Morphological, Histological and Molecular Identification of Some Cucurbits in Khartoum State, Sudan.

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قال تعالى:

ةَنَةَذَكَرُواُمَا تُسْأَرُونَ اِلَّٰذِيْنَ أَنْقُلُونَ 

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Dedication

This work is dedicated to the soul of my father, may Allah be pleased with him, my mother, Second father Mohammed, all Brothers and Sisters with love.
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Abstract

This study is an attempt to make a comprehensive systematic study of some Cucurbits in Khartoum State. The study includes a brief description of the study area: location, geology, geomorphology, soil types, climate, water resources and people.

Some Cucurbits were studied and systematic interrelationships among them were determined using morphological, histological and molecular identification.

In the morphological study, 27 Cucurbit species were collected from the study area belonging to 12 genera. Botanical names, synonyms and vernacular English and Arabic names have been presented. Brief species descriptions of the collected plants have also been provided. The results showed that the species were very similar in the above ground development and root habit, but were extremely diverse in fruit characteristics.

In the histological study, similarities were observed in the distribution, tissue differentiation and number of layers of cells and tissues in the root, stem and leaf transverse sections. There were, however, variations in the vascular bundles in the roots where they were found to be bicollateral in *Cucumis sativus* and *Luffa aegyptiaca*. In the other species, the bundle consists of four radial arms of primary xylem alternating with four arms of primary phloem.

In the molecular study thirteen RAPD primers were used to amplify DNA extracted from the leaves of 10 Cucurbit species using CTAB method. A total of 227 bands were amplified of which 225 showed polymorphism among the 10 accessions. PCR-RAPD analysis showed a number of differences in the size and number of bands among the accessions, which means that there are genetical differences among the studied Cucurbit species. Based on these markers, genetic similarity coefficients were
calculated and a dendrogram was constructed. The dendrogram analysis delineated three major clusters. The first cluster consisted of one group which comprised *Cucurbita moschata* and *C. pepo* at a level of 38.6 % genetic similarity. The second cluster consisted of four groups. Group I comprised *Luffa aegyptiaca* at a level of 20.6 % genetic similarity. Group II comprised the closely related species *Cucumis melo* var *reticulatus* and *C. melo* var *flexuosus* at a level of 62 % genetic similarity. Group III consisted of *Cucumis sativus* with about 37.8 % genetic similarity to group II. Group IV consisted of *Ctenolepis cerasiformis* at a level of 26.6 % genetic similarity. The third cluster consisted of two groups. Group I comprised *Citrullus lanatus* and *Colocynthis vulgaris* at a level of 36.2 % genetic similarity. Group II consisted of *Coccinia grandis* at the level of 19.4 % genetic similarity. The three clusters were similar to each other at a level of 15% genetic similarity. Genetic similarity ranged between 15 to 62 %. This study demonstrates that molecular markers are useful in assessing genetic diversity among Cucurbits.
ملخص البحث

هذه الدراسة هي محاولة لعمل نظام تقسيمي شامل لبعض أنواع الفربيات بولاية الخرطوم. تتضمن هذه الدراسة وصف موجز لمنطقة البحث من حيث الموقع، الجيولوجي، الجيومورفولوجي، أنواع النبات، المناخ، مصادر المياه والسكان.

تم دراسة بعض الفربيات وتحديد العلاقات المورفولوجية والتشريحية والجينية بينها. في الدراسة المورفولوجية، تم جمع 27 نوعا من الفربيات من منطقة الدراسة تنتمن إلى 12 جنساً. تم رصد الأسماء العلمية والأسماء المرادفة لها وكذلك الأسماء المحلية والإنجليزية والعربية. تضمنت الدراسة وصفاً مختصراً لكل نوع من الفربيات التي تم جمعها. أظهرت نتائج الدراسة المورفولوجية أن هناك تشابهاً كبيراً بين الأعضاء الهوائية والجذرية واختلافاً كبيراً بين الصفات الثمرة لتلك الفربيات.

في الدراسة التشريحية لوحظ أن هناك تشابهاً بين الأنواع من حيث التوزيع والتمايز النسيجي المتمثل في عدد طبقات الخلايا والأنسجة في القطعات العرضية للجذر والسيقان والأوراق. ووجدت أيضاً اختلافات في الحجم والوعائية في الجذر حيث لوحظ أنها ذات جانبين في جذور الفربيات الحرير والليف بينما هي في الأنواع الأخرى مكونة من أربع أذرع قطبية من الخشب الأولي متبادلة مع أربع أذرع من اللحاء الأولي.

استخدم في الدراسة الجينية عدد 13 بادئة لإكتشاف الحمض النووي الريبوزي RAPD منقوص الأوكسجين (DNA) المستخلص بطريقة CTAB. تم الحصول على عدد 227 قطعة من عملية الإكثار وأظهرت حوالي 225 من هذه القطع تبايناً بين العشرة أنواع. تم إجراء المقارنة بين الأنواع المختلفة وأظهرت المقارنة وجود اختلافات في حجم وعدد القطع في الحمض النووي الناتج من عملية الإكثار العشوائي مما يدل على وجود اختلافات وراثية بين أنواع الفربيات التي تم دراستها. تم عمل رسم شجري (dendrogram) لتوضيح العلاقات الوراثية بين هذه الأنواع وتم تحليل الرسم. أوضحت النتائج التحليل أن الورش الشجري يتكون من ثلاث أنواع من التجمعات النوعية الفربيات.

يتكون التجمع الأول من مجموعة واحدة تشمل نوعين من نبات الفروع بنسبة تشابه وراثي 38.6%. Cucurbita moschata & C. pepo

يتكون التجمع الثاني من أربع مجموعات نوعية حيث تكون المجموعة الأولى من نبات الليف بنسبة تشابه وراثي 20.6%. تكون المجموعة الثانية من نباتين وثني القربى الوراثي وهي الشمام والعجور بنسبة تشابه وراثي 62%. أما المجموعة الثالثة فهي تحتوي على نبات الخير بنسبة تشابه وراثي 37.8% مع المجموعة الثانية. تحتوى المجموعة الرابعة على نبات
بنسبة تشابة وراثي 26.6%.

Ctenolepis cerasiformis

يتكون التجمع الثالث من مجموعتين. المجموعة الأولى تضم نبات البطيخ والحنظل بنسبة تشابة وراثي 36.2% بينما تضم المجموعة الثانية نبات Coccinia grandis ووراثي 19.4% مع المجموعة الأولى.

الثالث تجمعات متشابهة وراثياً مع بعضها البعض بنسبة 15% وجد في هذه الدراسة أن التشابة الوراثي بين أنواع بعض الفرعويات ينحصر ما بين 15 إلى 62% وخلصت هذه الدراسة إلى أن الواسمات الجزئية أداة فعالة لتوضيح التنوع الوراثي بين الفرعويات.
CHAPTER ONE
INTRODUCTION

Cucurbits belong to the family Cucurbitaceae and consist of about 118 genera and 825 species, according to the last taxonomic treatment of Jeffrey (1990). Cucurbits are present in both the New and Old World and are among the most important plant families that supply humans with edible products and useful fibers. The most important cultivated genera are Cucurbita L., Cucumis L., Citrullus L., Colocynthis Mill, Lagenaria L., Luffa L., (Whitaker and Davis, 1962) and Coccinia Wight & Arn., Corallocarpus Welw., Ctenolepis Hook., Kedrostis Medik., and Momordica L., (Jeffrey.1975, 1978 and Meeuse. 1962). This review focuses on the most important species of the following genera: The genus Cucurbita is represented by pumpkins (C. maxima Duch, C. moschata (Duch .exLam.)Duch. & Poir and squash (C. pepo L). The genus Cucumis is represented by cucumber (C. sativus L.) and melon (C melo L.).The genus Citrullus includes the water melon (C. lanatus (Thunb ) Mate & Nak ). The genus Colocynthis includes the bitter apple (Colocynthis vulgaris Schrad.). The genus Lagenaria includes the bottle gourd (L .siceraria (Mol.) Standl). The genus Luffa includes the angled loofah (L .echinata (L) Roxb ), smooth loofah (L .aegyptiaca Mill. Syn. L. cylindrica (L.) Roem) or loofah (Luffa sp). The genus Adenopus includes the (Adenopus breviflorus Benth.). The genus Coccinia includes the (Coccinia diversifolia Sensu Andr.) and (Coccinia grandis (L.)). The genus corallocarpus includes the (Corallocarpus corallinus (Naud)) and (Corallocarpus gijef (J.F.Gmel.)). The genus Ctenolepis includes the (Ctenolepis cerasiformis (Stocks) Hook.). The genus Kedrostis includes the (Kedrostis foetidissima (Jacq.) Cogn.). The genus
Momordica includes the balsam apple (*M. balsamina* L.), balsam pear (*M. charantia* L.), (*M. foetida* Schumach). and (*M. schimperiana* Naud.).

Although cultivated Cucurbits are very similar in the above ground development and root habit, they are extremely diverse in fruit characteristics. Fruits are eaten when immature (summer squash) or mature (water melon). Fruits can be packed (squash), pickled (cucumber), candied (water melon), or consumed fresh in salads (cucumber) or dessert (melon). Also, seeds, flowers (squash, pumpkins) are consumed by humans. Cucurbits are also produced for other uses than food. Fruits of bottle gourd are used for storage, drinking containers, bottles, utensils, smoking pipes, musical instruments, gourd craft decoration, masks, floats for fish nets, and other items. The fiber of a mature loofah fruit can be used as a sponge for personal hygiene, household cleaning and various other purposes, including filtration. Seeds or fruit parts of some Cucurbits are reported to possess purgatives, emetics and anthelmintics properties due to the secondary metabolite cucurbitacin content (Robinson & Decker – Walters, 1997). Fruits and roots with high cucurbitacin content function as an insect attractant (e.g. cucumber beetle – *Diabrotica* sp.) or as an insect repellent (e.g. honey bee – *Aphis mellifera* L. and yellow jacket wasp – *Vespula* sp.) (Chambliss & Jones, 1966a). Ectopic application of cucurbitacin can function as a protectant against infection by *Botrytis cinerea* (Bar – Nun & Mayer, 1990).

Histologists have devised histological techniques from time to time to obtain precise information on the internal structure of plant parts.

**Objectives of this study are:**

1. To study the morphology of some Cucurbits in Khartoum State.
2. To study the histology of some Cucurbits in the study area.
3. To study the genetic diversity among some Cucurbits in the study area.
4. To compare morphological and anatomical investigation carried out on the living samples of Cucurbits.

