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KARYOTYPIC ANALYSES OF ACCESSIONS OF KERSTING'S GROUNDNUT

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ABSTRACT

Kersting's groundnut (*Macrotyloma geocarpa*) is an orphan crop of significant food and household income in sub-Saharan Africa. The objective of this study was to evaluate the chromosomal variability among the accessions of the species through analysis of the karyotypes. Six accessions of Kersting's groundnut were subjected to conventional cytological techniques, using root tip meristems. The somatic chromosome number was $2n = 20$ in all the accessions. The highest total chromosome length was obtained in Tkg 1 (1.739 ± 0.047); while the least was in Tkg 12 (1.014 ± 0.029). All the accessions had metacentric, submetacentric and subtelocentric chromosomes. However, there was a preponderance towards metacentric chromosomes, suggesting a symmetrical karyotype, which is known to be a primitive condition. Five of the accessions were grouped in karyotype 2A while one accession, Tkg 9 was grouped in karyotype 2B. The 2B karyotypes are known to have greater chromosomal asymmetry than 2A; thus Tkg 9 may be advancing evolutionarily faster than the other accessions. Hierarchical cluster analysis divided the accessions into three clusters. Cluster 1 comprised of four accessions (Tkg 1, Tkg 6, Tkg 9 and Tkg 11); while Cluster 2 comprised of one accession (Tkg 6); and cluster 3 had one accession (Tkg 12). Tkg 6 and Tkg 12 were observed to be distantly related to the other accessions; as such could serve as parents in hybridisation programmes to generate wider variability for the improvement of the species.

Key Words: Chromosome, hybridisation, *Macrotyloma geocarpa*, Metacentric

RÉSUMÉ

L'arachide de Kersting (*Macrotyloma geocarpa*) est une culture orpheline alimentaire et de revenus significatives des ménages en Afrique Subsaharienne. L'objectif de cette étude était d'évaluer la variabilité chromosomique parmi les accessions de l'espèce en utilisant les analyses des caryotypes. Les six accessions ont été soumises à des techniques cytologiques conventionnelles en utilisant des méristèmes de la pointe des racines. Le nombre de chromosomes somatiques était de $2n = 20$ dans toutes les accessions. La longueur totale de chromosome la plus élevée a été obtenue en Tkg 1 ($1,739 + 0,047$); tandis que le moins était en Tkg 12 ($1,014 + 0,029$). Toutes les accessions avaient des chromosomes métacentriques, submétacentriques et subtélocentriques. Cependant, il y avait une

prépondérance de chromosomes métacentriques, suggérant un caryotype symétrique qui est connu pour être une condition primitive. Cinq des accessions ont été regroupées dans le caryotype 2A tandis qu'une accession, Tkg 9 a été regroupée dans le caryotype 2B. Les caryotypes 2B sont connus pour avoir une plus grande asymétrie chromosomique que 2A; ainsi Tkg 9 peut évoluer plus vite que les autres accessions. L'analyse hiérarchique des clusters a placé les accessions en trois clusters. Groupe 1 composé de quatre accessions (Tkg 1, Tkg 6, Tkg 9 et Tkg 11); tandis que le groupe 2 comprenait une accession (Tkg 6); et le groupe 3 avait une accession (Tkg 12). On a observé que les Tkg 6 et Tkg 12 étaient éloignées des autres accessions; en tant que tel pourrait servir de parents dans les programmes d'hybridation pour générer une plus grande variabilité pour l'amélioration de l'espèce.

Mots Clés: Chromosome, hybridation, *Macrotyloma geocarpa*, métacentrique

INTRODUCTION

Kersting's groundnut (*Macrotyloma geocarpa*) is an orphan crop of significant food and household income in sub-Saharan Africa (SSA) (Coulibaly, 2020). The plant belongs to the leguminous family Fabaceae (ITIS, 2011) and is cultivated in West Africa, Tanzania, Mauritius and Fiji primarily for its edible seeds (Achigan-Dako and Vodouhe, 2006). The seeds are richer in amino acids such as lysine and methionine, than most of the other legume crops (Ajayi and Oyetayo, 2009).

