

Morphological traits as indicators of bitterness in traditional vegetables; the case of spider plant (*Cleome gynandra*) in Kenya

ABSTRACT

Aims: The study set to find out existence of variation in leaf bitterness of spider plant in six (6) agro ecological zones in Kenya and whether leaf bitterness levels correlated with morphological characters; colour of main stem including leaf blade colour, stem pubescence and leaf waxiness.

Study design: Field studies done using purposive sampling method

Place and duration of study: Agro-ecological zones; upper highlands, lower highlands, upper midlands, lower midlands, inland lowlands and coastal lowlands sampled between 2016-2017.

Methodology: Morphological characterization for *Cleome gynandra* was done using IPGRI descriptors in 18 sites representing agro-ecological zones. Mature healthy seeds of spider plant were collected from the accessions characterized and grown at Nairobi Botanic Garden for organoleptic characterization. Organoleptic testing was done on 40 spider plant accessions which germinated and matured. Qualitative analysis performed on four (4) qualitative traits; colour of main stem, stem pubescence, leaf waxiness and leaf blade colour was correlated to levels of bitterness using Pearson correlational analysis. Leaf blade colour strongly associated with leaf bitterness while others correlated weakly. *Cleome gynandra*

specimens were grouped into two (2) clusters which were further divided into eight (8) clusters in the dendrogram based on level of leaf bitterness.

Changes in agro ecology had a significant effect on level of bitterness and leaf blade colour was a strong indicator of level of leaf bitterness of spider plant. Leaf blade colour was recommended for distinguishing non-bitter types from bitter types of spider plant.

Key words; spider plant, variation, morphology, leaf bitterness

1. INTRODUCTION

Spider plant (*Cleome gynandra*) belongs to the plant family Cleomaceae [7] and is among the list of important traditional African leafy vegetables used as a relish in Kenyan households [15,16] and other African countries whose market demand is on the rise [17] due to its rich nutritional and medicinal value. It is commonly referred to as spider plant, spider flower in English or by its trade name 'saga' or 'managu' in Kenya [5]. It is a key vegetable available during relish-gap period hence plays an important role in household food security during lean times [22]. It is relished for its taste by various communities in Kenya such as the Luo, Luhya, Kisii and Mijikenda and also its perceived medicinal value [15]. In Kenya, managu or spider plant vegetable is regarded as a powerful 'medicinal food' or nutraceutical consumed by recuperating individuals such as pregnant and lactating mothers, circumcised boys and invalids [21]. Spider plant stimulates the restoration of blood after delivery by increasing the number of red blood cells and the corpuscular hemoglobin concentration and also by stimulating the synthesis of iron biomarkers such as transferrin and ferritin in the body [3].

Nutritional studies have shown that it is rich in micronutrients such as calcium, magnesium, iron, zinc, vitamin A, C and E, hence suitable for combating hidden hunger and management of lifestyle diseases [24]. It is an ingredient in a baby's weaning food where leaves are crushed and added to a meal to increase its nutritional value in Nigeria [9]. It is also reported that spider plant is a good source of vitamin A and Iron providing 50–75% RDA for children [26].

An infusion of the leaves is used to treat anaemia and used as an eye wash [5]. The vegetable has been a source of income for women in rural areas who sell it in the local markets in Africa [21]. Research shows that spider plant contributes 15-40% of the total income of small-scale farmers in Kenya with the price of fresh leaves selling at 0.40-0.50 USD/Kg when in plenty during wet season and sells twice as much in dry season [23].

Cleome gynandra is a herb [2], quite variable in its range [5] because it is adapted to a wide agro ecological range [14]. Variation has been reported in stem colour, stem pubescence, large or small leaves, flower colour, shape of pods, range in bitterness among others [21,11]. The bitter taste in spider plant is said to vary from slightly bitter to extreme bitterness [21]. Various cooking techniques are applied to reduce the bitter taste [11, 15]. It is reported that spider plant is referred to as *bilolo* which means bitter in Lingala language due to its bitter taste [14]. Some communities in Kenya (western and coast) and West Africa appreciate the bitter taste as it is said to be appetizing and also 'good for the stomach' [21]. The bitter taste of spider plant deters some people from consumption such as the youth, children and certain community groups in Kenya because it is considered unpalatable. Spider plant contains condensed tannins that cause the bitter taste [11]. More so the concentration of condensed tannins varies within *Cleome gynandra* genotypes and that bitterness is amenable by breeding by reducing concentration of condensed tannins [11].

Indigenous leafy vegetables such as spider plant are cooked traditionally by first boiling to eliminate unwanted non-nutrient bio-active compounds [1]. Extreme bitter types of spider plant are cooked for long periods (1-2 hours) with several water changes to reduce leaf bitterness which leads to extreme losses of thermo-labile vitamins beta carotene, vitamin C and other useful medicinal compounds [1,12]. Other spider plant preparation methods include boiling and fermenting for two (2) days with addition of fresh milk or coconut milk to neutralize the bitter taste [15]. It is also cooked mixed with other vegetables such as amaranth or Ethiopian kale to neutralize the bitter taste. Local communities in Kenya and Tanzania have developed knowledge on how to select spider plant types which are not bitter by use of morphological traits such as stem colour or colour of petiole [21]. Green types are preferred to purple types as they are reported to be less bitter and more tender [21]. The Duruma of coastal Kenya

distinguish two (2) types of spider plant; light green coloured leaves (*changani cheruhe*) and dark green coloured leaves (*changani chiru*) in which the former is light green leaves are more bitter than the dark green leaves [13]. This knowledge on selection of bitter and non-bitter types has not been verified scientifically.