5. To compare morphological, histological and molecular methods as tools of identification of Cucurbits. This provides a comparison between traditional Taxonomy which is based on morphology and modern Taxonomy which is based on DNA characterization.

6. To compare the utility of the DNA marker system for evaluating similarity.
CHAPTER TWO
THE STUDY AREA

1. Location

Khartoum State lies between longitudes 36° - 31′ and 25° - 34′ E and latitudes 8° - 15′ and 45° - 16′ N. It is 381 feet above the sea level. It shares boundaries with the River Nile State from the north, the Northern State from the north-west, the Northern Kordofan State from the west, the White Nile State from the south-west, Algadarif State from the south-east and Kassala State from the north-east. It has an area of about 20971 square kilometers. It is divided into seven provinces: Khartoum, Jebel Aulia, Umdorman, Umbada, Karary, Khartoum North and Eastern Nile.

2. Climate

The prevailing climate is semi-dry. It is hot, dry and rainy during the summer and cold dry in the winter.

2.1 Temperature

The maximum annual average temperature is 37.2°C with a maximum of about 42°C in May. The minimum average annual temperature is 23.6°C with a minimum of about 17.5°C in January. (Khartoum Meteorological Station-2003).

2.2 Rainfall

The rainy season usually begins in July and continues through, August and September. The annual average rainfall reported during the Year 2003 was 150 mm. (Khartoum Meteorological Station-2003).
Fig. (1): Location of the study area.
Legend:

- **Rivers**
- **closed trees**
- **open to way open shrubs and woody vegetation**
- **open to way open trees**
- **closed to open herbaceous vegetation**
- **closed to open herbaceous vegetation (incl. sparse trees and shrubs)** on permanently flooded areas
- **sparse vegetation**
- **Tree and shrubs savannah**
- **Khartoum shp**

Fig. (2): Vegetation of the study area.
2.3 Winds

The winds blow mainly from two directions: north and south. The northern winds prevail from late October until early May. These are dry and warm in the winter and hot in the summer. The southern winds dominate the region in March and early April.

2.4 Humidity

The average relative humidity in the study area is 29% (Khartoum Meteorological Station-2003). The daily evaporation rate is 7.7mm, the highest rate being recorded in April with an average of 9.3mm.

3. Geology

The study area is composed of three main formations. The first formation is the Basement complex which covers the northern part of the state. Most of this complex is from granite rocks which resist weathering. Secondly; the Nile forms a narrow strip of recent deposits from Elfaki Hashim passing through Elgaili and Wad Ramli. It also covers AbuDelig area. Thirdly, Adam (1975) reported that the Nubian sandstone complex covers most of the state with different forms liable to weathering forming the red gravel land. The strong current of the Blue Nile eroded the Nubian sandstone forming the old and recent deposits. This has resulted in a number of agricultural activities along the eastern bank of the Blue Nile. Many small Khors, big wadis (AbuHasheem and ElRawakeeb) and many hilly gravels and sand dunes are found along the western bank of the White Nile. Adam (1975) reported that the Gezira formation extends along the two Niles in the form of old deposits on the Nile channels.
4. Geomorphology

The geomorphology of this area consists of nine categories of geomorphological units identified by Elfadoll (2000). These are:-

1- Recent terraces, Gerf land and Nile islands.

2- Nile basin (Depression of the Blue Nile and channels on the old River Nile).

3- Higher old terraces.

4- Fan plains of the old Blue Nile channels.

5- Mobile sand dunes.

6- Surface red eroded plains being formed from the Nubian sandstones, rich in iron minerals.

7- Sloping deteriorated eroded plain, west of the River Nile and the White Nile.

8- Big and small wadis.

9- Mountains and their surroundings.

5. Soils

The soil in Khartoum state includes:

1- Recently deposited soils which include recent terraces and Nile islands. These soils are characterized by silt deposition and are known mostly by their
agricultural activities due to their location, ease of irrigation and higher potentialities especially for horticultural crops and vegetables.

2- Old deposits soils

These soils consist of different geomorphological units which include:-

1- Old higher and lower basins of higher terraces, east of the Blue Nile and River Nile.

2- Deposits of the Gezira clay plains and old channels of the Blue Nile.

These soils have bad drainage features. The studies conducted by Soba Research Station confirmed that these soils can be used for planting crops capable of resisting salinity.

3- Soils originated from the White Nile deposits. These include different geomorphological units as recent and old terraces contain more than 60% heavy clays and have low permeability. Presently, these soils are used for planting forage species (Abu70). The soils of higher terraces are characterized by medium to bad drainage. Irrigated agriculture can be practised by pumps for planting rice, cotton, dura and sometimes wheat.

4- Aeolion deposits:

There are generally sandy soils or sand dunes with low fertility and productivity. These are usually planted with millet in the rainy season. It can be planted with watermelon.

5- Soil originated from the flat red eroded plains:-
These are unaffected by salinity or alkalinity. They are of alluvial clays and have good permeability but poor in nutritive elements. Currently, these soils are used as natural pastures and also used as sand and gravel sources.

6- Soils originated from sloping eroded plains:-

These are soils formed from eroded materials resulting from weathering of Nubian sandstones. They are affected by gravels and stones which cover large areas of the eastern and western banks of the River Nile. These soils are used as natural forests, pastures and rainfed agriculture.

7- Wadis (AbuHasheem, Soba, ElRawakeeb). These wadis are partially flooded. They can be used for planting fast growing dura or natural pastures.

8- Soils originated from Basement complex.

The soils of this unit originated from the weathering of granite rocks. They are alluvial sands which are not affected by salts or soda. Also some wadis of medium depth have been formed which can be used as gravel source, residential areas and natural pastures or forests.

6. People

Khartoum state has an estimated population of 5.35 million people who represent about 15.9% of the total Sudan population (population census 2003). According to the Central Bureau of Statistics, people living in this state belong to most of the Sudanese tribes.
7. **Water resources**

The main water resources for drinking and other purposes are the Blue Nile, the White Nile, the River Nile, Wadis, (Wadi Elmagadam, Hasseb, Soba, Saidna, Elkabashi, Elhawad, AbuHasheem and ElRawakeeb), Khors (Shambat) and artesian wells.
CHAPTER THREE
MATERIALS AND METHODS

1. Morphological methods:-

Plant specimens were collected from the vicinity of the Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum, in addition to field trips to different parts of Khartoum State.

The collected specimens were air-dried, poisoned and deposited at the herbarium of the Department of Botany and Agricultural Biotechnology. A preliminary identification of the collected plant material was made using the key of Andrews (1950). This identification was later checked using the work of Oliver (1968), Jeffery (1962) and Hutch and Dalz. (1954-1958-1963). Scientific names and synonyms have been updated according to the most current literature of Wickens (1976), Gumaa (1988), El Awad (1995) and Mohammed (1997), Bebawi and Neugebhrn (1991) and Braun et al. (1991).

A key for the genera has been constructed for the family whereas keys for species have been provided for genera having two or more species. The genera and species have been arranged in alphabetical order. A brief description has been provided for each species including vegetative, floral and fruit characters and geographical distribution. Common uses have been given whenever available. Photograph illustrations have been provided for most of the specimens.

2. Histological methods

2.1 Preparation of plant material

Cuts of about 2.5 cm long were prepared using vegetative parts of roots, stems and leaves of the plant material.
2.2 Fixation

The prepared plant material was placed in vials containing formaldehyde Acetic Acid (FAA) which is represented by the following formula according to Sass (1958):

- Ethyl alcohol (95 %) 50 cc
- Glacial Acetic acid 5 cc
- Formaldehyde (37 – 40 %) 10 cc
- Distilled water 35 cc

It was observed that the plant cuts were fully immersed in the fixative. The fixation was repeated 2-3 times until the solution became transparent. The plant material was then labelled using a piece of paper and pencil. A key for labeling was made to avoid hazard.

2.3 Washing

The plant specimens were washed in distilled water three times allowing a time of 20 minutes for each wash. The washing was necessary in order to avoid the interference of acids in the staining process later on.

2.4 Dehydration

Series of different alcoholic concentrations (50 %, 70 %, 90 %, and 95%) were used for this purpose. In each concentration the plant specimens were left overnight or more to ensure complete dehydration.

2.5 Clearing

The plant specimens were cleared using two mixtures as in the following table:
Table (1): Composition of clearing solution (I & II)

<table>
<thead>
<tr>
<th>Time</th>
<th>Mixture I</th>
<th>Mixture II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute alcohol</td>
<td>Cedar wood oil</td>
</tr>
<tr>
<td>24 hrs</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>3 hrs</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>3 hrs</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>24 hrs</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

2.6 Embedding in paraffin wax

A well-insulated oven with thermostat-controlled electrical heating was used for infiltration. The oven was adjusted the day before embedding at 60 °C. Three containers were put in the oven containing melted wax. The plant specimens were placed in closed vials containing 1:1 xylene and melted wax and put in the oven. The wax used in this step was called W₁ which is later on replaced by a new pure wax called W₂. After 45 minutes, W₂ was replaced by a new pure wax called W₃. The vials were left open so as to get rid of the xylene vapour.

2.7 Blocking

The equipments used in this process were: a heating source (Spirit lamp), a spatula, molds, strips of paper, a pencil and trough containing cold water. The specimens were transferred from the vials to the mold containing pure melted wax. Each specimen was pressed gently against the peripheral part of the mold. The wax was left to consolidate.
2.8 Trimming

A scalpel was used for removing excess wax, and the remaining wax was left to support the plant specimen.

2.9 Blocking

A heating spatula was used to stick the wax block on a wooden block. The label paper was also stuck respectively.

2.10 Sectioning

A rotary microtome, a brush, a razor blade, distilled water, trays, slides, a hot plate and a cold atmosphere were used for this process. The stems sections were 14 microns thick while the leaves and the roots were 9 and 12 microns thick, respectively. The ribbons were mounted on slides flooded with distilled water and placed on a hot plate so as to flatten the sections. The slides were removed from the plate and left to cool. The sections were then separated using a razor blade and each was mounted on a separate slide. The slides were returned to the hot plate and left to dry. They were then put in a tray and left overnight to ensure complete drying. The slides were then labelled using a diamond pen.