The crop is also of immense medicinal importance, being reportedly effective against diarrhoea, poisoning, dysentery, venereal diseases, fever and diabetes (Achigan-Dako and Vodouhe, 2006; Amujoyegbe *et al.*, 2007). Furthermore, the plant plays a role in improving soil fertility in association with nitrogen fixing bacteria of the Bradyrhizobia type (Adazebra, 2013).

According to Amujoyegbe *et al.* (2007), Kersting's groundnut is on the verge of extinction due to lack of efforts to develop it. Similarly, Adu-Gyamfi *et al.* (2012) noted that the crop is likely to disappear from the food basket of households in sub-Saharan Africa unless efforts are engineered to rescue it. As of 2006, only 12 accessions were conserved in the seed gene bank of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria (Achigan-Dako and Vodouhe, 2006).

Moreover, Ayenan and Ezin (2016) quoting several authors, noted only about 100

accessions conserved in various gene banks in Benin, Ghana, France and Belgium. The authors advocated for extensive characterisation and evaluation of the available germplasm.

According to Achigan-Dako and Vodouhe (2006), little is known about the genetic diversity of *M. geocarpa* and no breeding programmes exist on them; thus there are no improved varieties thus far. In particular, complete knowledge and understanding of the genetic diversity among the available germplasm is necessary as a basis for meaningful development of the crop. Ghafor and Ahmad (2005) earlier reported that information about genetic diversity of germplasm is a useful tool in genebank management and in planning experiments as it assists researchers in identifying and/or eliminating duplicates in the genestock.

Baltisberger and Horandl (2016) and Kawano (2018) are of the opinion that karyotyping is a powerful method for characterising genomes of species. These authors further wrote that karyotypic features are important factors for delineating plant species because chromosomal differences establish immediate postzygotic crossing barriers. In other words, species with different karyotypes would not be able to cross; while those with same karyotype would be expected to produce hybrids. The distinctive feature of karyotyping is the ability to differentiate closely related species that have the same chromosome number (Saensouk *et al.*, 2019).

Prajitha and Thoppil (2018) noted that genetic variability and evolutionary status of the species are indices of survival of a species to changing environmental conditions.

The objective of this study was to provide the first karyotype of this species and to assess the chromosomal variability among the studied accessions.

MATERIALS AND METHODS

Six accessions of *M. geocarpa* used in this study were obtained from the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. The accessions were labelled by IITA as Tkg 1, Tkg 6, Tkg 8, Tkg 9, Tkg 11 and Tkg 12. This karyotypic study was done in the Cytology Laboratory of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Mitotic root tips of the respective accessions were used for the study.

Fifty seeds of each accession were chemically scarified by soaking them in beakers containing 96% concentrated anhydrous sulphuric acid. This was done for 5 minutes until gradual appearance of small black spots on the surface of the seeds (Garcia *et al.*, 2006). Subsequently, they were rinsed three times with sterile distilled water to ensure maximum removal of the sulphuric acid to avoid local heating that may damage the seeds.

A pinch of white sand was put into six labelled sieves, which were randomly placed on a laboratory bench and the rinsed seeds were placed in respective sieves. Sand was added to assist in the germination process. The sieves were later placed on beakers which were filled with distilled water. The base of the sieves slightly touched water in the beakers so as to keep the sand wet and to avoid drying out of the seeds. As the seeds began to germinate after five days, the roots penetrated through the sieves into the water in the beaker, thereby giving uncontaminated healthy roots.

On the 7th day between 7.30 and 8 am, the tap roots (1-2 cm) of the respective accessions

were chopped off using a small scissors and put into beakers containing distilled water to rinse them (From preliminary studies (unpublished) more dividing cells were observed between 7.30 and 8 am). By gentle shaking of the beakers for two minutes, the roots were cleaned off and the water decanted. The root tips (30 for each accession) were placed separately for a minute on blotting papers in order to remove adhering water before pretreatment.