A number of past research studies have characterized *Cleome gynandra* based on different criteria. It has been demonstrated that there is distinct genetic variation in five (5) *Cleome gynandra* genotypes tested for levels of bitterness and genetic variability on levels of condensed tannins [11]. A total of two (2) genotypes that had low levels of condensed tannins thus less bitter hence were recommended for direct use because they required less amount of cooking time and therefore thermo-labile vitamins were preserved [11]. This variation was explained as a result of evolutionary history in terms of pathogen/pest pressure in areas of origin of these genotypes. Such evolutionary adaptations are often genetically controlled, highly heritable and amenable to breeding [11].

The objective of this study was to evaluate existence of variation in leaf bitterness in spider plant and determine whether bitterness has a relationship with morphological characters; leaf blade colour, colour of main stem, stem pubescence and leaf waxiness in six (6) agro ecological zones.

Dar a conocer la información sobre los siguientes aspectos: a) la distribución y b) la importancia económica de spider plant in Kenya.

2. MATERIALS AND METHODS

2.1 Morphological characterization

Purposive method of sampling was used to sample only those sites such as the wild and farms which had spider plant growing naturally. The sites sampled are shown in figure 1 which represent the six agro ecological zones. The sites samples were Baringo, Bungoma, Elgeyo Marakwet, Garissa, Homabay, Kakamega, Kericho, Kilifi, Kitui, Kisii, Kwale, Makueni, Nakuru, Taita, Trans Nzoia, Uasin Gishu, West Pokot and Nyeri as shown in table 1.