2.11 Staining

The double staining process was employed for this purpose. The steps of this process included dewaxing, rehydration, staining, and dehydration of material. These were performed by passing the slides through a series of coupling jars containing the proper chemical solutions. In the dewaxing process the slides were passed twice through xylene, each for 3-5 minutes. Rehydration was carried by passing the dewaxed slides through a series of different concentrations of ethanol and safranine in this order: absolute, absolute, 95%, 90%, 70% and 50% and then in safranine, each one for 2-3 minutes. Dehydration was carried out by passing the slides through different
concentrations of ethanol: 50%, 70%, 90%, 95% and absolute, each for 2-3 minutes. The slides were then immersed in fast green stain for one and a half minutes. The slides were then cleaned by passing through absolute ethanol and xylene.

2.12 Mounting

The material was mounted in D.P.X and covered with cover slips. The slides were kept in an oven at 60 °C and left for 3 days, before examining under light microscope at x10.

Procedures used in this section were after Creedy (1977), Sass (1958), Esau (1959) and Metcalfe (1950).

3. Molecular methods

3.1 Preparation of solutions

3.1.1 CTAB (3x) (Cetyl trimethyl ammonium bromide)

It was prepared by mixing the reagents 100 mMTris (hydroxymethyl amino methane hydrochloride) - HCl (pH 8), 1.4 M NaCl, 20 mM EDTA (Ethylene diamine tetra acetic acid), 3% (w/v) CTAB and RNase 10 mg/ml.

3.1.2 Washing buffer

It was represented by mixing ethanol 70% and isopropanol.

3.1.3 TE buffer

It was prepared by adding the following reagents: 10 mM Tris-HCl and 1mM EDTA (pH 8).

3.1.4 Chloroform isoamyl alcohol

This was a mixture of chloroform (24 ml) and isoamyl alcohol (1ml). The ratio of chloroform and isoamyl alcohol was 24: 1 respectively.

3.1.5 TAE buffer (1x)

It was prepared by adding the 40 mM Tris base, 1 mM EDTA and acetic acid, and the pH was then adjusted to 7.6.
3.1.6 Liquid nitrogen

3.1.7 Loading buffer (10x)

It was prepared by adding the following reagents: 0.25% bromophenol blue, 50% glycerol and 60 mM EDTA (pH 8).

3.1.8 Preparation of agarose gel

Amplification products were separated by electrophoresis in 2% agarose gel which was prepared by the following method according to William et al. (1990):

The ends of perspex tray were sealed with masking tape and a comb was inserted. To prepare a 2% agarose gel, 2g of agarose were added to 100 ml of (1x) TAE buffer and the solution was slowly boiled and then cooled to 60°C. The resulting gel was poured gently on the tray. After the gel was completely set, the tape was removed and the gel with the tray was placed into an electrophoresis tank. Approximately 500 – 1000 ml of (1x) TAE buffer was poured into the tank to cover the gel and the comb was then carefully removed.

3.2 Preparation of plant tissue

Fresh leaves were collected from seedlings or adult plants of some genera of the family Cucurbitaceae and then washed twice with distilled water.

3.3 DNA extraction by 3x CTAB method

There are many DNA isolation methods depending on laboratory conditions. In this research we have adopted the DNA extraction by CTAB (3x) method. This was conducted according to Dellaporta et al. (1983):

Fresh plant tissues, with liquid nitrogen, or dry tissues without liquid nitrogen were crushed in a mortar and a pestle was used to disrupt the cell wall. Tubes (Eppendorf tubes 1.5) containing 500 µl (3x) CTAB buffer were
placed in a water bath (65°C). 0.5 – 1g of the crushed tissues were added to the preheated (3x) CTAB buffer and mixed vigorously by a vortex. The tubes were then heated for 15 minutes with mixing and shaking every 5 minutes. Five µl of RNase and 500 µl of chloroform isoamyl alcohol were added to each tube. The solution was gently mixed for 5 minutes and then centrifuged at 13000 rpm for 10 minutes, after which period two layers were observed. The upper layer (aqueous phase) was carefully transferred with a pipette into a new tube and 300 µl isopropanol were added. The solution was then centrifuged at 13000 rpm for 10 minutes after which period the DNA precipitated as a pellet. The supernatant was decanted and the DNA precipitate was washed with 200 µl of cold buffer (70% ethanol) and centrifuged at 13000 rpm for 10 minutes. The resulting supernatant was also decanted and the DNA pellet was bench-dried for 30 minutes. The DNA pellet was then dissolved in 200 µl of TE buffer and stored at -20°C.

3.4 Quantification of the DNA

To quantify the amount of DNA, readings were taken from a spectrophotometer (Eppendorf – Germany) at wavelengths of 260 and 280 nm to determine the DNA concentration and purity of the samples. This was done by the following method:

The spectrophotometer was adjusted by a blank (50 µl distilled water). A dilution factor was used where 1 µl of DNA was mixed thoroughly with 49 µl distilled water and the DNA concentration and purity was then determined.

3.5 RAPD analysis

A total of 13 primers were surveyed for detecting polymorphism among plant species according to William et al. (1990). Nine of these primers were 10-mers (Operon Technologies; OP, and University of British Columbia, UBC). While the other 4 were 12-mers (Bexnet, Co., Japan). The reaction
mixture (20 µl) for PCR was composed of 15.1 µl dd H₂O, 2 µl 10x PCR buffer with 1.5M MgCl₂, 0.5 µl mixed 0.2 mM dNTPs, 1 µl 10 mM primer, 1 µl of template DNA (50 ng) and 0.4 unit of Taq DNA polymerase. Amplification was performed using touchdown annealing temperature along two or three steps starting with the melting temperature of the primer and then reducing the temperature by 3 degrees for each step. Amplification was carried out in a programmed temperature control system with preheating for 5 minutes at 94°C. This was followed by DNA denaturation for 1 min at 94°C. The 9 10-mers primers were treated as follows: primer annealing for 1 min at 37°C, 34°C, 33°C and 30°C. This was followed by primer extension for 2 min at 72°C and recycling steps for 19 cycles twice. The other 4 12-mers primers were treated as follows: primers annealing for 1 min at 42°C, 40°C and 37°C, primer extension for 2 min at 72°C and recycling steps for 14 cycles third. All the primers were finally extended for 5 min at 72°C and the samples were kept at 4°C. After all cycles had been completed, 7 µl of the products were mixed with 1 µl of DNA loading buffer and loaded into the 2% agarose gel. The agarose gel was then run in (1x) TAE buffer at 100 V for one and a half hour. The gels were soaked in 3% ethidium bromide for 30-60 min, and then placed on the UV-transilluminator for band visualization.

3.6 Data analysis

The RAPD fragments (200 to 1500) base pairs (bp) were visually scored as present (1) or absent (0). A dendrogram was constructed based on similarity matrix data by applying the UPGMA (Sneath and Sokal, 1973) and cluster analysis using the software package NTSYS – PC version 2 (Rohlf, 1993).
CHAPTER FOUR
LITERATURE REVIEW

1. Morphological study

The family Cucurbitaceae belongs to the order Cucurbitales, class Magnoliopsida (subclass Rosidae). Although most have Old World origins (Whitaker and Davis, 1962), many species originated in the New World and at least seven genera have origins in both hemispheres (Esquinas – Alcazar and Gulick, 1983). There is tremendous genetic diversity within the family, and the range of adaptation for Cucurbit species includes tropical and subtropical regions, arid deserts and temperate locations. Archaeological evidence has indicated that Cucurbits were present in ancient and prehistoric cultures. Here is a brief literature review on the morphology and taxonomy of some genera of the family Cucurbitaceae.

*Coccinia*

This genus includes about 30 species, 29 of which are only found in Africa, while only one species has a wider distribution in the Old World. Seven species are indigenous to southern Africa (Meeuse, 1962 and Jeffrey, 1978).

*Citrullus*

This genus consists of eight species and subspecies. Cogniaux and Harms (1924) have divided the genus Citrullus into four species, *C. vulgaris* Schrad, *C. colocynthis* (L.) Schrad, *C. naudinianus* Hook. and *C. ecirrhosus* Cogn. All these species are endemic in the central African region. Watermelon (*C. vulgaris*) is the only cultivated species of the genus. The water melon ancestor is the bitter-fruit from *C. vulgaris* Schrader. (Mohr, 1986). Watermelon originated in Africa and India (Mallick and Masui, 1986). In the
New World, cultivation began in Massachusetts as early as 1629 (Mohr, 1986). *Citrullus colocynthis* relative of watermelon is native to tropical Africa and highly drought tolerant. The fruits are extremely bitter, but the seeds can be removed and roasted as an edible commodity (Soliman et al., 1985). *C. colocynthis* differs from *C. vulgaris* chiefly in the size of fruit and seed. Their vegetative characters are practically identical.

**Corallocarpus**

This genus has about 15 species, native to the Old World tropics but mainly in Africa. There are 7 species indigenous to southern Africa (Meeuse, 1962 and Jeffrey, 1978).

**Ctenolepis**

Two species of this genus occur in the Old World tropics, one of them (*Ctenolepis cerasiformis*) has a distribution extending into southern Africa (Jeffrey, 1978).

**Cucumis**

According to a recent comprehensive biosystematic monograph of Kirkbrid (1993), the genus Cucumis includes 32 annual and perennial species divided into two very distinct groups defined by geographic origin and chromosome number (African 2n = 24 and Asiatic group 2n = 14 chromosomes). The African group includes melon (*C. melo*) and the Asiatic group includes cucumber (*C. sativus*) and its probable ancestor *C. sativus* var. *hardwickii* Royle or simply C. hardwickii (Perl-Treves & Galun, 1985).

Cucumber originated in India about 3,000 years ago and was soon cultivated in the South and East of the Himalayas, forming the Asiatic group (Kroon Net al., 1979; Ramachandran and Narayan, 1985). The African group
(melon group) has 30 species divided into six subgroups (Kirkbreid, 1993). Melon and other species (2n = 24) were originally distributed across a large part of Africa and Middle East up to Pakistan and South Arabia.

**Cucurbita**

Cucurbita, the yellow flowered genus, is considered to be one of the most morphologically variable genera in the entire plant kingdom (Robinson et al., 1976). It includes 22 wild and 5 cultivated species which are extremely diverse in fruit color, size and shape. The cultivated species are reproductively isolated from each other by genetic barriers and can be identified using morphological characteristics (Whitaker & Bemis, 1964; Whitaker & Bemis, 1975; Nee, 1990). Archaeological records of the New World suggest that Cucurbita was one of the first plants to be domesticated (Nee, 1990).

