*Original Research Article*

# Karyological Studies and Chromosomal Analysis of Fifteen Accessions of *Trichosanthes cucumerina* L. (Snake Gourd).


## Abstract

This is to investigate variability of karyological and chromosomal numbers of fifteen accessions of *Trichosanthes cucumerina* var *anguina* and *Trichosanthes cucumerina* var *cucumerina* L. (Snake Gourd) from four geopolitical zones in Nigeria. The studies were carried out using the root tips from the plant seedlings. The fifteen ecotypes were germinated in glass petri dishes covered with moisten cotton wool in the laboratory of the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria and was observed for 6-11days. The glass petri dishes were kept in a dark cupboard for warmth needed for germination. Squashed and stained root tips with acetone orcein. The viewed somatic chromosome number of the fifteen ecotype of snake gourd (*Trichosanthes cucumerina* L.) studied from four geopolitical zone of Nigeria was 22. The karyological formular differ from each other. The karyological formular ranged from(5m+6sm-9m+2sm). Short arm(S) length ranged from (1.28±0.01-1.38±0.05). Long arm (L) length ranged from (1.79±0.02-1.90±0.03). The total length (S+L) ranged from (3.08±0.01-3.33±0.13). The arm ratio(L/S) ranged from (1.29-1.48). The relative value (S/L) demonstrated variability among the ecotypes which varied from (0.67 – 0.72um). Centrometric index (S/L+ S/µm) affirmed variability among the ecotypes which varied from (1.95 – 2.15). Coefficient of variation of the fifteen ecotypes varied from (18.29 – 28.63%). The total form in percentages ranges from (66 – 77%). Standard deviation among the ecotypes studied ranges from (0.29 – 0.46). The variance among the 15 ecotypes affirmed variability (0.08 – 0.22). The mean displayed differences among the ecotypes ranged from (1.54 – 1.67). The long arm and short arm lengths determined were more at zero (0.00) which demonstrated closest genotype with same karyotype composition in the four ecotypes were closely related. There were ecotypes closer to zero such as 0.02, 0.08, 0.04, - 0.04, have almost the same karyotype composition. Ecotypes within red colour lines have close karyotypes similarities (V2/V3). $V_3$, $V_{14}$, $V_{11}$, $V_6$ and $V_8$ were within yellow colour lines and shared karyotype similarities. V7, V14, V12, V15, were in the same blue lines and shared karyotype similarities. V10, V2, V5 and V4 were distinct from others in terms of their karyotype compositions. The chromosomal number of the fifteen accessions are the same 22. The karyotypes have similarities such as V2, V3, V14, V11, V6 and V8. Snake gourd from Edo State, Kadroko, Nassarawa State, Ukwa, Abia State and Ilorin, Kwara State shared karyotype similarities. V10, V2, V5 and V4 displayed variabilities among ecotypes studied.



Keywords: *Trichosanthes cucumerina*, Chromosomal studies, Karyology, long arm, short arm, Ecotypes.

## Introduction

In Nigeria, indigenous people traditionally use a wide range of plants as food and medicine. *Cucurbitaceae* family is genetically diverse, it contains about 130 genera and approximately 900 species, mainly distributed in tropical and sub-tropical regions [1]. The cucurbits family is as one of the economically groups, including the most species providing human with edible products and beneficial fibers [2]. Plants of this group have high genetic diversity for fruit shape and other fruit characteristics as a result of their above ground development [2].

Snake Tomato (*Trichosanthes cucumerina*), also known as viper gourd or long tomato, is a well-known plant, with fruit mainly consumed as a vegetable [3]. Snake gourd originated in India or the Indo-Malayan region in tropical Asia commonly cultivated in Sri Lanka, India, Malaysia, Peninsula, and Philippine [3]. *Trichosanthes cucumerina* is a natural antibiotic expectorant, laxative, cure constipation and has been shown to be excellent for diabetes [4]. *T. cucumerina* is used in treatment of wounds, boils, sores, skin eruptions, such eczema and dermatitis [5].