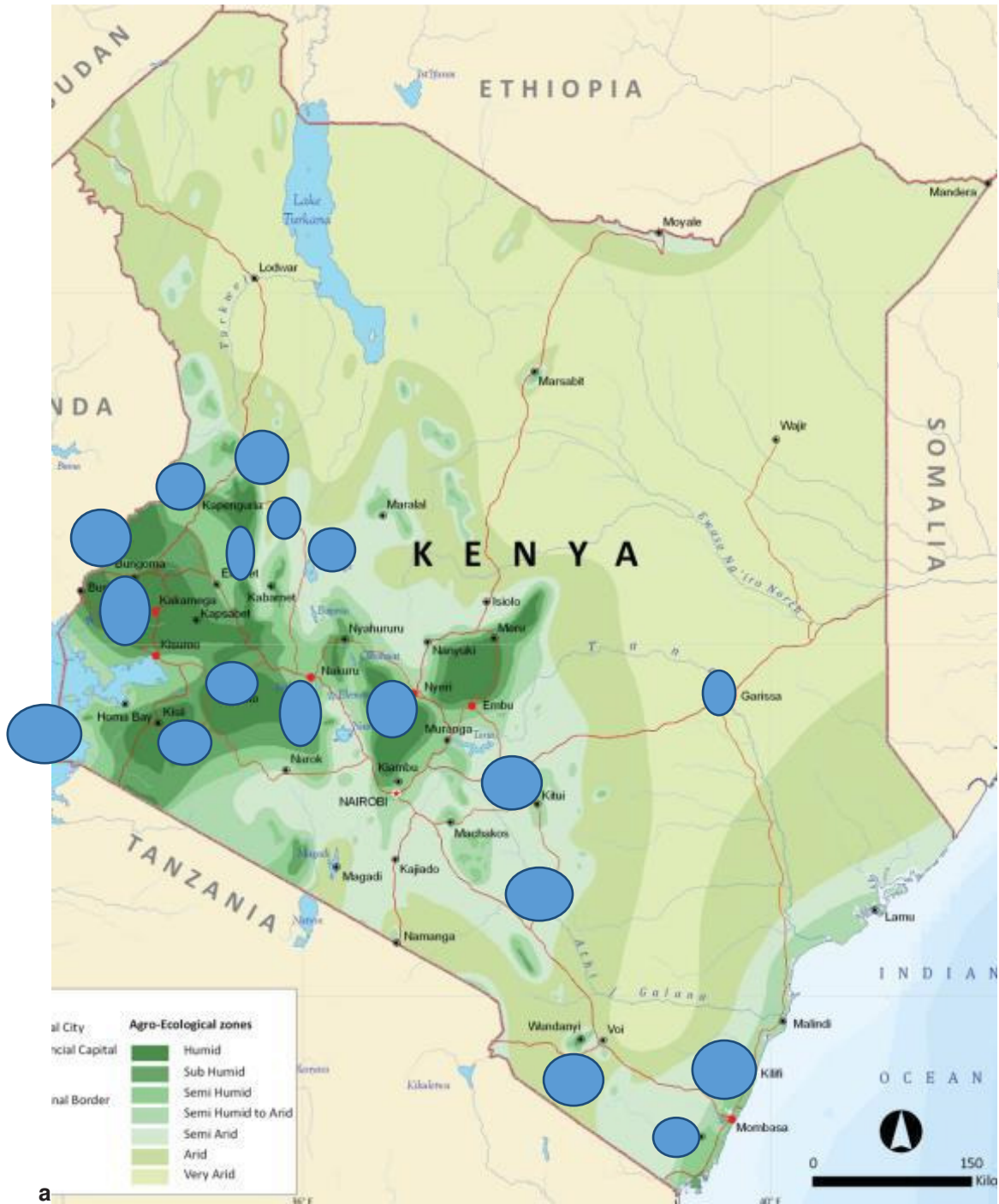


Figure 1: Map of collection sites for *Cleome gynandra*

● -denotes collection sites

Table 1: Collection sites for *Cleome gynandra*

Accession number	Agro-ecological zone	Area	Elevation (m)	Habitat	Cultivated/non-cultivated
BAR 1807B	Inland lowlands	Baringo	1012	Main farm	Natural
BAR 1807A	Inland lowlands	Baringo	1013	Main farm	Natural
BGM 2107A	Upper midlands	Bungoma	1713	Main farm	Natural
BGM 2107C	Upper midlands	Bungoma	1506	Main farm	Natural
ELG/1907B	Upper midlands	Elgeyo Marakwet	1247	Main farm	Natural
ELG/1907C	Upper midlands	Elgeyo Marakwet	1253	Main farm	Natural
ELG/1907A	Upper midlands	Elgeyo Marakwet	1247	Main farm	Natural
GA-01	Inland lowlands	Garissa	1035	Main farm	Cultivated
HBY 2307A	Lower midlands	Homabay	1155	Kitchen garden	Natural
HBY 2307B	Lower midlands	Homabay	1155	Kitchen garden	Natural
KK 2207	Upper midlands	Kakamega	1568	Kitchen garden	Natural
KRC 2507B	Lower highlands	Kericho	2063	Kitchen garden	Natural
KRC 2507A	Lower highlands	Kericho	1948	Main farm	Natural
KF-11	Coastal lowlands	Kilifi	282	Grassland area near a lake	Natural
KF-05A	Coastal lowlands	Kilifi	50	Main farm	Natural

KF-01	Coastal lowlands	Kilifi	10	Kitchen garden	Natural
KF-05	Coastal lowlands	Kilifi	50	Kitchen garden	Natural
KF-09	Coastal lowlands	Kilifi	236	Main farm	Cultivated
KF-07	Coastal lowlands	Kilifi	29	Kitchen garden	Natural
KIS/2407A	Upper midlands	Kisii	1787	Main farm	Natural
K1-01	Lower midlands	Kitui	1240	Main vegetable farm	Natural
K1-02	Lower midlands	Kitui	1240	Main farm of pigeon peas	Natural
KW-02	Coastal lowlands	Kwale	20	Kitchen garden around the homestead	Natural
MK-01	Lower midlands	Makueni	920	Kitchen garden at the edge of the compound	Natural
MK-02	Lower midlands	Makueni	925	Rubbish pit	Natural
NKR 1807	Upper highlands	Nakuru	2016	Kitchen garden	Natural
KIS/2407B	Upper midlands	Kisii	1756	Kitchen garden	Natural
TT-01	Upper midlands	Taita	864	Kitchen	Natural

				garden	
TT-02	Upper midlands	Taita	867	Main farm	Natural
TT-00	Lower midlands	Taita	188	Main farm	Natural
TNZ 2107B	Upper midlands	Trans Nzoia	1791	Main farm	Natural
UAG 1907C	Lower highlands	Uasin Gishu	2252	Main farm	Natural
UAG 1907A	Lower highlands	Uasin Gishu	2252	Main farm	Natural
UAG 1907B	Lower highlands	Uasin Gishu	2252	Main farm	Natural
WPK 2007A	Lower midlands	West Pokot	1722	Main farm	Natural
WPK 2007D	Lower midlands	West Pokot	1722	Main farm	Natural
WPK 2007E	Lower highlands	Kapenguria	2016	Kitchen garden	Natural
NYR 2107C	Upper highlands	Nyeri	2016	Main farm	Natural
NYR2107B	Upper highlands	Nyeri	2016	Main farm	Natural
NYR2107A	Upper highlands	Nyeri	2016	Main farm	Natural

Proporcionar la información sobre las coordenadas geográficas de las localidades correspondientes a las seis zonas agro-ecológicas. También proporcionar la información climática y edáficas de los sitios muestreados.

An IPGRI descriptor based on FAO standards was used to evaluate morphological qualitative characters across six (6) agro ecological zones as shown in table 2. A total of four (4) qualitative traits were evaluated for morphological variation were colour of main stem, stem pubescence, leaf colour and leaf waxiness.

Table 2: Descriptor codes for qualitative traits of spider plant accessions

Character	Descriptor and code
Colour of the main stem	Green (1), green tinged purple (2), purple tinged green (3), light purple (4), purple (5), dark purple (6), mixed (7)

Stem pubescence	Slightly hairy (1), hairy (2), very hairy (3), wooly (4)
Leaf blade colour	Light green (1), green (2), dark green (3)
Raw leaf bitterness	Not bitter (1), mild (2), bitter (3), very bitter (4), extremely bitter (5)
Leaf waxiness	Not waxy (1), fairly waxy (2), waxy (3), very waxy (4)

Explicar el proceso de germinacion de 56 accessions of spider plant seeds, asi mismo el sustrato utilizado para este fin.

2.2. Organoleptic characterization

Mature healthy seeds of spider plant were harvested from the accessions in which morphological variation had been done and were grown in the field at the Nairobi Botanic Garden. Fresh leaves of spider plant were harvested from these accessions and used for organoleptic testing for evaluation of differences in levels of bitterness which ranged from 1-5. **Especificar el diseño y el tamaño de muestreo de las plantas.**

The spider plant leaves were ready for harvesting at 50% flowering to conduct the organoleptic test. Of the 56 accessions of seeds of spider plant that were collected, only 40 accessions germinated and grew to maturity. After two (2) months, organoleptic test was carried out on raw leaves of 40 accessions. The raw leaves were tested for level of bitterness by chewing in the mouth.

Leaves from three (3) plants in each accession were randomly collected, put in a clean container and labelled. Only a handful of the leaves was collected. The three selected plants were tagged for evaluation.