The origin and early spread of all Cucurbita species was in the Americas. The cultivated species of Cucurbita can be divided into mesophytic annuals (*C. maxima*, *C. argyrosperma*, *C. moschata* and *C. pepo*) or mesophytic perennial (*C. ficifolia*) (Whitaker & Bemis, 1964). From a taxonomical point of view, *C. pepo* can be classified in three subspecies, established according to the allozyme variation and the morphology of its seeds and fruits (Decker – Walters et al. 2002). These are *C. pepo* L ssp. *pepo*, *C. pepo* L. ssp. *ovifera* (L.) Decke, and *C. pepo* L. ssp. *fraterna* (L.H. Bailey) Andres. Taxonomically, the species *C. maxima* is divided into two subspecies, *C. maxima* Duch. ssp. *maxima* and *C. maxima* Duch. ssp. *andreana* (Naud.) Filov. Filov (1966) classified more than 20 varieties of *C. moschata* into geographical subspecies.
**Kedrostis**

This genus consists of 25 species, native to the Old World tropics, with 9 species in southern Africa (Meeuse, 1962 and Jeffrey, 1978).

**Lagenaria**

A total of six species have been recognized as belonging to the genus lagenaria, the white-flowered gourd. One of these is the domesticated monoecious species *L. siceraria* while the other five species are wild perennial, dioecious forms from Africa and Madagascar. *L. siceraria* was domesticated in Asia and at the same time indigenous to Africa (Whitaker & Davis, 1962).

**Luffa**

The genus Luffa is comprised of seven species, four of which belong to the Old World (*L. echinata* Roxb. *L. acutangula*, *L.aegyptiaca*, and *L. graveolens* Roxb) and the other three species belong to the new World (*L. quinquefida* (Hook. & Arn.) Seem. *L. operculata* (L) Cogn, and *L. astorii* (Svens.) (Heiser & Schilling, 1990).

The early spread of the genus Luffa was in the New and Old World, but both cultivated species originated in India (Heiser & Schilling, 1990). *Luffa cylindrica* and *L.acutangula* Roxb. have well differentiated morphological characters. They differ in floral structure, time of anthesis, direction of flower opening, and fruit and seed structures.

**Momordica**

This genus comprised 40 species, native to the Old World with most of the species in the African tropics. There are 9 species native to southern Africa (Meeuse, 1962 and Jeffrey, 1978).
2. Histological study

Various investigations have been made of the anatomy of Cucurbits, most of them prior to 1940 (Zimmerman, 1922). Although differences exist between Cucurbit species, Holroyd (1924) had shown that, in general, there was considerable similarity among them. The most thorough and informative investigations were made with the genera Cucurbita and Citrullus. Hayward (1938), Hufford (1938), and Whiting (1938), issued detailed accounts of their studies on the internal anatomy of the vegetative parts of the species belonging to these two genera. Unless otherwise stated, the ensuing information will be concerned with Cucurbita and Citrullus, and based upon the work of the above investigators, along with our own observations. Though the economic potentials of these species are immense, information on their morphology and anatomy is either scanty or completely lacking. There are some reports by Agbagwa and Ndukwu (2001, 2004) on epidermal and vegetative characteristics of the three species *Cucurbita moschata*, *Cucurbita maxima* and *Cucurbita pepo*. There are also more reports on *Cucurbita moschata* (Okoli, 1984; Ndukwu, 1988; Ndukwu and Okoli, 1992). Few anatomical studies were carried out on the species above and dealt mainly with seed-coat anatomy (Vaughan, 1970; Ndukwu and Okoli, 1992). The latter studies, however, did not examine aspects of stem, leaf and petiole anatomy of the species.

**Roots**

Whitaker and Davis (1962) reported the following information on the root anatomy of Cucurbits. The meristem consists of about seven layers of cambium-like cells, the number diminishing towards the periphery of the root
apex. The tissue directly in the center forms the vascular cylinder, while adjacent cells form the cortical region. Approximate delimitation of the cortex and vascular cylinder is soon possible, because the cells of the innermost layer of the cortex continue to divide tangentially for a longer period of time than the adjacent cells of the vascular cylinder. Within the vascular cylinder, the first tissue to mature is the protophloem. It consists of four strands, each two or three cells wide, extending tangentially for ten or more cells. In the centre of each strand, usually wedged between two larger pericyclic cells, a single cell differentiates to form a duct which can be distinguished by its less dense contents. This protophloem element, although small in diameter, may be considerably elongated. Similar cells quickly differentiate in the procambium adjacent to the pericycle, among which are the large parenchyma cells of the protophloem.

In contrast with the first-formed phloem, the metaphloem develops sieve tubes and companion cells in addition to parenchyma. The major portion of the metaphloem is composed of parenchymatous cells that are larger in diameter than the sieve tubes and several times longer than their radial dimension. There are four protoxylem vessels, one at the apex of each protoxylem point. The protoxylem vessels soon become stretched and crushed, forming lacunae which later may become conspicuous. Further development of the xylem elements creates vessels which are intermediate between the spiral, reticulate, and scalariform patterns, so that it is difficult to distinguish between protoxylem and metaxylem. In general, the metaxylem vessels are conspicuously larger than the first-formed protoxylem elements, and for the most part they have closely reticulated thickenings.
Development of the vascular cylinder up to this point results in the formation of four radial arms of primary xylem alternating with four zones of primary phloem. The central portion of the axis remains parenchymatous. Because of the delayed differentiation of the central portion of the vascular cylinder, the Cucurbit root has been described as having pith. Between the primary xylem and phloem, there is a zone of parenchyma which later gives rise to the cambium. As the vascular cylinder matures, the cortical region also develops. The innermost layer is the endodermis, with a narrow Casparian strip. The cells of the endodermis are more elongated and somewhat larger than those of the pericycle. The parenchymatous cells of the cortex are still larger; they are elongated and show conspicuous intercellular spaces. Completing the axis is the piliferous layer, composed of elongated tubular cells, many of which form root-hairs.

**Stems**

The stem of Cucurbita is that of a herbaceous vine with discrete vascular bundles. In Hayward’s (1938) description, the bundles are arranged in two rings. The outer ring is composed of the often smaller bundles which are located at the angles of the stem. The inner ring contains the often larger bundles which alternate with those of the outer ring. The basic number of bundles is ten, each cycle consisting of five, although occasionally additional smaller ones may be present. The bicyclic character of the vascular cylinder is related to the mode of insertion and downward divergence of the leaf-traces. Each vascular strand extends downward through the stem for an average of two internodes, before anastomosing with another bundle. Within the inner ring, the pith disintegrates and a large, irregularly-shaped cavity is formed.
The vascular region is delimited on the outside by a broad zone of parenchyma. This is limited externally by an uninterrupted band of sclerenchyma, five or more cells in width. The parenchyma and adjacent fibers together constitute the pericyclic zone. There is a narrow band of chlorenchyma outside the sclerenchyma ring, and between this and the epidermis is a discontinuous band of collenchyma, several cells in width, which is interrupted by the chlorenchyma at regular intervals. The epidermis is regular, with a thin, smooth cuticle, and stomata occur where it is subtended by chlorenchyma. Numerous rigid, multicellular hairs are produced on somewhat raised bases.

In the stem the bundles are bicollateral. There is frequently a well-defined inner cambium in the larger bundles which produces some phloem and parenchyma.

**Leaves**

Whitaker and Davis (1962) reported the following information on the anatomy of Cucurbit leaves. These have a single layer of epidermal cells on both the upper and lower surfaces. The thickness of the palisade parenchyma is not uniform in the different species. The spongy parenchyma consists of two to six layers of cells.

The median line of the upper surface of the midribs is always raised into a ridge where chollenchyma is well-developed. The vascular bundles of the midrib in the proximal portion of the leaf are arranged in six ways, as follows: the first type has a single bundle situated at the centre of the midrib; the second type has two bundles, one above the other; the third type has three
bundles, a larger one at the centre and a smaller one on either side; the fourth type also has three bundles, but they are arranged in a straight line, from above downwards (Cucumis, Lagenaria), the uppermost bundles is the smallest, while the lowest is largest; the fifth type has four bundle (Luffa), being distinguished from the third type by having one additional small bundle near the upper part the central one; the sixth type has seven bundles (Citrullus, Cucurbita), the largest being the undermost and the sixth smaller one lying above or on each side.

The number of stomata on both the upper and lower leaf surfaces differs remarkably between species. According to Yasuda (1903), the average number on the upper surface varies from 76 per sq.mm. in \textit{Luffa cylindrica} to 160 per sq.mm. in \textit{Cucumis sativus}, and on the lower surface from 221 per sq.mm. in \textit{Luffa cylindrica} to 347 per sq.mm. in \textit{Cucurbita pepo}.

The trichomes on the leaves follow the same pattern as on the stem. Their shapes are the same on both the upper and lower surfaces.

3. Molecular study

3.1 DNA markers

Advances in molecular biology during the last decade have provided a new class of genetic markers, namely DNA markers. The major DNA marker assays being applied are: restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat lengths polymorphism (SSRLP), inter. SSR amplification (ISA) and amplified fragment length polymorphism (AFLP). These markers reflect heritable differences in homologous DNA sequences between individuals.
The differences result from base pair changes or rearrangements (e.g. translocation and inversion) or insertions or deletions at the homologous DNA region. These properties make DNA markers extremely useful genetic markers compared with morphological or even protein markers.

Different methods are available to obtain DNA markers. They are based either on southern hybridization techniques or on polymerase chain reaction (PCR) techniques. The southern hybridization technique aims to exploit variation in the lengths of restriction fragments (as in the case of restriction fragment length polymorphism). The PCR technique aims to detect variations in amplified DNA regions (as in the cases of sequence tagged site polymorphism (STSP) and random amplification of polymorphic DNA). It is hoped that these new makers will accelerate genome analysis in crop species, particularly in the less polymorphic species.

3.2 Uses of DNA markers
1. Fingerprinting crop varieties and pathogens.
2. Tagging genes for indirect selection.
3. DNA marker for phylogenetic analysis
4. Comparative mapping for determining genome homologies among crop species.

3.3 Random Amplified Polymorphic DNA (RAPD) Marker

DNA amplification based markers, RAPDs (William et al., 1990), DAF markers, and AP-PCRs (Welsh and McClelland, 1990) are based upon the fact that a short oligonucleotide or randomly chosen sequence (primer), when mixed with genomic DNA (template) and a thremostable DNA polymerase (Taq polymerase from *Thermus aquaticus*) and subjected to the temperature cycling condition of PCR (Innis et al., 1990), will allow the amplification of several DNA fragments.
The reaction products can be separated on standard agarose gels and made visible via ethidium bromide staining. Polymorphisms between individuals result from sequence differences in one or both of the primer-binding sites and from small insertion or deletions in the region of the genome that is amplified, or from both. They are visible as the presence or absence of a particular RAPD band.