For the pretreatment, 0.05% colchicine was prepared and poured into six different specimen bottles. Colchicine helps to break spindle fibres and thus arrest cell division at metaphase stage. The seeds were subsequently put into the labelled bottles and left there for 5 hours at the laboratory room temperature (25 °C). After 5 hours, they were removed and put into beakers and rinsed under running tap water to wash off the pretreatment solution. The excess water on the root tips were blotted off before putting the seeds in Carnoy's fixative solution in labelled specimen bottles, for duration of 24 hours. The fixative consisted of 1:3 glacial acetic acids to absolute alcohol. The fixed root tips were hydrolysed by placing them in 18% HCl for 8 minutes. The HCl was first heated to 60 °C in a water bath, within 1 hour before placing the root tips in it.

The respective fixed root tips were placed in 2% aceto-orcein stain and heated on a burner until the stain boiled. The heating process enabled the maceration of root tips, as well as allowing the stain to penetrate into the cells.

With the aid of a scalpel, 2 mm pieces of the root tips were cut from the hydrolysed root tips and placed on slides, one root tip per slide. The root tips were macerated with the scalpel. The samples were covered using coverslips without enclosing air bubble. A piece of blotting paper was gently used to absorb the excess stain spilling from the sides of the cover slips. By carefully placing the thumb on the cover slip without moving the cover slip,

the cells were squashed and spread under the cover slip to avoid chromosome overlap.

The slides were viewed under a microscope at $\times 10$, then $\times 40$ and the good and clear stages were viewed under oil immersion ($\times 1000$) for photomicrography (with Motic camera 2.0 version). At least 10 metaphase plates were examined for the derivation of chromosome numbers, but the measurements of the individual chromosome lengths (short arm and long arm lengths) were obtained from three well spread metaphase plates of each accession and these were taken in microns using the software Karyo-Type 2.0 version.

The arm ratio (AR) (long arm LA/short arm SA), the centromeric index, F% for the estimation of karyotype asymmetry (the short arm length /the length of the chromosome $\times 100$) were calculated using the method of Huziwara (1962). Karyotype symmetry nomenclature was determined based on Stebbin's (1971) classification.

The chromosomes were described according to the methods of Levan *et al.* (1964) who proposed a standard nomenclature for centromeric position as follows: "The chromosome with an arm ratio of 1.0-1.7 = metacentric, 1.7-3.0 = submetacentric, 3.0-7.0 = subtelocentric". Arm ratios at the borders defining these chromosome types were described according to the method of Baltisberger and Horandl (2016). Thus, the karyotypic formulae were derived from these descriptions and the length of the chromosome arms were used to draw the ideograms.

Hierarchical cluster analysis was performed using Wards Linkage with squared Euclidean distance (SPSS Version 20). Subsequently, a dendrogram was drawn to depict the relationships among the accessions. The data on short arm lengths, long arm lengths and total chromosome lengths were subjected to Analysis of Variance in Completely Randomised Design (CRD). Significant means were separated by using Least Significant difference tests at the probability of 5%.

RESULTS

The Chromosome number of each of the accessions was found to be $2n = 20$ (Fig. 1). Table 1 shows that the short arm lengths ranged from 0.374 μm for Tkg 12 to 0.717 μm for Tkg 1. There were significant differences ($P < 0.05$) among the values for the accessions. Tkg 1 exhibited the maximum short arm length but its length did not differ significantly ($P > 0.05$) from those of Tkg 8 and Tkg 11. The least short arm length was displayed by Tkg 12 and it differed significantly from those of the other accessions.

The long arm length ranged from 0.640 μm for Tkg 12 to 1.022 μm for Tkg 1 (Table 1). There were also significant differences among the lengths for the various accessions ($P < 0.05$). Tkg 1 again exhibited maximum long arm length but its length did not differ significantly from those of Tkg 8, 9 and 11. The least long arm length was displayed by Tkg 12 and it differed significantly from all the other accessions.

The longest total chromosome length was shown by Tkg 1 but this did not differ significantly ($P > 0.05$) from those of Tkg 8 and Tkg 11. On the other hand, Tkg 12 had the least total chromosome length, which differed significantly from those of the other accessions (Table 1).