*Trichosanthes cucumerina* commonly called snake gourd, viper gourd, long tomato or snake tomato [3]. *Trichosonthes cucumerina* is an annual climber with a diploid chromosome of twenty-two (22) (2n).[6]. *Trichosanthes cucumerina* L. belongs to the family Trichosanthes which is the one of the important family of Cucurbitaceae [7]. Trichosanthes L. is the largest genus of the cucurbitaecae with 918 species [8][9][10][11] [12][13][14] [15] [16][17]. *Trichosanthes* has two cultivated species Trichosanthes anguina L. and Trichosanthes dioca Roxb and several wild species[18]. Trichosanthes cucumeriodes (ser). Maxim (Japanese sake gourd) is cultivated in Japan and China as a source of starch[17]. Trichosanthes cucumerina L have a wide variability, *Trichosanthes lobota, Trichosanthes wallichiana, Trichosanthes cordata Roxb, Trichosanthes laponica and Trichosanthes nervifolia* L. [18].

The objective of this study enumerates cytological characters by counting the number of chromosomes and karyological characteristics of the root tips of the plant.

## MATERIALS AND METHODS

Viable seeds of snake gourd (*Trichosanthes cucumerina)* ecotypes were sourced and collected from different from the following locations (Table 1).

Table 1 : Viable seeds of snake gourd (Trichosanthes cucumerina) ecotypes

| S/N | SOURCE LOCATION | ECOTYPE | DESIGNATION |
|---|---|---|---|
| 1 | Oshogbo Osun State 1 | Rainforest | V1 |
| 2 | Iwo, Osun State 2 | Rainforest | V2 |
| 3 | Makurdi, Benue State 1 | Derived savannah | V3 |
| 4 | Adikpo, Benue State 2 | Derived savannah | V4 |
| 5 | NHST – 0588 | Guinea savannah | V5 |
| 6 | Ikwuano, Abia State 1 | Rainforest | V6 |
| 7 | Ukwa, Abia State 2 | Rainforest | V7 |

| | | | |
|---|---|---|---|
| 8 | NAGRAB – 00753 | Guinea savannah | V8 |
| 9 | Ikom, Cross River State | Rainforest | V9 |
| 10 | Rumibekwe, River State | Rainforest | V10 |
| 11 | Oye-Ekiti, Ekiti State | Rainforest | V11 |
| 12 | Nasarawa State | Derived savannah | V12 |
| 13 | Elelenwo – Rivers State | Rainforest | V13 |
| 14 | Benin, Edo State | Rainforest | V14 |
| 15 | Ilorin, Kwara State | Guinea savannah | V15 |
| | **TOTAL** | | **15** |

The fifteen ecotypes were germinated in glass petri dishes covered with moisten cotton wool in the laboratory of the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike and was observed for 6-11days. The glass petri dishes were kept in a dark cupboard for warmth needed for germination. Young healthy roots (about 15minutes) were carefully collected at two-hour interval from 7:00 and 9:00am after two hours of germination for all the genotypes. Clean sterilized scalpel was used to excise the growing root tips of *Trichosanthes cucumerina* L. The harvested root tips of *Trichosanthes cucumerina* L genotypes were rinsed twice in distilled water and pre-treated in 0.002ml solution of 8-hydroxylquinoline 0.058g dissolved in 100ml of distilled water for four (4) hours. The Pretreated root tips were rinsed twice in distilled water and fixed in 3:1 glacial acetic alcohol (3-part glacial acetic, to 1 parts ethanol). The fixative was freshly prepared for use and the root tips were fixed at room temperature for 24 hours, after which they were stored in 70% ethanol for further use. The fixed root tips were again rinsed twice in distilled water then hydrolyzed in water bath and controlled at 60°C for six (6) minutes.

