discriminated between accessions. The percentage variation explained by the first six Principal Components (PC) and the vector loadings for each character and PC are presented in Table 3. The first six

PCs explained 94.2% of the total variation among the study accessions. Stem hairiness and petiole hairiness were the main traits that contributed positively to PC1. 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. Leaf shape was the most important character that contributed to the second principal component in field grown accessions. All characters that made significant contributions to a particular principal component were important contributors to another principal component.

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 characters which were stem colour, stem hairiness, petiole colour, petiole hairiness, leaf hairiness and leaf shape. Future collections and characterization to broaden the Kenyan and South African spider plant resource base could focus on the characters identified above.

CONCLUSION

A range of observations was made in the current analyses of phenotypic diversity of spider plant using both their qualitative traits. In overall, 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 better possibilities 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 is likely to have a better chance of success than the use of accession 2289 from the same This study also revealed that some region. morphological traits discriminated more efficiently between the accessions than others. It would therefore be very important to identify beforehand the morphological characters with a high discrimination capacity before undertaking any genetic diversity studies based on morphological traits.

RECOMMENDATIONS

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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