Organoleptic testing of leaf bitterness in spider plant accessions was done based on protocol used earlier by Kutsukutsa and Carbonell-Baracchina [11, 4]. A Panel of 10 people; five (5) men and five (5) women aged 20-55 years were selected based history of consuming the vegetable and those who did not consume it/youth. Each person collected the leaves from each accession, then cleaned it in water and tasted. The level of bitterness was recorded, then the tester rinsed mouth and waited for five (5) minutes before tasting the next accession. The taste of the leaves was ranked as shown in table 3.

Table 3: Range of leaf bitterness levels

Taste description	Value
--------------------------	--------------

1.	Not bitter	1
2.	Mild	2
3.	Bitter	3
4.	Very bitter	4
5.	Extremely bitter	5

The Data was entered in an excel file; then mode and mean were conducted on the results of each accessions.

2.3 Data analysis

Data collected were analyzed using descriptive and inferential statistics which included frequencies, chi-square test using SPSS (Statistical Package for Social Sciences) version 21. **Tomando en cuenta la información proporcionada en la table 1 sobre “Collection sites for *Cleome gynandra*” donde cuenta con un gran numero de accession (56) provenientes de 6 agroecological zones, el uso de la prueba estadística no paramétricas de Chi-cuadrada no es suficiente. De mi punto de vista conviene la aplicación de un ANOVA no paramétrica (Kurskal-Wallis test for exemple) en lugar chi cuadrada.**

A correlation analysis was done using Pearson correlation chi-square method to estimate relationship between levels of bitterness (dependent variable) and leaf blade colour, leaf waxiness, colour of the main stem and stem pubescence (predictors).

Qualitative data collected was analyzed using descriptive and inferential statistics such as frequencies, chi-square test using SPSS (Statistical Package for Social Sciences) version 21. La aplicación de la estadística inferencial para qualitative data collected esta totalmente confuse y sin entender. Requiere mayores especificaciones.

Hierarchical clustering analysis based on levels of leaf bitterness was performed using SPSS Software version 21. UPGMA (unweighted pair-group method using averages) was used which distributes the

accessions into a reasonable number of groups. It calculates differences between clusters as the average of all the point-to-point distances between a point in one cluster and a point in the other. The clusters and relationships were displayed on a dendrogram. In cluster analysis, two (2) most similar accessions will be clustered together in a group and similarities of this group calculated. Two closest groups are combined until a single group remains. The results were expressed in a dendrogram or a 2-dimensional hierarchical tree diagram which represent multivariate relationships among accessions

3. RESULTS AND DISCUSSION

3.1 Propagation of spider plant

A total of 56 accessions of spider plant seeds were collected from the field mission as shown in table 2 in the methodology. Of the 56 accessions, only 40 accessions (71.4%) germinated and grew to maturity. Accessions that did not grow included; KF-02, KF-03, KF-04A, KF-04B, KF-06, KF-08, KF-10, KF-12, KW-01, KF-13, KIR-1707, WPK-2007B, WPK-2007C, BGM-2107B, KIS-2407A, KRC-2507C.

Lack of germination for the 16 accessions could be attributed to the fact that spider plant seed experiences poor germination [27] due to dormancy and dormancy is broken after six (6) months of storage [28]. In this study, *Cleome gynandra* seeds were planted two (2) months after harvesting and processing which may have caused germination failure. The second reason is due to lack of storing spider plant seeds under low temperatures (sub-zero) probably -20°C for six months which enhances seed quality [29]. The seeds may not have been also mature enough when harvested due to variation in fruit set and development in different accessions [24,10]. **Es importante discutir y compara los resultados sobre “propagation of spider plant” con los resultados similares de otros autores, asi evitando una discusion de tipo de revision bibliográfica (números).**

3.2 Variation in leaf bitterness

Organoleptic tests demonstrated that there is variation in level of bitterness in *Cleome gynandra* within and among different agro ecological zones which concurred with Schippers' and Chweya and Mnzava's findings [11, 21]. Levels of leaf bitterness in *Cleome gynandra* varied across six (6) agro ecological zones in Kenya as shown in table 4. The accessions ranged in bitterness with the highest at 4.2 (very bitter) in lower midlands to the lowest in the upper highlands which were not bitter (1.0). Accessions with the

highest level of bitterness were KF-05, KI-01, KW-02, GA-01 and TT-01 which were termed as very bitter to extremely bitter (4-5) while the accessions which had the lowest level of bitterness were UAG1907A, UAG1907B, UAG1907C, KRC2507A, HBY2307B and KF-07 which were termed as not bitter to mild (1-2). KF-07 was not bitter (1.4) yet it was from coastal lowlands because it was a cultivated type sourced from another region. Agro ecological zones which exhibited high bitterness levels were lower midlands and coastal lowlands while upper and lower highlands exhibited non bitter and mild types. The upper midlands accessions had bitterness which was highly varied from mild, bitter and very bitter (1.7-4.1). The very bitter types in upper midlands were from Taita region. The bitterness level in the lower midlands ranged from 1.6 in Homabay to 4.2 in Kitui, while those in the coastal lowlands had bitterness levels ranging from 1.4-4.0. Inland lowland accessions were also considered as bitter and the bitterness level was 2.0-3.5. Accessions from lower highlands ranged from 1.7-2.4 and upper highlands 1.0-2.4 and were regarded as not bitter to mild in taste while the midlands are bitter to extreme bitter.