The root tips were stored in 70% ethanol solution, and preserved in a refrigerator until it was needed for squashing. The hydrolyzed root tips were rinsed twice in distilled water, placed on a clean grease free slides (one root tip per slide) and excess fluid was removed using drying paper. The apical 1mm (whiter and denser) portion of the root tips were carefully cut off on the slide. One to two drops of 1% acetone orcein was added to the specimen and the material was macerated thoroughly. A thin cover slip was laid on top of the specimen and the slide was placed in a folded filter paper, and thumb pressure was applied to remove excess stain. The cover slip was tapped gently with the blunt end of a biro. Tapping continued until the materials became well spread out and hardly visible. The corner slip was sealed with nail vanish and then viewed under the low and high power digital AMSCOPE 3000 camera microscopes. The slides were placed on the stage of the microscope and then adjusted for proper and clearer views at various magnifications; Photomicrographs of the cells at various stages of mitosis were taken at x4 x10, x40 and x100 with oil immersion.

Fresh root tips of 1 – 1.5 cm long was cut from rapidly growing seedlings (2 – 3 weeks after planting). The root tips was pretreated with 0.5 g of 8 – hydroxyquionolene in 100 ml of distilled water at room temperature for 3 hours. Samples of the root tips were subsequently washed three

times with distilled water for 5 minutes at room temperature. The root tips were then fixed in Carnoy's fixative (Glacial acetic acid: Ethanol 3:1) overnight or for 24 hours at room temperature. Thereafter there was a thorough washing with distilled water after which a 1 NHCl was added using a dropper for hydrolysis. The root tips were placed in 1NHCl solution in a test tube and place in a water bath at $60^0$ C for 6 – 8 minutes. Thermometer was used to measure and ensure that the needed temperature was achieved. The hydrolyzed root tips were allowed to stand for 10 minutes to hydrolyze the cells. The HCl was then removed from the test tubes using the dropper. The petri dish was refined with distilled water twice. The root tips were removed and placed in a glass slide with the help of a forcep the root tips were squashed properly. The squashed root tips were then stained using 2% aceto orcein carmine. The stain was added at the centre of the slide (microscopic slide) and allowed to stand for 10-15 minutes to enable the root tips pick up the stain.

The best metaphase plates in terms of clarity were photographed using an external camera Amscope digital Camera 3000MA to the microscope and laptop and scanned at 1000 – resolutions.

All measurements were recorded using the software images and measurement options. Chromosomal morphology was described using nomenclatures proposed by [19] while numerical characterization was done using other parameters.

**Formulae used in the study**

The arm ratio (AR) was computed as the ratio of long arm length of a chromosome pair to the short arm length of the chromosome pair using the formulas;

1. AR = L/S
   where L = long arm length of chromosome
   S = short arm length of chromosome
2. TL = L + S
   Where L = long arm length of chromosome
   S = short arm length of chromosome
3. R – Value = S/L
   where L = long arm length of chromosome
   S = short arm length of chromosome
4. % TF = ΣS/ΣL X 100
   % TF = percentage total form
   ΣS = Sum of short arm length
5. Centrometric index (CI) = (S/L + S)
   where L = long arm length of chromosome
   S = short arm length of chromosome
6. Centrometric gradient (CG) = SX/TLX x 100
   where SX = Length of median short arm chromosome
   TLX = Total length of long arm of chromosome

7. CV = SD/Mean x 100
   CV = Coefficient of variability
   SD = Standard deviation

Karyotype asymmetry was estimated using two numerical parameters described to[20] as follows

8. Intrachromosomal index ($A_1$)

8. Interchromosomal index ($A_2$)

A1 = ΣSX/LX / N

A2 = SD/X

where SX = Length of median short arm chromosome
LX = Length of long arm of chromosome
SD = Standard deviation
X = Mean Chromosome length
N = Number of homologue (n=12)

**Statistical analysis of data**

The data generated from the study on short arm length, long arm length, arm ratio, R-value and centrometric index were analyzed using the PAST 3 software procedure and test with the [21].