One of the first practical uses of RAPD markers allowed the creation of high density genetic maps and saturation of already existing genetic maps as in *Arabidopsis thaliana* (Reiter et al, 1992), tomato (Klein-Lankhorst et al., 1991), pine (Chaparro et al, 1992) and sugar cane (Aljanabi et al, 1993).

### 3.4 Polymerase chain reaction (PCR)

It is powerful, extremely sensitive technique with applications in fields of molecular biology, medical diagnosis, population genetics and forensic analysis (Saiki et al, 1985, 1988; White et al, 1989). PCR is based on the enzymatic amplification of a DNA fragment that is flanked by two oligonucleotide primers hybridizing to opposite strands of the target sequence. Repeated cycles of heat denaturation of the template, annealing of primers to their complementary sequences and the extension of annealed primers with a DNA polymerase, result in the amplification of the segment defined by the 5 – end of the PCR primers. Since the extension products of each primer can serve as a template for the other primer, each cycle essentially doubles the number of DNA fragments produced in the previous cycle.

### 3.5 Molecular analysis of Cucurbits

Molecular markers can be an effective means to determine genetic relatedness among cultivars and selections used in Cucurbits breeding
programs. A number of studies have been designed to examine genetic diversity and phylogenetic relationships among Cucurbit cultivars. Some of these studies used biochemical methods such as isoenzyme (Vallejos, 1983) while others used molecular approaches based on DNA (Evett and Weir, 1998) to evaluate genetic diversity in crop species. Most of the isoenzymes tested produced monomorphic patterns (Biles et al. 1989; Zamir et al., 1984).

Several methods are now available concerning molecular markers studies and scientists can choose the method of particular interest, depending on available material and objectives. Among the existing molecular approaches, random amplified polymorphic DNA (RAPD) is one of the most widely used molecular methods in genetic studies and has been applied for the less-known species. However, this method is less reproducible and show a lower degree of variability as compared to other methods. RAPD procedure provided a sufficient number of informative markers that could distinguish among water melon cultivars (Hashizume et al., 1993; Zhang et al., 1994). In a recent study (Levi et al., 2000), genetic diversity and relatedness were examined among Citrullus lanatus var lanatus, C. lanatus var citroides, and C. colocynthis using RAPD analysis. RAPD markers were also used in construction of an initial genetic linkage map for water melon (Hashizume et al., 1996), and to determine genetic relatedness among Asian water melon cultivars and breeding lines (Lee et al., 1996). Toshiharu et al. (1996) used RAPD to study polymorphism and construct a linkage map for water melon (Citrullus lanatus (Thunb.) Matsum & Nakai) by using 148 primers. Amnon Levi et al., (2001) used RAPD markers to study genetic diversity among accessions of some American cultivars of water melon (Citrullus lanatus var lanatus). He used 138 RAPD primers that were initially screened among some cultivars of water
melon. Of the 138 RAPD primers only 35 primers produced polymorphic RAPD patterns. Of the later, 25 primers produced 288 reproducible RAPD bands that ranged in size from 100 to 3000 bp. They also used the same technique for studying genetic diversity between *Citrullus lanatus* and *C. colocynthis* with 30 RAPD primers that were used for genetic diversity among the two species. The primers produced 662 bands that could be rated with high confidence and ranged in size from 100 to 2650 bp. The number of bands produced by each primer in the above study was relatively high (an average of 22 bands per primer). Leah et al., (1999) studied the molecular variation in melon (*Cucumis melo* L.) by 18 RAPD primers and found a total of 176 bands. The numbers of polymorphic bands were 75 in *Cucumis metuliferus* and 96 in *Cucumis melo*.

Dje Y et al. (2006) applied a molecular approach using inter-simple sequence repeat (ISSR) marker to analyse genetic diversity among three African edible-seeded Cucurbits (*Citrullus lanatus* L. Matsumra & Nakai, *Cucumeropsis mannii* L. Naudin and *Cucumis melo* var *agrestis* L. Naudin.). They applied 11 ISSR primers on DNA extracted from an accession of the three species and observed 66 bands with 4 to 11 bands per primer.

María, et al. (2004) used two molecular systems to analyse diversity among Cucurbit species. The first molecular system is the SRAP (sequence – related amplified polymorphism) marker a method which is modified from Li and and Quiros (2001). They successfully analysed diversity in *Cucurbita maxima* Duchesne and *C. pepo* L. with 11SRAP primers and reported a total of 148 reproducible bands. Among these, 98 were polymorphic (66.2 %) and ranged in size from 140 to 950 bp. The total number of bands per primer
ranged between 9 to 21, with an average of 13.5 bands per primer. The second molecular system is the AFLP (amplified fragment length polymorphism) marker (Vos et al., 1995), which had also been successfully used in the study of diversity among many Cucurbits (Ferriol et al., 2003a; 2003b; Garcia-Mas et al., 2000), with 6 AFLP primers and identified a total of 156 reproducible bands which ranged in size from 60 to 380 bp. Of these, 134 (85.9 %) were polymorphic. A range of 14 to 41 amplified bands per primer, with an average of 26 bands.
CHAPTER FIVE
RESULTS AND DISCUSSION

Cucurbitaceae

Herbs or rarely under shrubs, with watery juice, often scabrid; stems climbing or prostrate or trailers. Leaves simple, alternate, very variable in shape. Inflorescences axillary solitary; flowers mostly unisexual, monoecious or dioecious, actinomorphic, epigynous. Male flowers: calyx tubular, the lobes imbricate or open; corolla polypetalous or gamopetalous; stamens 1 – 5 (usually 3), free, or variously united. Female flowers: calyx tube often produced beyond the ovary; corolla polypetalous or gamopetalous; ovary inferior, with commonly 3 parietal placentas; styles 1 or rarely 3, with thick stigmas; ovules numerous. Fruits pepo, variable in shape; seeds numerous, variable in color and shape, often flattened.

1. Genera and Species Descriptions

The following genera of Cucurbits are encountered in the study area: Citrullus, Colocynthis, Luffa, Cucumis, Lagenaria, Cucurbita, Coccinia, Corallocarpus, Ctenolepis, Kedrostis, Adenopus, and Momordica these can be classified according to the following key below:

Key to the Genera

A. Filaments of the stamens united into a column..................... Coccinia

AA. Filaments of the stamens free or united at the base or in pairs:

B. Anther-loculi straight:

C. Male flowers solitary or in clusters or heads:
   (a) Stigma 1; ovules few.......................... Corallocarpus
   (aa) Stigmas 3-5; ovules numerous............... Cucumis

CC. Male flowers in racemes:
   (b) Petiole with a stipule-like leaf at the base ......Ctenolepis
(bb) Petiole without a stipule-like leaf at the base......

.................. Kedrostis

BB. Anther-loculi U- or S-shaped:

D. Calyx-tube of male flowers cylindric or funnel-shaped:

E. Anthers connate; female flowers without staminodes......

............... Adenopus

EE. Anthers free; female flowers with staminodes........

............... Lagenaria

DD. Calyx-tube of male flowers campanulate:

F. Stamens inserted at the throat of the calyx-tube.........

............... Momordica

FF. Stamens inserted in the calyx-tube:

G. Male flowers in racemes; ripe fruit dry....... Luffa

GG. Male flowers mostly solitary or in clusters; ripe fruit not dry:

H. Leaves pinnatifid:

I. Leaves thin; seeds black to white............

............... Citrullus

II. Leaves thick; seeds light brown............

............... Colocynthis

HH. Leaves not pinnatifid:

J. Corolla campanulate, gamopetalous,
lobed half-way down............ Cucurbita

JJ. Corolla rotate, deeply 5-parted, small......

............... Cucumis
**Adenopus Benth.**

**Adenopus breviflorus** Benth. in Hook., Niger Fl.: 372 (1849); F.T.A. 2: 528 (1871); F.W.T.A. 1:176, fig. 77 (1927) & the ed. 2, 1:206, fig. 81 (1954); F.P.S. 1:164, fig. 97 (1950). Fig. (3).


Perennial climbing herbs. Leaves simple, alternate, scabrid, mostly distinctly 5-lobed, cordate. Inflorescences axillary solitary; flowers regular, monoecious, epigynous; sepals 5, united; petals 5, united; stamens 3, united, epipetalous; gynoecium of 5 carpels, syncarpous; ovary inferior, 5-locular. Fruit oblong-ellipsoid, mottled-green when young, scarlet when ripe, edible. Distribution: Nile banks.

**Citrullus** Eck. & Zeyh.


**Vernacular names**: Water melon (Eng.); Batiekh (Ar.).

Climbing or sprawling herb. Leaves thin, simple, alternate, divided, 3 – 4-lobed, ovate to obovate, deeply pinnatifid, pubescent. Inflorescences axillary solitary; flowers regular, monoecious, epigynous; sepals 5, united, valvate; petals 5, united, valvate; stamens 3, united, epipetalous; gynoecium of 3 carpels, syncarpous; ovary inferior, 3 – locular. Fruits pepo, globose to oblong, light or dark green; seeds numerous, black to white or greenish, pitted, glabrous.
Fig. (3): *Adenopus breviflorus* Benth.
Fig. (4): *Citrullus lanatus* (Thunb.) Mansf.
Distribution: widespread.
Edible. Dried parched seeds are chewed; oil extracted from the seed is used in cooking and as an illuminant; the seed-cake can be used as livestock feed.

**Coccinia Wight & Arn.**

Key to the Species

A. Twiners; leaves pentagonal, scabrid; fruits ellipsoid, green with white mottling, becoming scarlet on ripening. C. grandis

AA. Prostrate or climbing herbs; leaves variable in shape, the upper ones ovate, the lower ones deeply 3-5-lobed, hispid; fruits ovoid, red, yellow or white. C. diversifolia

*Coccinia diversifolia* sensu Andr., F.P.S. 1:166 (1950), non (Naud.) cogn.


Prostrate or climbing herbs. Leaves simple, alternate, upper ones ovate, lower ones palmately deeply 3-5-lobed, hispid. Male flowers regular; corolla broadly campanulate; stamens 3. Fruit ovoid, marbled, red, yellow and white. Distribution: Nile banks.