Arm ratios ranged from 1.00 up to 3.00 for Tkg 1 and 1.00 up to 5.00 for Tkg 6, 8, 9, 11 and 12 (Table 1). Centromeric index or F% varied from 16.67 up to 50% for Tkg 11 and 25 up to 50% for Tkg 1. On the other hand, Total Form percent (TF%) varied from 37% (Tkg 6 and Tkg 12) to 42% (Tkg 1) (Table 2).

In terms of Stebbin's Asymmetry (Table 2), five accessions (Tkg 1, 6, 8, 11 and 12) were classified as 2A; while only one accession (Tkg 9) was classified as 2B. Phylogenetic relationships were depicted in a dendrogram with three clusters of accessions recognised. Cluster 1 comprised of Tkg 1, 8, 9 and 11;

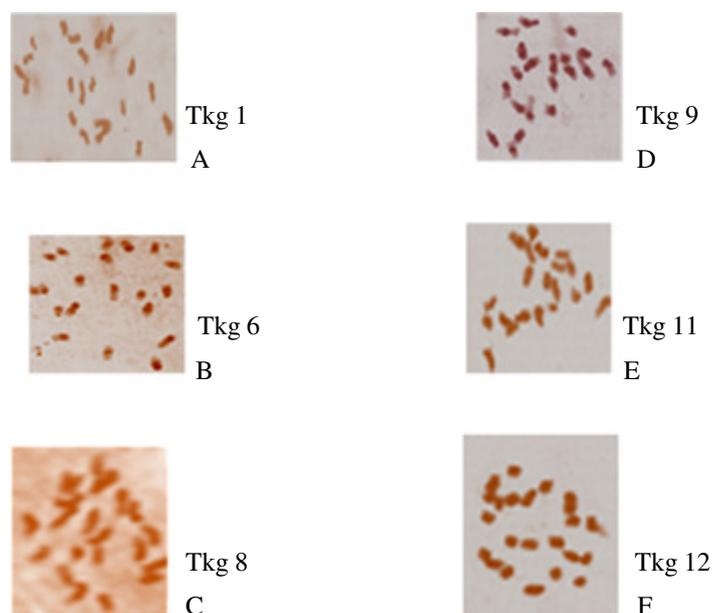


Figure 1. The metaphase plates of *Macrotyloma geocarpa* ($\times 1000$) ($2n = 20$).

TABLE 1. Chromosomal characteristics of six accessions of *Macrotyloma geocarpa*

Accessions	Mean short arm lengths (μm)	Mean long arm lengths (μm)	Mean total chromosome (μm)	Range of total chromosome lengths (μm)	Range of arm ratios
Tkg 1	0.717 ± 0.034	1.022 ± 0.048	1.739 ± 0.047	$1.19 \pm 0.012 - 2.00 \pm 0.003$	1.00 - 3.00
Tkg 6	0.534 ± 0.040	0.863 ± 0.036	1.397 ± 0.054	$1.10 \pm 0.003 - 1.83 \pm 0.005$	1.08 - 5.00
Tkg 8	0.668 ± 0.046	1.006 ± 0.036	1.674 ± 0.063	$1.00 \pm 0.003 - 2.00 \pm 0.003$	1.00 - 4.14
Tkg 9	0.603 ± 0.035	0.978 ± 0.049	1.581 ± 0.071	$0.85 \pm 0.012 - 2.00 \pm 0.003$	1.25 - 3.25
Tkg 11	0.647 ± 0.043	0.988 ± 0.047	1.635 ± 0.059	$1.00 \pm 0.003 - 2.00 \pm 0.000$	1.00 - 5.00
Tkg 12	0.374 ± 0.015	0.640 ± 0.037	1.014 ± 0.029	$0.85 \pm 0.012 - 1.25 \pm 0.003$	1.00 - 4.00
LSD (0.05)	0.103	0.120	0.155		
CV (%)	34.2	25.6	20.2		

while Cluster 2 had only one accession, Tkg 6, cluster 3 also one accession, Tkg 12.