Very bitter types dominated in the coastal lowlands and lower midlands. Lower highlands had types which were not bitter and mild because spider plant is highly commercialized in these areas where a lot of selection work has been done according to preferential criteria of the consumers and producers [3]. The producers in the lower highlands produce spider plant for the large market town centers thus select and propagate spider plant types with less or no bitterness. On the other hand, spider plant types were bitter in the coastal lowlands due to the hot temperatures in lowlands which causes increase in concentration of condensed tannins making the plant bitter which protects the plant against herbivores and pests for survival purposes [30]. In addition to these, local communities in coastal lowlands have a rich food culture of consuming wild vegetables in which spider plant is among them [13]. They conserve both bitter and non-bitter landraces of spider plant as they are accustomed to bitter taste and hence appreciate the flavor as it is associated with high medicinal benefits [6]. They also have a well-developed indigenous knowledge on how to prepare the bitter types using traditional vegetable recipes which tone down the bitter taste hence making the vegetable palatable [6]. Spider plant types from lower midlands had a wide range of bitterness because spider plant is still a wild growing vegetable in Kitui (less consumed) while it is semi-domesticated in Homabay where it is largely consumed [13].

Table 4: Range in leaf bitterness in six (6) agro ecological zones

		Mode	Maximum	Minimum
Agro-ecological zones	Coastal lowlands	3.1	4.0	1.4
	Inland lowlands	2.1 ^a	3.5	2.0
	Lower highlands	1.8	2.4	1.7
	Lower midlands	2.7	4.2	1.6
	Upper highlands	1.0	2.4	1.0
	Upper midlands	2.9, 2.0 ^a	4.1	1.7

a. Multiple modes exist. The smallest value is shown

3.3 Variation in four qualitative characters

3.3.1. Colour of the main stem

Spider plant accessions varied in the colour of the main stem which ranged from purely green to dark purple. Green colour dominated in the accessions with 30% purely green and 25% being green with a tinge of purple. 5% were dark purple while purple and pink (light purple) were both 12.5% of the accessions. One accession had main stems with some populations being green and some purple (mixed) which made 5% of the accessions. Colour variation displayed in spider plant tissues such as stems varied from pink to purple is due to accumulation of plant pigment; anthocyanins in plant tissues which are environmentally controlled by factors such as stress, nutrients and temperature [10]. Anthocyanins are

flavonoids responsible for plant-animal interactions which include attraction of pollinators and chemical repellence of herbivores and pests (plant defense mechanisms [25]). Anthocyanins also camouflage plant parts against their background protecting them from predation. Accumulation of anthocyanins in plant tissues (stems and petioles) therefore increases the ability of spider plant ecotypes to grow and thrive under diverse environmental conditions [10]. Anthocyanins being flavonoid in nature, have anti-bacterial, anti-viral, antifungal [25], anti-tumor, anti-oxidant [24] properties which are powerful health promoting compounds in the body when consumed [18].

3.3.2 Stem pubescence

The stems for the spider plant accessions ranged from being slightly hairy to being woolly (densely hairy). 77.5% were mainly slightly hairy, 12.5% were hairy, 2.5% were very hairy and 7.5% were woolly.

Stem pubescence variation recorded in this study concurs with Chweya and Mnzava's findings which in addition also recorded glabrous stems [5]. Glabrous character was reported to be rare which concurs with this study as they were no glabrous stems found in the accessions. Stiff and irritating hairs on petioles and stems of plants protect plants against browsing by herbivorous animals and insects [19] hence very hairy spider plants are better protected to enhance chances of survival.

3.3.3 Leaf blade colour

The colour of the leaves varied from light green, green and dark green. 42.5% of the accessions were green, 35% were dark green while 22.5% were light green in colour. This leaf blade colour diversity has not been evaluated in any other study. Green colour was most common among the accessions. However, another study recorded brown coloured leaves which were not documented in the study [5]. Leaf blade colour was found to negatively correlate with bitterness, whereby bitterness levels decreased with increase in concentration of the green colour. This is also an important character that can be used for selecting bitter types from non-bitter types according to consumer or farmer preferences.

3.3.4 Leaf waxiness

The leaf waxiness varied across different agro ecological zones. They were either not waxy, fairly waxy, waxy and very waxy. Almost half of the accessions (45%) were very waxy, 40% fairly waxy, 12.5% not waxy and the least were waxy (2.5%). Most of the spider plant accessions were waxy and this insulates

plant surfaces by preventing them against excessive loss of water caused by transpiration, protection against pest damage and air pollutants [20].

3.4 Correlation between qualitative characters and level of leaf bitterness

The four (4) predictors; leaf blade colour, colour of main stem, leaf waxiness and stem pubescence when combined together were strongly positively correlated at $P=0.05$ to leaf bitterness (0.001) as shown in table 5. However, when the predictors were separated, colour of the main stem, stem pubescence, leaf waxiness showed moderately positive correlation with leaf bitterness as shown in table 6 but was not significant. Colour of the main stem, leaf waxiness and stem pubescence showed 0.102, 0.301 and 0.955 respectively which were positive correlations but not significant. However, leaf blade colour showed a strong negative correlation at $P=0.05$ with leaf bitterness (-0.000) as shown in table 6. This meant that bitterness levels increased as the leaf green colour became lighter (light green) while it decreased as leaf green colour became darker. However, GA-01 had dark green leaves yet bitter because it was a cultivated type.

Various reports indicate that farmers determine or distinguish bitter types from non-bitter types of spider plant using colour of the stems [21,24]. This does not concur with this study because colour of the main stem alone did not correlate with bitterness levels in *Cleome gynandra*. However, this study found out that only leaf blade colour correlated negatively with bitterness levels indicating that as the leaf's green colour decreased the bitterness levels increased while as the green colour increased the bitterness levels decreased. An exception is accession GA-01 which had dark green leaves yet bitter because it was a cultivated type.