**Results**

The cytological investigation revealed that meristematic cells of *Trichosanthes cucumerina* L was a diploid plant 2n=22 chromosomes and the principal chromosomes x=11 for all the snake gourd ecotypes. This is the somatic metaphase (Plate 1).

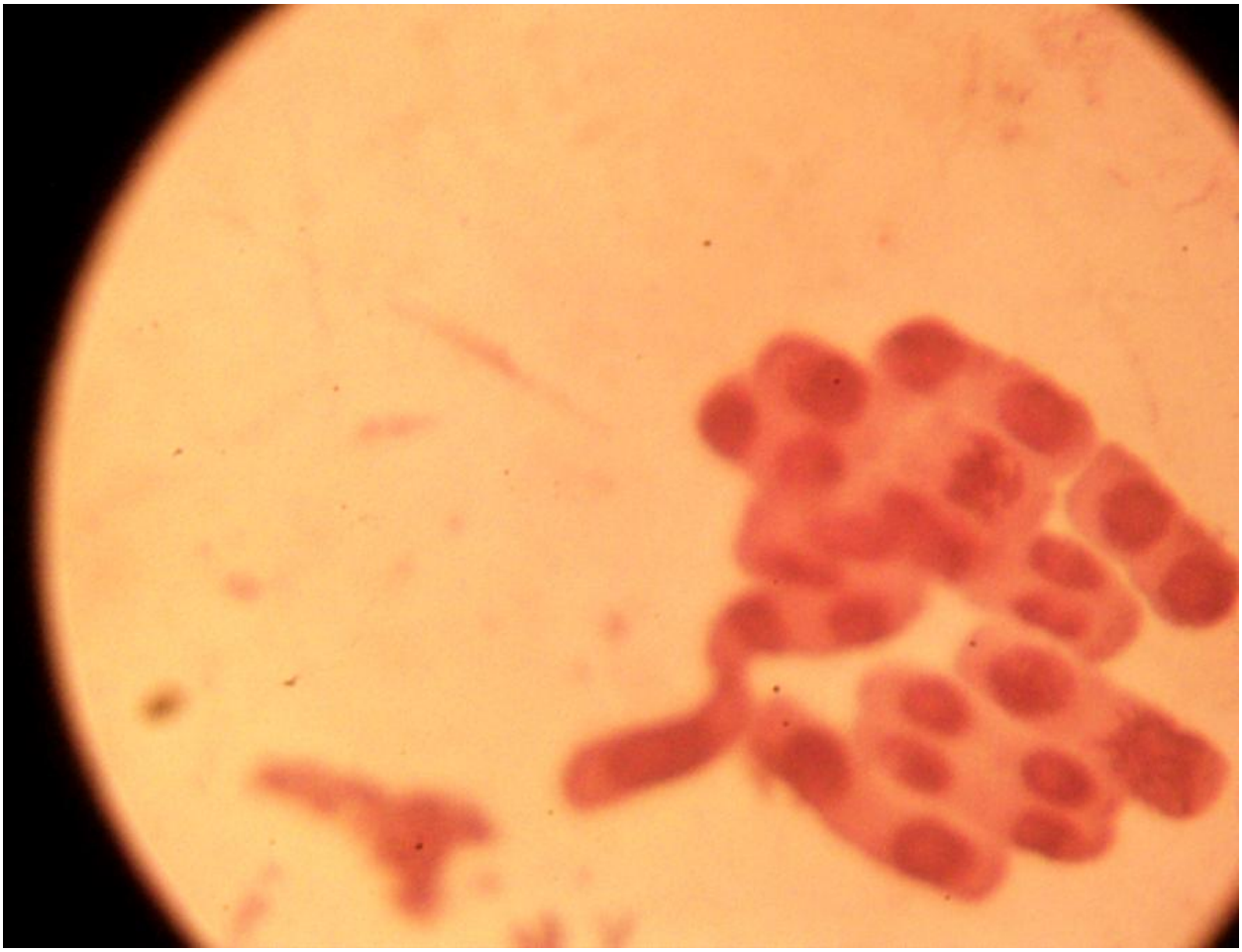
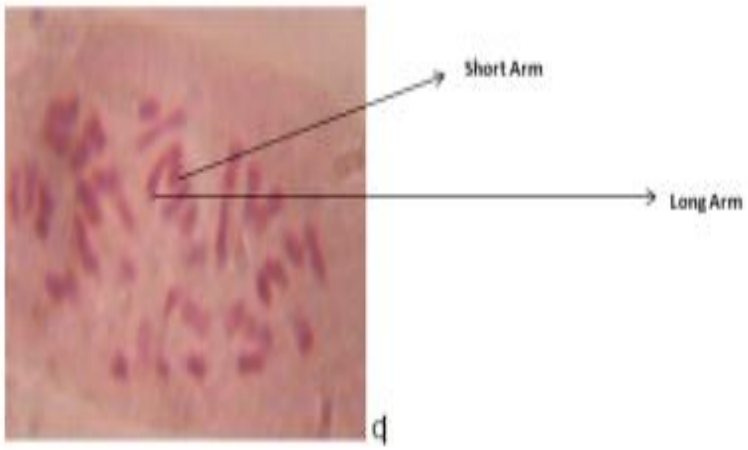