The Karyotypic formulae are shown on Table 2. The chromosomes of each of the accessions consisted of more of metacentrics and few submetacentric and subtelocentrics. The metaphase plates of each of the accessions are shown in Figure 1; while the ideograms are shown in Figures 2 to 7.

DISCUSSION

This karyotypic study has shown clear evidence that the studied *M. geocarpa* is diploid with a somatic chromosome number of $2n = 20$ (Fig. 1). This observation is in agreement with an earlier report of Pasquet *et al.* (2002) who revealed a count of $2n = 20$ for the wild taxon; while Miede (1962) gave a count of $2n$

TABLE 2. Karyotypic features of six accessions of *Macrotyloma geocarpa*

Accessions	Range of F % (CI)	TF %	Stebbin's Asymmetry	Karyotype formulae
Tkg 1	25.00 - 50.00	42	2A	$2n = 20 = 7m + 1m/sm + 2sm/st$
Tkg 6	16.67 - 48.15	37	2A	$2n = 20 = 6m + 2sm + 2st$
Tkg 8	19.44 - 50.00	40	2A	$2n = 20 = 7m + 1sm + 1sm/st + 1st$
Tkg 9	23.53 - 44.44	38	2B	$2n = 20 = 7m + 1sm + 1sm/st + 1st$
Tkg 11	16.67 - 50.00	40	2A	$2n = 20 = 7m + 2sm/st + 1st$
Tkg 12	20.00 - 50.00	37	2A	$2n = 20 = 6m + 2sm + 2st$

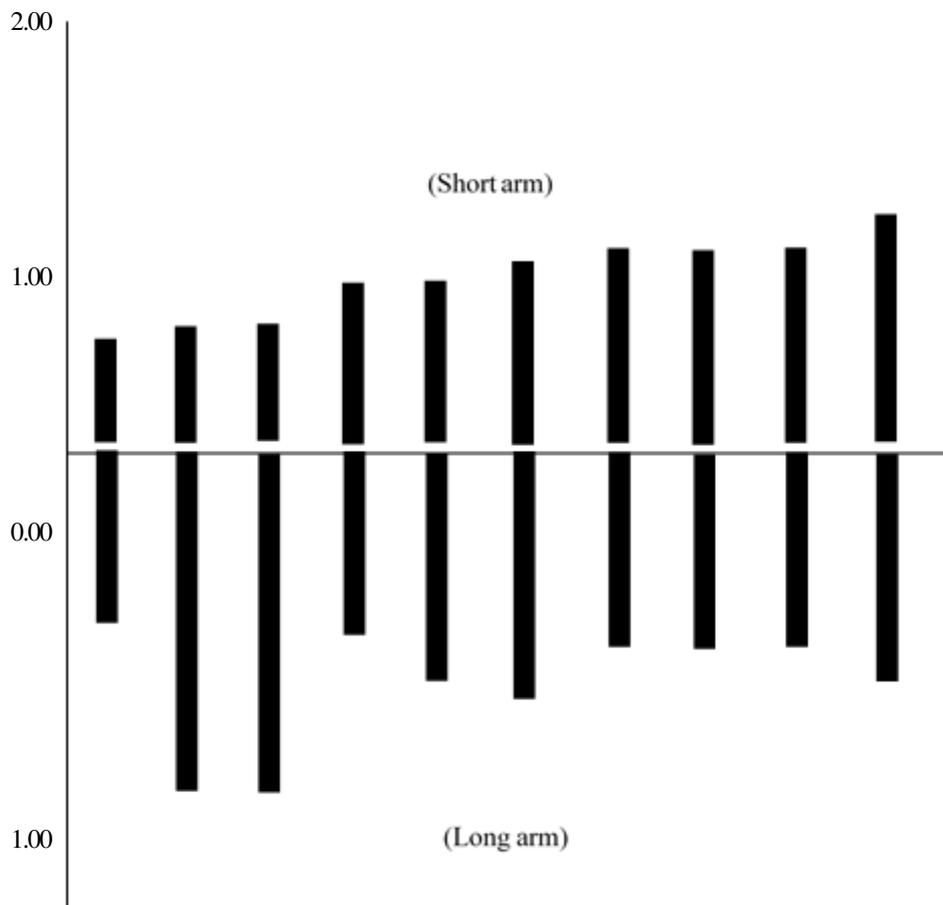


Figure 2. The ideogram of Tkg 1 representing diagrammatically its chromosomes with the aim of showing their relative sizes. The line separating the short and long arms represents the centromere.