The difference in bitterness levels in different spider plant accessions is attributed to difference in concentration of condensed tannins due to genetic variability in quantity of condensed tannins as bitter taste increases with increase in quantity of condensed tannins [11]. Condensed tannins are involved in biochemical plant defense mechanisms with the variation arising from evolutionary history in terms of pathogen/pest pressure in areas of origin. These adaptations are genetically controlled and highly heritable, amenable by breeding [8]. Cultivated types such as accession KF-07 from the coastal lowland did not express bitter taste like other accessions from this agro ecological zone due to the fact that seeds were sourced from another agro ecological zone.

Las variables cualitativas sobre la Variation in leaf bitterness y la Variation in four qualitative characters no fueron aplicadas ningún análisis estadísticos (Chi-cuadrada por ejemplo) como indica la metodología.

Todos los resultados presentados no fueron adecuadamente discutidos con los resultados similares de otros autores. Cabe mencionar que los resultados fueron interpretados como una revision bibliograficas sin una discusión adecuada.

Table 5: Relationship between level of bitterness and combined four (4) morphological traits

ANOVA ^a						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	11.057	4	2.764	6.037	.001 ^b
	Residual	16.027	35	.458		
	Total	27.084	39			

a. Dependent Variable: avc_Leaf bitterness

b. Predictors: (Constant), Leaf blade colour, leaf waxiness, stem pubescence, colour of the main stem

Table 6: Relationship between level of bitterness and four (4) morphological traits

Coefficients ^a						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	3.141	.440		7.143	.000
	Leaf waxiness	.108	.103	.150	1.050	.301
	Colour of the main stem	.105	.063	.233	1.681	.102
	Stem pubescence	.007	.131	.008	.056	.955
	Leaf blade colour	-.586	.144	-.532	-	.000
					4.067	

a. Dependent Variable: avc_Leaf bitterness

En relacion con la table 6, especificar los modelos correspondientes a a) Leaf waxiness; b) Colour of the main Stem; c) Stem pubescence; d) Leaf blade colour ($\hat{Y} = a + \beta X_i \dots$).

UNDER PEER REVIEW

3.5 Cluster analysis

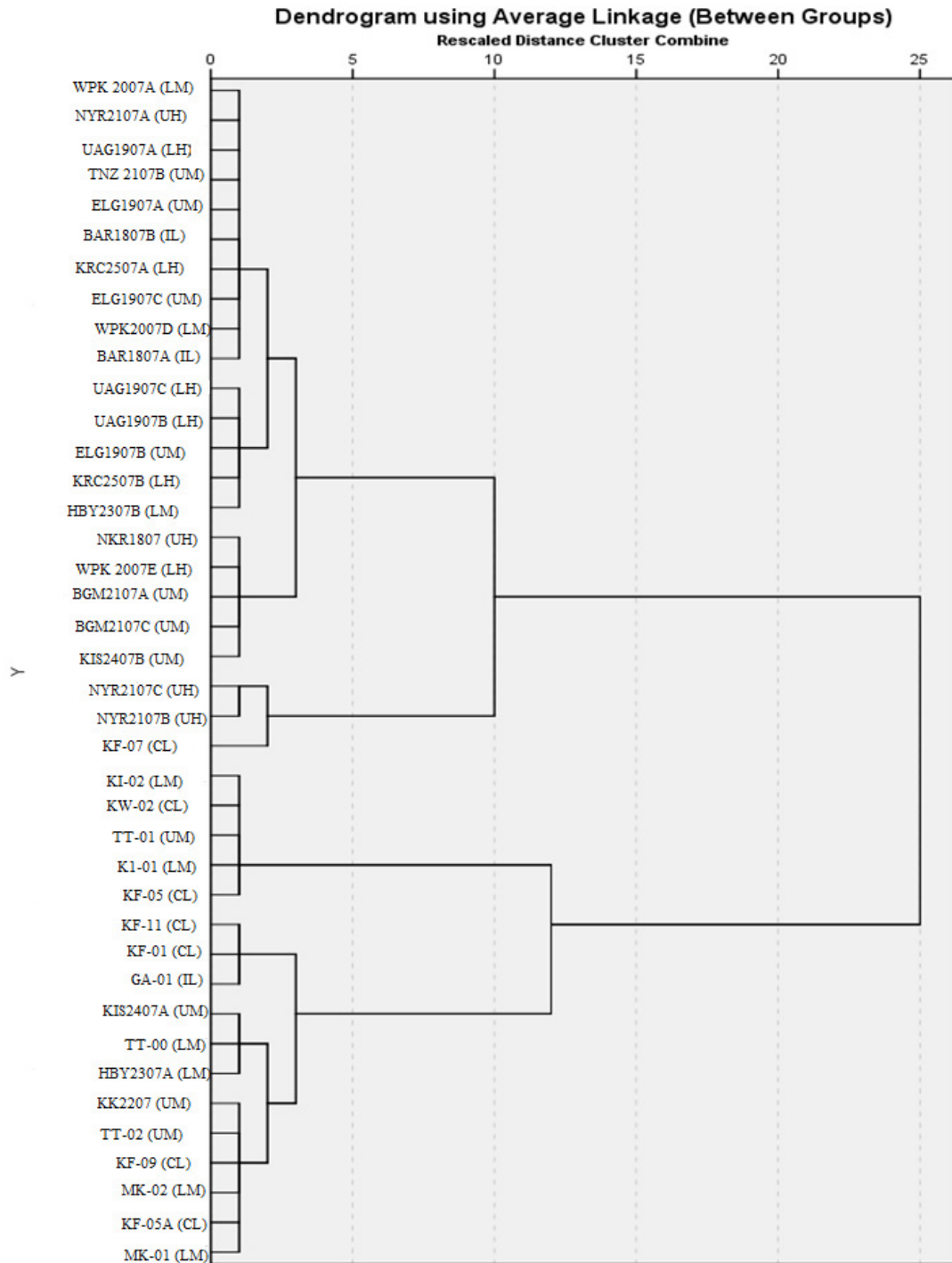


Figure 2: Diversity relationship of leaf bitterness levels

The above dendrogram is based on relationship on level of bitterness among accessions from different agro ecological zones. The dendrogram separated the 40 accessions into two (2) major clusters by