*Coccinia grandis* (L) Voigt, Hort. Suburb. Calc.:59 (1845); F.P.S. 1: 165, fig.98 (1950); F.W.T.A., ed 2,1: 215,fig.85 (1954). Fig. (5)


Slender twiner; stem angular, dotted. Leaves simple, alternate, broadly ovate to subpentagonal, glabrous, shallowly to deeply-palmately 3-5-lobed. Flowers regular, solitary, white or yellowish-cream, pedicellate. Male flowers pedicels about ¾ in. long. Female flowers pedicels a little shorter. Fruit green with white mottling, scarlet when ripe, ellipsoid.

Distribution: Nile banks.
Fig. (5): *Coccinia grandis* L.
Colocynthis Mill.


Fig. (6).


Vernacular names: Bitter Apple (Eng.); Handal (Ar.).

Climbing or prostrate herbs. Leaves thick, simple, triangular, deeply divided, three-lobed, alternate, and hirsute. Inflorescences axillary solitary; flowers regular, unisexual, epigynous; sepals 5, united, valvate; petals 5, united, valvate; stamens 3, thick, united, epipetalous, slightly bilobed; gynoecium of 3 carpels, syncarpous; ovary inferior, 3 – locular. Fruit globose pepo; seeds numerous, light brown, glabrous.

Distribution: Nile banks, wadis and valleys in Khartoum.

Fruit edible by camels; the pulp is extracted and used in medicine; the powdered pulp, either alone or mixed with black pepper, is used as preventive against the clothes moth; fodder plant.

Corallocarpus Welw.

Key to the Species

A. Stem stout; leaves orbicular, palmately 3-5-lobed; fruit ¾ in. long, long- beaked................................................................. C. corallinus

AA. Stem branched, twisted; leaves broadly subreniform-ovate, obscurely 3- lobed; fruit ½ in. long, very shortly –beaked ................. C. gijef

Corallocarpus corallinus (Naud.) Cogn. in DC., Monogr. Phan. 3:647 (1881); F.P.S. 1:168 (1950).

Fig. (6): *Colocynthis vulgaris* Schrad.
Glabrous prostrate stout herbs. Leaves simple, alternate, orbicular. Fruit fleshy, ovoid, scarlet, long-beaked; seeds grey-brown.
Distribution: Nile banks.

*Corallocarpus gijef* (J.F. Gmel.) Hook. f., F.T.A. 2:566 (1871); A. Zimm., Cucurbitac. 2:2 (1922); W.F.K.: 30 (1948); F.P.S. 1:168, fig. 100 (1950).

Distribution: Nile banks.

**Ctenolepis** Hook.

*Ctenolepis cerasiformis* (Stocks) Hook. f., F.T.A. 2:558 (1871); F.P.S. 1:170, fig. 101 (1950); F.W.T.A., ed.2,1:208 (1954). Fig. (7).


Slender twiners; stem ribbed, slightly scabrid. Leaves simple, alternate, broadly ovate-cordate, digitately-3 to 5- partite, thin, scabrid-pubescent. Flowers creamy-white. Male flowers very small on axillary peduncles. Female flowers solitary, subsessile. Fruit globose, scarlet, 2-seeded.
Distribution: Nile banks.

**Cucumis** L.

Key to the Species

A. Trailers:

B. Stem setose; fruit globose or ovoid, villous when young............

......................... *C. melo*

BB. Stem scabrid; fruit tubercled or shortly bristly:

C. Leaves 3-5-lobed; fruit shortly bristly ............*C. prophetarum*
Fig. (7): *Ctenolepis cerasiformis* (Stocks) Hook.
CC. Leaves not as above; fruit tubercled............ *C. pustulatus*

AA. Climbers:

D. Stem bristly:

E. Stem 4- angled; leaves triangular-ovate; fruit variable, prickly.

......................... *C. sativus*

EE. Stem not as above; leaves rounded; fruit broadly ovoid, bristly...................... *C. dipsaceus*

DD. Stem prickly.............................................. *C. metuliferus*

*Cucumis dipsaceus*. Spach, Hist. Nat. Veg. Phan. 6:211 (1838); F.T.A. 2:543 (1871); F.W.T.A. 1:182 (1927); W.F.K.:29 (1948); F.P.S. 1:172, fig.102 (1950).Fig. (8).

Slender annual climbers, stem with pale-green, long stiff jointed bristles. Leaves simple, alternate, rounded, scabrid. Fruit broadly ovoid, densely bristly; seeds elliptic.

Distribution: Nile banks.

A fodder plant.

*Cucumis melo* var *flexuosus* Naud. in Ann. Sc. Nat., ser. 5,6 : 12 (1866),non De Notar. (1848), nom. illegit. Fig. (9).

Vernacular names: Snake melon (Eng.); Ajur (Ar.).

Trailing or climbing herbs. Leaves simple, alternate, orbicular or ovate to reniform, hirsute. Inflorescences axillary solitary; flowers regular, monoecious, and epigynous; sepals 5, united, valvate; petals 5, united, quincuncial; stamens 3, thick, united, epipetalous; gynoecium of 3 carpels, syncarpous; ovary inferior, 3 – locular. Fruit pepo, oblong, green, edible; seeds numerous, white – cream to yellow, glabrous.

Distribution: widespread.
Fig. (8): *Cucumis dipsaceus* Spach.
Fig. (9): *Cucumis melo var flexuosus* Naud.
**Cucumis melo** var *reticulatus* L., Sp.Pl. :1011 (1753); F.T.A. 2:546 (1871); Hassib, Cucurbitac. in Egypt: 78, figs. 30-64; U.O.P.Z. : 220 (1949); Andr., F.P.S. (1950). Fig. (10).

**Vernacular names:** Musk melon (Eng.); Shammam (Ar.).

Trailing or climbing herbs. Leaves simple, alternate, shallowly 5 – 7 – lobed, orbicular or ovate to reniform, hirsute. Inflorescences axillary solitary; flowers regular, monoecious, epigynous; sepals 5, united, valvate; petals 5, united, quincuncial; stamens 3, thick, united, epipetalous; gynoecium of 3 carpels, syncarpous; ovary inferior, 3 – locular. Fruit pepo, globose, smooth or sutured, yellow – brown or greenish - yellow; seeds numerous, white, glabrous.

Distribution: widespread.

The fresh flesh of the fruit is eaten as dessert; seeds edible and chewed and they yield edible oil.

**Cucumis metuliferus** E. Mey. ex Naud. in Ann. Sc. Nat., ser. 5,6 : 12 (1866),non De Notar. (1848), nom. illegit. Fig. (11).

Annual climbers; stems setose. Leaves simple, alternate, broadly ovate-cordate, setose. Flowers yellow. Fruit orange to scarlet when ripe, prickly; seeds glabrous.

Distribution: Nile banks.

**Cucumis prophetarum** L. Cent. 1:32 (1755); C. Jeffrey in K.B. 15:350 (1962). Fig. (12).

Ground trailers; stems densely scabrid. Leaves simple, alternate, 3-5-lobed. Flowers yellow. Fruit with longitudinal coloured stripes, covered with very short bristles, yellow when ripe; seeds glabrous.

Distribution: Nile banks.

A fodder plant.
Fig. (10): *Cucumis melo* var *reticulatus* L
Fig. (11): *Cucumis metuliferus* E.
Fig. (12): *Cucumis prophetarum* L.

**Syn. Cucumis figurei** (non Del) Broun & Massey, F.P.S. (1929).

Ground trailers; stems scabrid. Leaves simple, alternate, cordate, scabrid. Flowers yellow. Fruit uniformly grey-green, oblong-ellipsoid, smooth.

Distribution: Nile banks.

**Cucumis sativus** L., Sp.Pl: 1012 (1753). Fig. (13).

**Vernacular names**: Cucumber (Eng.); Khiar (Ar.).

Trailing or climbing herbs. Leaves simple, alternate, triangular – ovate, 3 – lobed, spiny, hirsute. Inflorescences axillary solitary; flowers regular, monoecious, epigynous; sepals 5, united, valvate; petals 5, united, quincuncial; stamens 3, thick, united, epipetalous; gynoecium of 3 carpels, syncarpous; ovary inferior, 3 – locular. Fruit pepo, prickly, mostly oblong or cylindrical, green; seeds numerous, white, glabrous.

Distribution: widespread.

Fruits edible as salad or pickled; the seed kernels yield edible oil; leaves eaten as salad or cooked.

**Cucurbita** *L.*

**Key to the Species**

A. Stem soft, round; peduncle soft, terete.......................... *C. maxima*

AA. Stem hard, angular; peduncle hard, angular, grooved:

B. Foliage speculate..................................................... *C. pepo*

BB. Foliage non speculate:

C. Peduncle flared at fruit attachment................. *C. moschata*

CC. Peduncle not flared at fruit attachment.......... *C. mixta*
Fig. (13): *Cucumis sativus* L.
**Cucurbita maxima** Duch. in Lam., Encyc. 2,151, (1786).  
Fig. (14).

Trailers rarely bushy; stems soft, round in cross-section. Leaves simple, alternate, usually reniform, serrate, not lobed. Inflorescences axillary solitary; flowers regular, monoecious; sepal lobes linear; corolla bright yellow; stigmas small, yellow, smooth. Fruit variable, top-shaped, globular, oblong-cylindrical or flattened-cylindrical; seeds white or buff-coloured or pale brown.

Distribution: Nile banks.

The immature fruits eaten as a fresh vegetable; mature fruits used for baking, making jam and for pies or forage for livestock.


Pilose annual vines; stems hard, 5-angled. Leaves simple, alternate, large, cordate, shallowly to moderately-lobed, usually with white blotches. Inflorescences axillary solitary; flowers regular, monoecious; sepals linear; corolla yellow to orange-yellow; stigmas bright orange to yellow or green, rough; mature peduncle hard, not flared at fruit attachment. Fruit variable, hard, or soft-shelled, usually dull in colour; flesh white to pale tan or yellow; seeds plump, white or tan.

Distribution: Nile banks.

Uses similar to *C. maxima*.

Fig. (15).