Plate 1: Somatic metaphase phase

Plate 2: This is the somatic metaphase plate of *T.cucumerina* 2n=22 showing the long arm and short arm of the chromosome.




*T. cucumerina* short arm (S) ranged from (1.29±0.05 – 1.38±0.05µm), long arm (L) ranged from (1.79±0.02 – 1.98 ±0.00µm), total length (S+L) revealed the variability among the ecotypes (3.08±0.03 – 3.33±0.13µm). The arm rate (L/S) um among the ecotypes confirmed that it ranged from (1.37 – 1.48um). The relative value (S/L) demonstrated variability among the ecotypes which varied from (0.67 – 0.72um). Centrometric index (S/L+ S) affirmed variability among the ecotypes which varied from (1.95 – 2.15µm) Table 2.

Table 2: Short arm (S), long arm (L), total length (S+L), arm ratio (L/S), relative value (S/L) and centrometric index (S/L + S) of 15 genotypes of *Trichosanthes cucumerina* L

| *Trichosanthes cucumerina* | Short arm (S) µm | Long arm (L) µm | Total Length (S +L) µm | Arm Ratio (L/S) µm | R-Value (S/L) µm | Centrometric index (S/L +S) µm |
|---|---|---|---|---|---|---|
| OSH, OSU | 1.29± 0.05 | 1.79±0.02 | 3.08± 0.01 | 1.38 | 0.72 | 2.01 |
| NAGRAB | 1.33±0.01 | 1.87±0.11 | 3.20±0.03 | 1.41 | 0.71 | 2.04 |
| NHST 0588 | 1.35±0.03 | 1.98±0.10 | 3.33±0.13 | 1.51 | 0.66 | 1.96 |
| IWO, OSU | 1.32±0.10 | 1.89±0.03 | 3.21±0.01 | 1.43 | 0.69 | 2.01 |
| IKWUANO | 1.35±0.03 | 1.98±0.01 | 3.33±0.13 | 1.46 | 0.68 | 2.03 |
| CRS-IKM | 1.32±0.00 | 1.88±0.10 | 3.20±0.03 | 1.42 | 0.70 | 2.02 |
| RIV-RUM | 1.33±0.01 | 1.84±0.01 | 3.17±0.03 | 1.38 | 0.72 | 2.05 |
| ABI-UKWA | 1.31±0.02 | 1.89±0.03 | 3.20±0.01 | 1.42 | 0.69 | 2.00 |
| EKI-OYE | 1.31±0.01 | 1.88±0.05 | 3.19±0.019 | 1.37 | 0.70 | 2.01 |
| NAS-KDK | 1.38±0.05 | 1.79±0.03 | 3.17±0.03 | 1.29 | 0.71 | 2.15 |
| KWA-ILO | 1.32±0.01 | 1.87±0.01 | 3.19±0.03 | 1.42 | 0.72 | 2.03 |
| RIV-ELE | 1.28±0.01 | 1.90±0.03 | 3.18±0.03 | 1.48 | 0.69 | 1.95 |
| EDO-BEN | 1.32±0.03 | 1.89±0.01 | 3.21±0.01 | 1.43 | 0.69 | 2.01 |
| BEN-MKD | 1.32±0.03 | 1.86±0.01 | 3.18±0.05 | 1.45 | 0.71 | 2.03 |
| BEN-MKD | 1.29±0.01 | 1.89±0.03 | 3.18±0.01 | 1.47 | 0.68 | 1.97 |