grouping accessions which share similar bitterness levels. The two (2) clusters represent bitter and non-bitter taste and are further divided into eight (8) clusters, with each cluster having accessions related to each other in terms of bitterness levels.

The first cluster was the largest and was divided into 10 clades consisting of two (2) accessions from lower highlands, lower midlands and inland lowlands, three (3) accessions from upper midlands and one (1) from upper highlands. The two accessions from inland lowlands (BAR 1807A and BAR 1807B) scored 2 and 2.1 respectively were more closely related to those from lower midlands, lower highlands, upper highlands and upper midlands which consisted of mild types. This shows that there is variation in levels of bitterness in the inland lowland zone.

The 2nd cluster was divided into five (5) clades with three (3) accessions drawn from lower highlands and one (1) from upper midlands and lower midlands respectively. This cluster was made up of accessions of a mild taste. The 3rd cluster was divided into five (5) clades and had accessions from upper highlands (one), lower highlands (one) and upper midlands (three). Cluster 3 (three) was considered to vary from mild taste to bitter taste.

The 4th cluster is a unique cluster which was divided into three (3) clades in which two (2) accessions were from upper highlands and one (1) from coastal lowlands and were considered as having a mild taste. NYR 2107B is a subgroup of NYR 2107C. KF-09 was closely related to NYR 2107C because KF-09 is a cultivated type with a mild taste (1.4) yet most of the accessions from coastal lowlands were bitter. KF-09 was purchased from another locality.

The 5th cluster was divided into five (5) clades and was made up of two (2) accessions from lower midlands, and coastal lowlands respectively and one (1) from upper midlands. Upper midland zone was represented by TT-01 which had bitterness level of four (4) thus was closely related to accessions from the lowlands.

The 6th cluster was divided into three (3) clades which consisted of those from coastal lowlands (two) and inland lowlands (one) with a bitterness level mean of three (3) (bitter) thus considered bitter. GA-01 from inland lowland scored 3.5 thus was closely related to those from coastal lowlands.

7th cluster was divided into 3 clades and had accessions from upper midlands (one) and lower midlands (two) with a bitterness level means of 2.7 hence bitter.

8th cluster was divided into six (6) accessions from upper midlands (two), lower midlands (two) and coastal lowlands (two) which had a bitterness level of 2.4-3, thus considered less bitter to bitter

The clustering analysis clearly showed that accessions were related to each other based on level of leaf bitterness. It also shows that there was variation in agro ecological zones. The close resemblance of accessions KF-07, NYR2107C and NYR2107B indicated that they came from the same genetic background which can be attributed to seed trade or exchange. Accession GA-01 expressed bitter taste (3) yet it was a cultivated type and therefore could have been sourced from a lowland region (associated with bitter types) through seed trade.

CONCLUSION

A number of observations and conclusions were made from this study using qualitative characters used; sensory and observational. The study showed that there was significant variation in leaf bitterness levels across six (6) agro ecological zones and correlation analysis proved that there was a significant relationship between level of leaf bitterness and leaf blade colour in spider plant. This means that leaf blade colour can be used as indicator to distinguish non-bitter types from bitter types in vegetable species; *Cleome gynandra*. This can be used by consumers and farmers for selection of bitter types from non-bitter types depending one's preference. The study therefore recommends;

- Use of leaf blade colour as an indicator of leaf bitterness in spider plant
- further characterization to be done to validate variation in leaf bitterness by growing the plants in multiple sites under different environments. This can further be supplemented by use of molecular markers such as simple sequence Repeats (SSRs) to identify polymorphism that is not due to environmental conditions
- further characterization to determine levels of bitterness at different stages of spider plant growth i.e. juvenile and maturity stage
- farmers and consumers be taught how to use leaf blade colour to discriminate types with low levels of bitterness which will reduce boiling or cooking time and hence save nutrients

REFERENCES

1. Agbemafle R, Obodai E, Adukpo G, Amprako D. Effects of boiling time on the concentrations of vitamin c and beta-carotene in five selected green vegetables consumed in Ghana. *Advances in Applied Science Research*. 2012; 3(5): 2815-2820.
2. Agnew A, Upland Kenya Wild Flowers; A Flora of the Ferns and Herbaceous Flowering Plants of Upland Kenya. 3rd ed. Nature Kenya—The East Africa Natural History Society; 2013
3. Bosire K. Effects of Chinsaga (*Gynandropsis gynandra*) Haematological Profile and Markers of Iron Metabolism in Kenyan Breastfeeding Women. PhD thesis. University of Nairobi; 2014.
4. Carbonell-Barrachina A. Application of sensory evaluation of food to quality control in the Spanish food industry. Alicante, Spain: Department of Agro-Food Technology, Miguel Hernández University; 2007.
5. Chweya J and Mnzava N. Cat's whiskers, Spider plant: Promoting the conservation and use of underutilized and neglected crops. Rome, Italy: Institute of plant Genetics and crop plant Research, Gatersleben/International Plant Genetic Resources Institute; 1997.
6. Chweya J, Eyzaguirre P. Biodiversity of African Leafy vegetables. Rome, Italy: IPGRI, pp.51-83; 1999.
7. Elffers R, Graham R, Dewolf P. Flora of Tropical East Africa; Capparaceae. Kew: Royal Botanic Gardens; 1964.
8. Franziska B. Importance of tannins for responses of aspen to anthropogenic nitrogen enrichment. [ebook] Sweden: Umeå Plant Science Centre Fysiologisk Botanik, Umeå University; 2016.
9. Hassan S, Umar R, Maishanu H, Matazu I, Faruk U, Sani A. The effects of drying method on the nutrients and non-nutrients composition of leaves of *Gynandropsis gynandra* (Capparaceae). *Asian Journal of Biochemistry*. 2007;349-353.