**Vernacular names:** Pumpkin (Eng.); Gara' assaly (Ar.).

Prostrate or creeping herbs, stem hard, 5-angled. Leaves simple, alternate, shallowly – lobed, with white blotches, globular or reniform, hairy – prickly. Inflorescences axillary solitary; flowers regular, monoecious, epigynous; sepals 5, free, valvate; petals 5, united, valvate; stamens 3, thick, united, epipetalous; gynoecium of 3 carpels, syncarpous; ovary inferior, 3 –
Fig. (14): *Cucurbita maxima* Duch.

a. leaf, b. plant, c. and d. variability of seeds, e. detail of fruit peduncle and f. morphological variability of fruits.
locular, mature peduncle hard, flared at fruit attachment. Fruit pepo, variable in shape, usually large, globular, cylindrical, or flattened, yellow; seeds numerous, smooth, flat, white. Distribution: widespread. Uses similar to *C. maxima*.

**Cucurbita pepo** L., Sp. Pl.: 1010 (1753). Fig. (16).

**Vernacular names:** Squash (Eng.); Cossa (Ar.).

Trailing herbs, stem hard, 5-angled. Leaves simple, alternate, with or without white blotches, broad, triangular, deeply – lobed, spiny – hairy. Inflorescences axillary solitary; flowers regular, monoecious, epigynous; sepals 5, free, valvate; petals 5, united, valvate; stamens 3, thick, united, epipetalous; gynoecium of 3 carpels, syncarpous; ovary inferior, 3 – locular; peduncles hard, sharply 5-angled. Fruit pepo, edible, mostly oblong, of various sizes and colour; seeds numerous, flat, smooth, tan to dingy white. Distribution: widespread. Uses similar to *C. maxima*

**Kedrostis Medik**

**Kedrostis foetidissima** (Jacq.) Cogn. in DC., Monogr. Phan. 3:634 (1881); F.W.T.A. 1:179 (1927); F.P.S. 1:175 (1950).

Fig. (15): *Cucurbita moschata* Poir.

a. and b. morphological variability of leaves, c. plant, d. fruit peduncle, e. morphological variability of fruits and f. seeds.
Female flower (left) and male flower (right) of *Cucurbita* genus

Fig. (16): *Cucurbita pepo* L.

a. leaf, b. bushy plant, c. long vine plant, d. mottled leaves, e. seeds, f. and g. variability in fruits and h. detail of the fruit peduncle
**Lagenaria** Ser.


**Vernacular names:** Bottle gourd (Eng.).

Long-running or climbing, musky-scented herbs. Leaves simple, alternate, widely ovate, white hairy beneath. Inflorescences axillary solitary; flowers regular, white, monoecious, epigynous; sepals 5, free, valvate; petals 5, free, white, valvate; stamens 3; anthers white, epipetalous; gynoecium of 3 carpels, syncarpous; ovary inferior, 3 – locular. Fruit pepo, variable in shape and size, globular, flattened, bottle- or club-shaped; seeds numerous, white or tan.

Distribution: widespread.

Used in making bowls, bottles, lades, milk-pots, churn, spoons, work-baskets and containers of many types, also used for floats for fishing nets, pipes, musical instruments.

**Luffa** Mill.

Key to the Species

A. Stem herbaceous, 5-angled; leaves orbicular-reniform; flowers ca 1 across; fruit oblong or subglobose, scabrid. .................... **L. echinata**

AA. Stem not as above; leaves digitately-lobed; flowers ca 2 across; fruit ellipsoid, smooth .......................................................... **L. aegyptiaca**


Fig. (18).
Fig. (17): *Lagenaria siceraria* Molina.

**Vernacular names:** Smooth loofah (Eng.); Leef (Ar.).

A vigorous annual climbing herb; stems 5-angled. Leaves simple, alternate, broadly ovate to reniform, dark-green, dentate, scabrous. Inflorescences axillary solitary; flowers regular, yellow, monoecious, epigynous; sepals 5, free, valvate; petals 5, free, yellow, valvate; stamens 5, free, epipetalous; gynoecium of 3 carpels, syncarpous; ovary inferior, 3 – locular. Fruit pepo, mottled-green when young, brown when ripe, nearly cylindrical, normally with light furrows or stripes; seeds numerous, black, flat, smooth.

Distribution: widespread.

Used as bath sponges and for cleaning purposes and in the manufacture of potholders, table mats, door and bath mats, insoles, sandals and gloves.


Herbaceous climbers; stems 5-angled. Leaves simple, alternate, orbicular-reniform, 5-7-lobed, scabrid. Inflorescences axillary solitary; flowers regular, yellow to white. Fruit oblong or subglobose, scabrid, with soft ciliate spines.

Distribution: Nile banks.

**Momordica L.**

Key to the Species

A. Leaves palmately-lobed:

B. Leaves 5-9-lobed; ripe fruit orange, tubercled; seeds scarlet........

......................... *M. charantia*

BB. Leaves 3-7-lobed; ripe fruit yellow, warted when young; seeds red-brown.......................... *M. balsamina*
Fig. (18): *Luffa aegyptiaca* Mill.
AA. Leaves ovate:
   C. Ripe fruit scarlet, bristly. \(M. \) \textit{schimperiana}\n   CC. Ripe fruit yellow or pale-orange, prickly. \(M. \) \textit{foetida}\n
\textbf{Momordica balsamina} \(L.\), Sp. PI. :1009 (1753); FI. Cap. 2:491 (862); F.T.A. 2:537 (1871); F.W.T.A. 1:181 (1927) & ed. 2,1:212 (1954); F.P.S. 1: 181, fig. 106 (1950).

Glabrous or pubescent herbaceous climbers; Leaves simple, alternate, palmately 3-7-lobed. Inflorescences axillary solitary; flowers regular, monoecious, pale yellow or white, with dark centers. Fruit orange-yellow, edible, warty when young, ovoid-ellipsoid, fleshy; seeds ovate-oblong. Distribution: Nile banks.

\textbf{Momordica charantia} \(L.\), Sp. PI. :1009 (1753); F.T.A. 2:537 (1871);
F.W.T.A. 1:181, fig. 80 (1927) & ed. 2,1:212, fig. 84 (1954); F.P.S. 1:181, fig. 105 (1950).

Herbaceous climbers; stems ridged, glabrous or hairy. Leaves simple, alternate, palmately 5-9-lobed. Inflorescences axillary solitary; flowers regular, monoecious, yellow. Fruit orange when ripe, edible, ovoid-fusiform, with longitudinal tubercled lines; seeds enclosed in scarlet pulp. Distribution: Nile banks.

Ingredient of curries; fruit pickled and often used in native medicines.


Fig. (21).


Climbers; stems glabrous. Leaves simple, alternate, broadly ovate, scabrid-puberulous beneath. Flowers regular, dioecious, white or yellowish.
Fig.(19): *Momordica balsamina* L.
Fig. (20): *Momordica charantia* L.
Fig. (21): *Momordica foetida* Schumach.
Male calyx bristly. Fruit yellow or pale-orange when ripe, with soft prickles; seeds in a red pulp.

Distribution: Nile banks.


Herbaceous climbers. Leaves simple, alternate, ovate. Flowers regular, yellow. Fruit scarlet when ripe, covered with soft bristles.

Distribution: Nile banks.

2. **Histological Analysis**

The present study is based on comparative morphological and anatomical investigation carried out on fresh samples of Cucurbit species. It is intended that the results will provide additional evidence for the delimitation of the species in the family. Results from the anatomy will also provide a guide and basis for studies leading to further exploitation of these important vegetable species.

A summary of the anatomical features of the root, stem and leaf of the some Cucurbit species have been presented in photomicrographs of the transverse sections of these parts.

Trichomes on the leaves and stems in the family are similar in forms, multicellular and nonglanular (Fig. 22).

Roots usually consist of epidermis, cortex, endodermis and vascular bundles in most of the genera of the family Cucurbitaceae. Epidermis of the root of most genera is one cell thick. The root cortex collenchyma is 2 – 4 cells thick and cortex parenchyma is 5 – 7 cells thick in most of the genera. The endodermal and pericyclic layers are inconspicuous. The root vascular bundles consist of four radial arms of primary xylem alternating with four zones of primary phloem in most species except *Cucumis sativus* and *Luffa*.
Fig. (22): Leaf surface of *Cucumis melo* L. (x4)

Note: The nonglandular multicellular trichomes.
aegyptiaca where the vascular bundles are bicolateral and arranged in a ring (Figs. 23 and 24).

Stem epidermis of Cucurbits is regular, with thin cells and scattered collenchyma cells in most of the species except *Cucurbita moschata* (Fig. 25) where there are no scattered collenchyma. Stem cortex collenchyma is 4 - 6 cells thick in *Cucurbita moschata* while it consists of a narrow band of sclarencyma cells in most of the other species eg. *Colocynthis vulgaris* (Fig. 26). Stem vascular bundles in most of species are arranged in two rings. The outer ring is composed of the often smaller bundles which are located at the angles of the stem. The inner ring contains the often larger bundles which alternate with those of the outer ring as in *Colocynthis vulgaris* (Fig. 27). However, in *Cucurbita moschata* the vascular bundles are arranged in one ring (Fig. 28). The basic number of bundles is ten, each cycle consisting of five. Stem vascular bundles are bicolateral (Fig. 29).

Leaves usually have a single layer of epidermal cells on both the upper and lower surfaces in all species with hairs present on both surfaces. Collenchyma tissues are seen in the median line of the upper surface of the leaf midrib. The thickness of the palisade parenchyma is not uniform in the different species. The spongy parenchyma consists of 2 to 6 layers of cells. The vascular bundles are bicolateral and arranged in different ways depending on the genera. The genus Cucurbita and Citrullus had seven bundles which are arranged so that the largest being the undermost and the six smaller ones lying above or on each side. The genus Cucumis has three bundles, which are arranged in a straight line, from above downwards; the uppermost bundle is the smallest, while the lowest is the largest. The genus Luffa has four bundles, being distinguished from the genus Cucumis by an
Fig. (23): T. S Root of *Cucurbita moschata* L. (X4).
Fig. (24): T. S Root of *Luffa aegyptiaca* Mill. (X4).
Fig. (25): T. S Stem of *Cucurbita moschata* L. (X4).
Fig. (26): T. S Stem of *Colocynthis vulgaris* Schrad. (X4).
Fig. (27): T. S Stem of *Colocynthis vulgaris* Schrad. (X4).
Fig. (28): T. S Stem of *Cucurbita moschata* L. (X4).
Fig. (29): T. S Stem of *Cucurbita moschata* L. (X4).
additional small bundle near the upper part of the central bundle (Figs. 30 and 31).