**Legend:** ABI-UKW= Abia-Ukwa, KWA-ILO= Kwara-Ilorin, OSU-OSH-1=Osun-Oshogbo 1, BEN-MKD1=Benue-Makurdi 1, RIV-RUM= Rivers-Rumibekwe, ABI-IBE= Abia- Iberenta, EKI-OYE= Ekiti-OyeEkiti, BEN-MKD2= Benue-Makurdi 2, RIV-ELE= Rivers-Elelenwo, OSU-OSH-2= Osun-Oshogbo 2, ABI-IKW=Abia-Ikwa, EDO-BEN= Edo-Benin, NAS-KDK= Nasarawa-Kardarko, CRS-IKM= Cross Rivers-Ikom

Table 3: Coefficient of variation of the fifteen ecotypes varied from (18.29 – 28.63%). The total form in percentages ranges from (66 – 77%). Standard deviation among the ecotypes studied ranges from (0.29 – 0.46). The variance among the 15 ecotypes affirmed variability (0.08 – 0.22). The mean displayed differences among the ecotypes which ranged from (1.54 – 1.67). Karyotype formular differs among the ecotype which was (8m + 3sm – 5m + 6sm).

| *Trichosanthes Cucumerina* GENOTYPES | CV (%) | TOTAL FORM (%) | STD DEV | VARIANCE | MEAN | KARYOTYPE FORMULA (Xm+Ysm) |
|---|---|---|---|---|---|---|

Table 3: *T. cucumerina* coefficient of variation total form, standard deviation, variance, mean and karyotype formula of the fifteen ecotypes was revealed in this table.

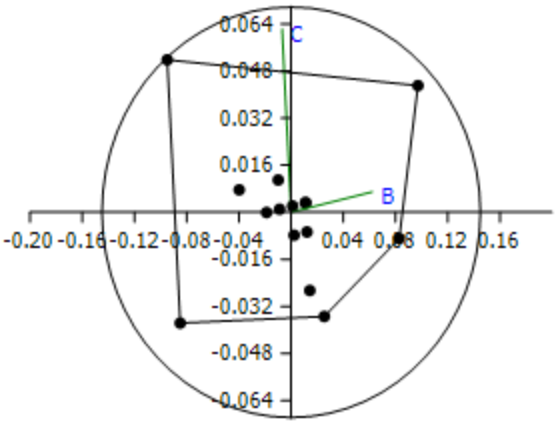
| | | | | | | |
|---|---|---|---|---|---|---|
| OSH, OSU | 25.11 | 69 | 0.40 | 0.16 | 1.61 | 8m+3sm |
| OSH, OSU | 22.95 | 72 | 0.35 | 0.13 | 1.54 | 7m+4sm |
| NAGRAB | 23.86 | 71 | 0.38 | 0.14 | 1.60 | 6m+6sm |
| NHST | 28.63 | 66 | 0.46 | 0.22 | 1.63 | 7m+5sm |
| ABI-IKW | 26.75 | 68 | 0.45 | 0.19 | 1.67 | 5m +6sm |
| CRS-IKM | 24.75 | 70 | 0.39 | 0.16 | 1.60 | 7m +4sm |
| RIV-RUM | 22.75 | 72 | 0.36 | 0.13 | 1.58 | 6m +sm |
| ABI-UKW | 25.63 | 69 | 0.41 | 0.17 | 1.68 | 7m+4sm |
| EKI-OYE | 25.27 | 70 | 0.40 | 0.16 | 1.59 | 8m+3sm |
| NAS-KDK | 18.29 | 77 | 0.29 | 0.08 | 1.58 | 9m+2sm |
| KWA-ILO | 24.38 | 71 | 0.38 | 0.15 | 1.59 | 7m+4sm |
| RIV-ELE | 27.57 | 67 | 0.43 | 0.19 | 1.59 | 6m+5sm |
| EDO-BEN | 25.12 | 69 | 0.40 | 0.16 | 1.60 | 8m+3sm |
| BEN-MKD | 24.01 | 71 | 0.38 | 0.14 | 1.59 | 8m+3sm |
| BEN-MKD | 26.68 | 68 | 0.42 | 0.18 | 1.59 | 6m+5sm |