10. K'Opondo F. Morphological characterization of selected *Cleome gynandra* types from western Kenya. *Annals of Biology Research*. 2011; 54-64.
11. Kutsukutsa R, Gasura E, Mabasa S, Ngadze E. Variability in condensed tannins and bitterness in spider plant genotypes. *African Crop Science Journal*. 2014; 22(4):275 – 280.
12. Mathooko F, Imungi J. Ascorbic acid changes in three indigenous Kenyan leafy vegetables during traditional cooking. *Ecology of Food and Nutrition*. 1994; 32:239-245.
13. Maundu P. Mboga za watu wa Pwani. *Bioversity International*;2011.
14. Maundu P, Achigan-Dako E, Morimoto Y. Biodiversity of African vegetables. In: C. Shackleton, M. Pasquini and A. Drescher, ed., *African Indigenous Vegetables in Urban Agriculture*. London, UK: Earthscan, 2009; 65–104.
15. Maundu P, Ngugi G Kabuye C. *Traditional Food Plants of Kenya*. Nairobi, Kenya: KENRIK National Museums of Kenya; 1999.
16. Oluoch M, Pichop G, Silué D, Abukutsa-Onyango M, Diouf M. Production and harvesting systems for African indigenous vegetables. In: M. Shackleton and D. Pasquini, ed., *African Indigenous Vegetables in Urban Agriculture*. London, UK: Earthscan. 2009;145–175.
17. Omondi E, Debener T, Linde M, Abukutsa-Onyango M, Dinssa F, Winkelmann T. Molecular Markers for Genetic Diversity Studies in African Leafy Vegetables. *Advances in Bioscience and Biotechnology*. 2016;7(03):188–197.
18. Opole M, Chweya J, Imungi, J. Indigenous vegetables of Kenya; indigenous knowledge, agronomy and nutritive value. *Field and Laboratory Experience Report*; 1995.
19. Payne W. A Glossary of Plant Hair Terminology. *Brittonia*. 1978;30(2), pp.239–255.
20. Reicosky D, Hanover J. *Physiological Effects of Surface Waxes*. East Lansing, Michigan: Department of Forestry, Michigan State University; 1977
21. Schippers R. *African indigenous vegetables: An overview of the cultivated species*. Chatham, UK: Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation; 2000.

22. Smith F, Eyzaguirre P. African leafy vegetables: their role in the World Health Organization's global fruit and vegetables initiative. *Africa Journal of Food Agriculture and Development*. 2007;1–8.
23. Sogbohossou D, Achigan-Dako E, Maundu P, Solberg S, Deguenon M, Mumm R, Hale I, Van Deynze A, Schranz M. A roadmap for breeding orphan leafy vegetable species: a case study of *Gynandropsis gynandra* (Cleomaceae). *Journal of Horticulture Research*. 2018;5(1).
24. Wasonga D, Ambuko J, Chemining'wa G, Odeny D, Crampton B. Morphological Characterization and Selection of Spider Plant (*Cleome gynandra*) Accessions from Kenya and South Africa. *Asian Journal of Agricultural Sciences*. 2015.
25. Lev-Yadun S, Gould K. Role of anthocyanins in plant defense. University of Haifa, Oranim; 2008.
26. Van Jaarsveld P. (2014). Nutrient content of eight African leafy vegetables and their potential contribution to dietary reference intakes. *Journal of Food Composition and Analysis*. 2014; 33:77–84.
27. Ochuodho J. Physiological basis of seed germination in *Cleome gynandra* (L.). PhD thesis. University of KwaZulu-Natal, Pietermaritzburg, South Africa; 2005.
28. Muasya M, Simiyu J, Muui C, Rao N. Overcoming seed dormancy in *Cleome gynandra* L. to improve germination. (2009). *Seed Technology*. 2009; 31:34–143
29. Kamothe GN, Mathenge PW, Muasya RM, Dulloo ME, Effects of packaging and storage conditions on quality of spider plant (*Cleome gynandra*) seed. *African Journal of Food, Agriculture, Nutrition and Development*. 2013
30. Schweitzer, J., Madritch, M., Bailey, J., LeRoy, C., Fischer, D., Rehill, B., Lindroth, R., Hagerman, A., Wooley, S., Hart, S. and Whitham, T. (2008). From Genes to Ecosystems: The Genetic Basis of Condensed Tannins and Their Role in Nutrient Regulation in a Populus Model System. *Ecosystems*. [online] Available at: <https://doi.org/10.1007/s10021-008-9173-9> [Accessed 10 Apr. 2019].

31. Agro ecological zones map has been adapted from Agro-ecological zones in Kenya (National Environment Management Authority; 2010: 112.

UNDER PEER REVIEW