3. Molecular analysis

The DNA concentration of the 10 accessions in this study ranged between 80.8 to 352.1 µg/ml. The above concentrations were too high to run a PCR reaction and thus they were diluted to 50µg/ml. The range of DNA purity was from 1.7 to 2.3 (Table 2; Fig. 32). This range of purity was sufficient to achieve the PCR reaction. Sambrock et al. (1989) reported that pure DNA samples had values of 1.8 to 2.0. He added that samples contaminated with protein or phenol usually had values which differed significantly from the values given above and hence accurate quantification of the DNA of such samples would not be possible.

The analysis of the 10 accessions with 13 RAPD primers produced a total of 227 reproducible bands (Table 3). Among these, 225 bands were polymorphic (99.1%) and ranged in size from 200 to 1500 bp. The total number of bands per primer ranged between 9 to24, with an average of 17.5 bands per primer (Table 3). The number of polymorphic bands per primer varied from 9 to 24, with an average of 17.3 bands (Table 3). The number of polymorphic bands produced per primer was found to be relatively high among the species (Fig 33,34,35,36 and 37). A cluster analysis of the accessions was performed using the software package NTSYS-PC version 2 (Rohlf, 1993) and UPGMA method (Unweight pair-group method with arithmetic average). To estimate genetic diversity among the accessions, genetic similarity coefficients were calculated, and a dendrogram was constructed (Fig 38). The range of DNA similarity in the dendrogram was between 0.15 and 0.62. The dendrogram consisted of three major clusters.
Fig. (30): T. S Leaf of *Cucumis sativus* L. (X4).
Fig. (31): T. S Leaf of *Luffa aegyptiaca* Mill. (X4).
Table (2): DNA concentration and purity of plant accessions.

<table>
<thead>
<tr>
<th>No</th>
<th>Species</th>
<th>Concentration µg/ml</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Coccinia grandis</em></td>
<td>80.8</td>
<td>1.9</td>
</tr>
<tr>
<td>2</td>
<td><em>Colocynthis vulgaris</em></td>
<td>154.6</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td><em>Ctenolepis cerasiformis</em></td>
<td>193</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td><em>Cucumis melo var flexuous</em></td>
<td>186</td>
<td>1.8</td>
</tr>
<tr>
<td>5</td>
<td><em>Cucumis sativus</em></td>
<td>352.1</td>
<td>1.8</td>
</tr>
<tr>
<td>6</td>
<td><em>Cucumis melo var reticulatus</em></td>
<td>228</td>
<td>1.8</td>
</tr>
<tr>
<td>7</td>
<td><em>Luffa aegyptiaca</em></td>
<td>201.2</td>
<td>1.8</td>
</tr>
<tr>
<td>8</td>
<td><em>Cucurbita pepo</em></td>
<td>294.3</td>
<td>2.1</td>
</tr>
<tr>
<td>9</td>
<td><em>Citrullus lanatus</em></td>
<td>274.8</td>
<td>1.8</td>
</tr>
<tr>
<td>10</td>
<td><em>Cucurbita moschata</em></td>
<td>152.1</td>
<td>1.7</td>
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</table>

Fig.(32): Total genome of accessions
Table (3): Number of total and polymorphic fragments using RAPD marker. Primer names are according to manufacturer’s identification system (Operon technology; OP, and University of British Columbia, UBC).

<table>
<thead>
<tr>
<th>No</th>
<th>Primer</th>
<th>Sequence (5’→3’)</th>
<th>Temperature (°C)</th>
<th>Total number of bands</th>
<th>Number of polymorphic bands</th>
<th>Percentage of polymorphism</th>
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<td>15</td>
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<tr>
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<td>A02</td>
<td>GCC AGC TGT ACG</td>
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<td>18</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>A03</td>
<td>TGC CTC GCA CCA</td>
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<td>17</td>
<td>17</td>
<td>100</td>
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<tr>
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<td>100</td>
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<td>UBC228</td>
<td>GCTGGGGCGGA</td>
<td>37.1</td>
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<td>9</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>227</td>
<td>225</td>
<td>99.1</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td>17.5</td>
<td>17.3</td>
<td>98.9</td>
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</tbody>
</table>
Fig. (33): Agarose gel showing polymerase chain reaction (PCR) products of 10 accessions of Cucurbits using UBC 106 primer.
Fig. (34): Agarose gel showing polymerase chain reaction (PCR) products of 10 accessions of Cucurbits using UBC 157 primer (left) and UBC 155 primer (right).

Fig. (35): Agarose gel showing polymerase chain reaction (PCR) products of 10 accessions of Cucurbits using UBC 222 primer (left) and UBC OPB 05 primer (right).
Fig. (36): Agarose gel showing polymerase chain reaction (PCR) products of 10 accessions of Cucurbit using A02 primer (left) and A01 primer (right).
Fig.(37): Agarose gel showing polymerase chain reaction (PCR) products of 10 accessions of Cucurbits using A0 4 primer (left) and A0 3 primer (right).
Fig. (38): Dendrogram analysis of 10 accessions species of the family Cucurbitaceae.
The first cluster consists of one group (Group I) which comprises the species: *Cucurbita moschata* and *C. pepo* which both have a DNA of a genetic similarity of 38.6 %.

The second cluster consists of four groups as follows: Group I comprises the species *Luffa aegyptiaca* which has a genetic similarity of 20.6 %. Group II comprises the closely related species *Cucumis melo* var *reticullatus* and *C. melo* var *flexuous* with a genetic similarity of 62 %. Group III consists of *Cucumis sativus*. This group is similar to group II in their genetic similarity of (37.8 %). Group IV consists of *Ctenolepis cerasiformis* with a genetic similarity of 26.6 %.

The third cluster consists of two groups. The first group comprises the species *Citrullus lanatus* and *Colocynthis vulgaris* which have a genetic similarity of 36.2 %. The second group consists of *Coccinia grandis* which has a genetic similarity of 19.4 %. Marría et al. (2004) analysed accessions of *Cucurbita moschata* with 11SRAP primers and reported a total of 148 reproducible bands. Among these, 98 were polymorphic (66.2 %) and ranged in size from 140 to 950 bp. The total number of bands per primer ranged between 9 to 21, with an average of 13.5 bands per primer.

He also analysed accessions of the same plant with 6 AFLP primers and identified a total of 156 reproducible bands which ranged in size from 60 to 380 bp. Of these, 134 (85.9 %) were polymorphic. He found a range of 14 to 41 amplified bands per primer, with an average of 26 bands.

DNA extracted from an accession of the three species and observed 66 bands with 4 to 11 bands per primer.

Amnon Lvei et al. (2001) analysed accessions of Citrullus lanatus var lanatus using 138 RAPD primers that were initially screened among some cultivars of watermelon. Of the 138 RAPD primers only 35 primers produced polymorphic RAPD patterns. Of the later, 25 primers produced 288 reproducible RAPD bands that ranged in size from 100 to 3000 bp.

Amnon Lvei et al. (loc.cit) also analysed accession of Citrullus lanatus and C. colocynthis with 30 RAPD primers that were used for genetic diversity among the two species. The primers produced 662 bands that could be rated with high confidence and ranged in size from 100 to 2650 bp. The number of bands produced by each primer in the above study was relatively high (an average of 22 bands per primer).

Leah et al. (1999) analysed of molecular variation in accession of Cucumis melo L. and C. metuliferus by 18 RAPD primers and found a total of 176 bands. The numbers of polymorphic bands were 75 in Cucumis metuliferus and 96 in Cucumis melo.

In general the cluster analysis grouped the accessions according to many morphological traits and showed a clear separation between the species. The RAPD marker revealed considerable genetic diversity among species, a finding which strongly agrees with the great morphological variability observed among the Cucurbits in this study. Similar results were observed for the oriental Cucurbita moschata using RAPD marker (Jeon et al., 1994). RAPD marker systems have proved to be useful for analyzing the genetic diversity of some genera of the family Cucurbitaceae. The knowledge of the
diversity of this germplasm will facilitate its use in breeding programs and the improvement of management of large collections of the species of this family.
CONCLUSIONS & RECOMMENDATIONS

Conclusions
1. The studied Cucurbits are morphologically similar in their aerial parts and root habit but they are extremely diverse in their fruit characters.
2. Cucurbits are similar in tissue distribution and differentiation with respect to number of layers of cells and tissues in their roots, stems and leaves.
3. Cucurbits differ in their root vascular bundles. The vascular bundles are bicollateral in *Cucumis sativus* and *Luffa aegyptiaca*. However, in the other species they consist of four radial rays of primary xylem alternating with four rays of primary phloem.
4. Vascular bundles of most Cucurbits are arranged in two rings (outer and inner) except for *Cucurbita moschata* where the vascular bundles are arranged in only one ring.
5. Cluster analysis of extracted DNA of 10 accessions of Cucurbit species revealed the following:
   a. *Cucurbita moschata* and *C. pepo* are closely genetically related at a level of 38.6% genetic similarity.
   b. *Luffa aegyptiaca* has a genetic similarity of 20.6% to *Cucumis melo* var *reticulatus*, *C. melo* var *flexuosus*, *C. sativus* and *Ctenolepis cerasiformis*.
   c. *Cucumis melo* var *reticulatus* and *C. melo* var *flexuosus* are very closely genetically related with 62% genetic similarity.
   d. *Cucumis sativus* is genetically similar to *Cucumis melo* var *reticulatus*, *C. melo* var *flexuosus* at a level of 38.8% genetic similarity.
   e. *Ctenolepis cerasiformis* is closely related to *Cucumis melo* var *reticulatus*, *C. melo* var *flexuosus* and *C. sativus* at a level of 26.6% genetic similarity.
   f. *Citrullus lanatus* and *Colocynthis vulgaris* are closely genetically related with 36.2% genetic similarity.
g. *Coccinia grandis* is closely related to *Citrullus lanatus* and *Colocynthis vulgaris* at a level of 19.4% genetic similarity.

**Recommendations**

Cucurbits are of high economic importance in the Sudan. Thus the author recommends the following:

1. More official financial and technical support is needed to encourage vertical and horizontal cultivation of Cucurbits.
2. The utilization of new biotechniques to upgrade and improve Cucurbit production.
3. National research projects are needed for further studies on Cucurbits to cover areas that have not been covered in this project.
4. DNA markers are more specific tools in the identification and characterization of species especially at the variety levels. It can be seen from this study that cluster analysis by RAPD markers confirmed and coincided with the traditional classification of some Cucurbits eg. *Cucurbita moschata* and *C. pepo*; *Cucumis melo* var *reticulatus* and *C. melo* var *flexuosus*.
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