**Legend:** ABI-UKW= Abia-Ukwa, KWA-ILO= Kwara-Ilorin, OSU-OSH-1=Osun-Oshogbo 1, BEN-MKD1=Benue-Makurdi 1, RIV-RUM= Rivers-Rumibekwe, ABI-IBE= Abia- Iberenta, EKI-OYE= Ekiti-OyeEkiti, BEN-MKD2= Benue-Makurdi 2, RIV-ELE= Rivers-Elelenwo, OSU-OSH-2= Osun-Oshogbo 2, ABI-IKW=Abia-Ikwa, EDO-BEN= Edo-Benin, NAS-KDK= Nasarawa-Kardarko, CRS-IKM= Cross Rivers-Ikom

**CN = Chromosome number, 2n = diploid; M = metacentric chromosome and Sm = Sub metacentric chromosomes**
CV = Coefficient of variation
TF = Total form
KF = Karyotype formula XM + YSm
**Legend:** ABI-UKW= Abia-Ukwa, KWA-ILO= Kwara-Ilorin, OSU-OSH-1=Osun-Oshogbo 1, BEN-MKD1=Benue-Makurdi 1, RIV-RUM= Rivers-Rumibekwe, ABI-IBE= Abia- Iberenta, EKI-OYE= Ekiti-OyeEkiti, BEN-MKD2= Benue-Makurdi 2, RIV-ELE= Rivers-Elelenwo, OSU-OSH-2= Osun-Oshogbo 2, ABI-IKW=Abia-Ikwa, EDO-BEN= Edo-Benin, NAS-KDK= Nasarawa-Kardarko, CRS-IKM= Cross Rivers-Ikom

The scatter diagram revealed that long arm and short arm length for the determined the proximities for karyotyping.

The long arm and short arm length which determined was more at zero (0.00) which demonstrated closest genotype with same karyotype composition in the diagram four ecotypes were closely related. There were ecotypes closer to zero such as 0.02, 0.08, 0.04, - 0.04, have almost the same karyotype composition.

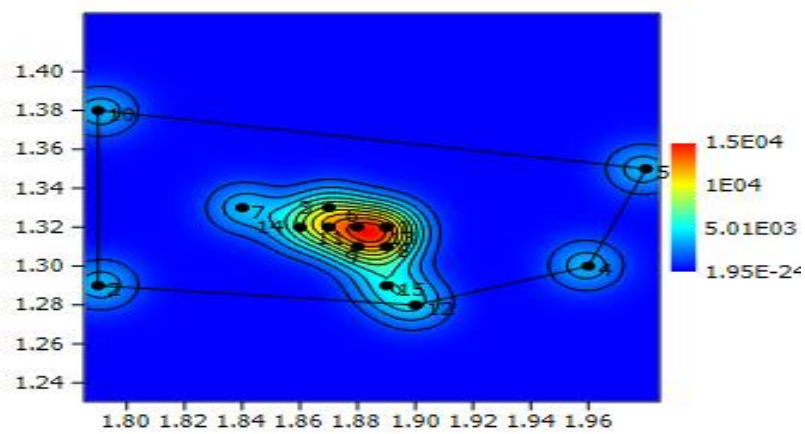



Scatter diagram incorporating long arm and short lengths for the genotype chromosomes for determining proximities for karyotyping

Fig I: Iillustrated the relationship between fifteen *T. cucumerina* ecotypes investigated.

Karyotypes mapping using kernel density chord confirmed. *T. cucumerina* ecotypes and how they were related to each other. Ecotypes within red colour lines have close karyotypes similarities (V2/V3). $V_4$, $V_{14}$, $V_{11}$, $V_6$ and $V_8$ were within yellow colour line share karyotype similarities. V7, V14, V12, V15, were in the same blue lines and share karyotype similarities. V10, V2, V5 and V4 were distinct from others in terms of their karyotype compositions.

Figure 2: **Kernel density chart showing 15 *T. cucumerina* genotype karyotypes mapping**



Y – axis = Short arm lengths

X – axis = Long arm lengths

## DISCUSSION

*Trichosanthes cucumerina* (L) is underutilized crop in Nigeria and is disappearing from the home stead of our various garden. The cytological studies of *T.cucumerina* was investigated to appropriate their taxonomic evidences.The chromosome number of the fifteen of *T. cucumerina* (snake gourd) (Plate 1 – 2) were 2n = 2x = 22 which is in agreement with the work of [22] who reported that *T. cucumerian var anguina* and *T. cucumerian var cucumerina* had the same chromosome number but differs in their karyotype. The karyotype of 15 ecotypes of *T. cucumerina* varied considerably depending on the position of metacentric and submetacentric chromosomes and its ranged from short arm (S) (1.29±0.05-1.38±0.05) long arm (1.79±0.02-1.98+0.00) total length (S + L/Nm ( 3.08±0.03 – 3.33 ± 0.134m), arm ratio (1.37 – 1.48Nm), relative value (0.67 – 0.72µm, centrometric index (1.95 – 2.15µm). This is in agreement with the findings of [23] who reported that *Syngonium podophyllum* chromosome length ranged from (1.44 + 0.08µm – 3.13+ 0.18µm), short arms (0.99 ± 0.10 – 1.83 ±0.11µm), arm ratio (1.23 – 2.12µm) Karotype formula of 7m + 5sm. This work is not in agreement with the work of [6] who reported on the chromosome of six commercial cultivated cucuribits ranged from 2n = 26, 26, 22, 24,14 and 22. The karyotype formula differs from the karyotype of six edible cucurbits. The karyotype formular ranged from (8m + 3sm – 6m + sm) while that of [6] ranged from 26m – 2Sm + 20m. This work is not in agreement with the mean length of chromosome of genus Ornithogalum L. which ranged from 1.20 – 7.63µm as reported by [24] .

This work is not in agreement with [25] who reported that *Centaurear species* has different basic chromosomes numbers which were 2n = 18, C demirizii 2n = 16, C. leptophylla, 2n = 36, C. Baligna 2n = 36. The karyological formular ranged from 4m + 5sm – 3m + sm. [26] investigated chromosome number and karyotype of sixteen taxa of genus *Cirsium mill* and observed that chromosome number were the same but their karyotype varied. [27] reported that *Trichosantes*

*borenenssis, T.motanna, T.ovigera, T.pubera, T.quinquanglata, T.tricupspidta, T.villosa, T.warwrae* and *T.cucumeriina var anguina* have the same somatic chromosome number of (2n=22).

CONCLUSION: Chromosome and karyotype composition and formular of the fifteen accessions of *T. cucumerina*. The chromosome number of twenty-two (22) was confirmed by this work inspite of picking the *T.cucumerina* ecotypes from different locations . The karyotype formular varied considerably. The following ecotypes karyotypes was distinct Rumibekwe, River State, Iwo, Osun State, NHST-0588 and Adikpo, Benue State.

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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