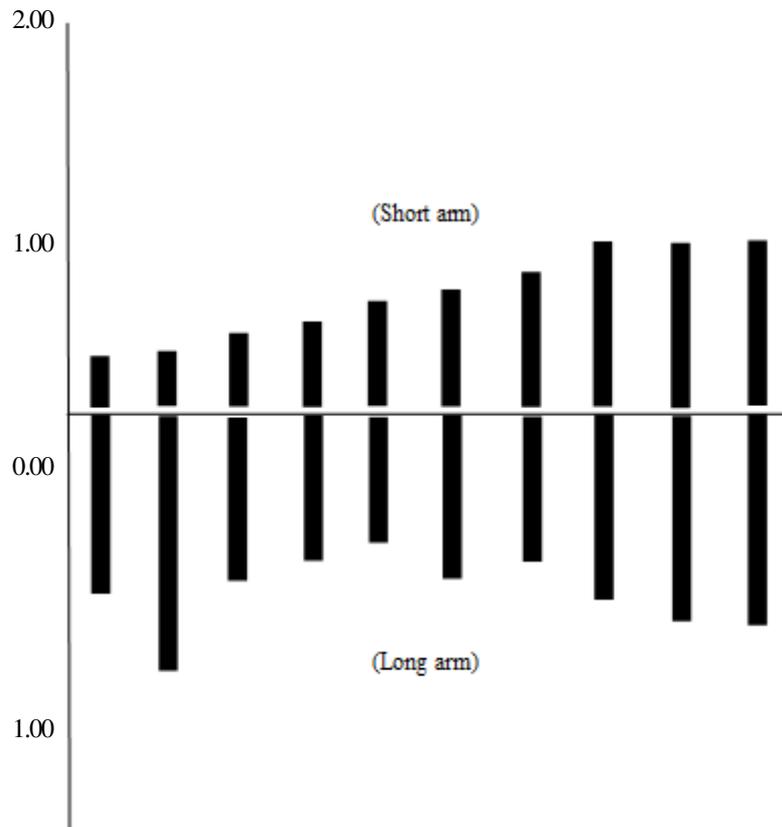


Figure 3. The ideogram of Tkg 6 representing diagrammatically its chromosomes with the aim of showing their relative sizes. The line separating the short and long arms represent the centromere.

= 22 for the domesticated taxon. Cook *et al.* (2005) cited in Morris (2008) reported that *M. axillare* has a diploid chromosome number of $2n = 20$; while *M. uniflorum* has a diploid number of $2n = 20, 22, 24$. Halder *et al.* (2012) confirmed a diploid chromosome number of $2n = 20$ for *M. uniflorum*. The positive implication of these reports for the improvement of *M. geocarpa* is the possibility of successful hybridisation among the accessions since they all had the same chromosome number. Regular chromosome pairing and bivalent formation at meiosis are expected, thus assuring successful hybrid formation as well as hybrid fertility.

There is also the likelihood of successful interspecific hybridisations between *M. geocarpa* and *M. axillare*, and *M. uniflorum* of $2n = 20$. Interspecific crossing assures the

introgression of useful traits such as environmental stress tolerance, disease resistance, yield and quality increases in addition to the overcoming of certain agricultural deficiencies (Pink and Keane, 1993; Cao and Deng, 2014).

Uguru *et al.* (2002) noted that information on the chromosome number of a species can only be of value when considered in conjunction with information on the morphology of the chromosomes as these might show some variations. This fact was authenticated by the report of Seiji and Fernandez (2003) that large differences in chromosome size played a very significant role in the evolution of *Lathyrus* species, in spite of the stability in chromosome number. Uguru *et al.* (2002) further noted that, in spite of observable morphological differences in

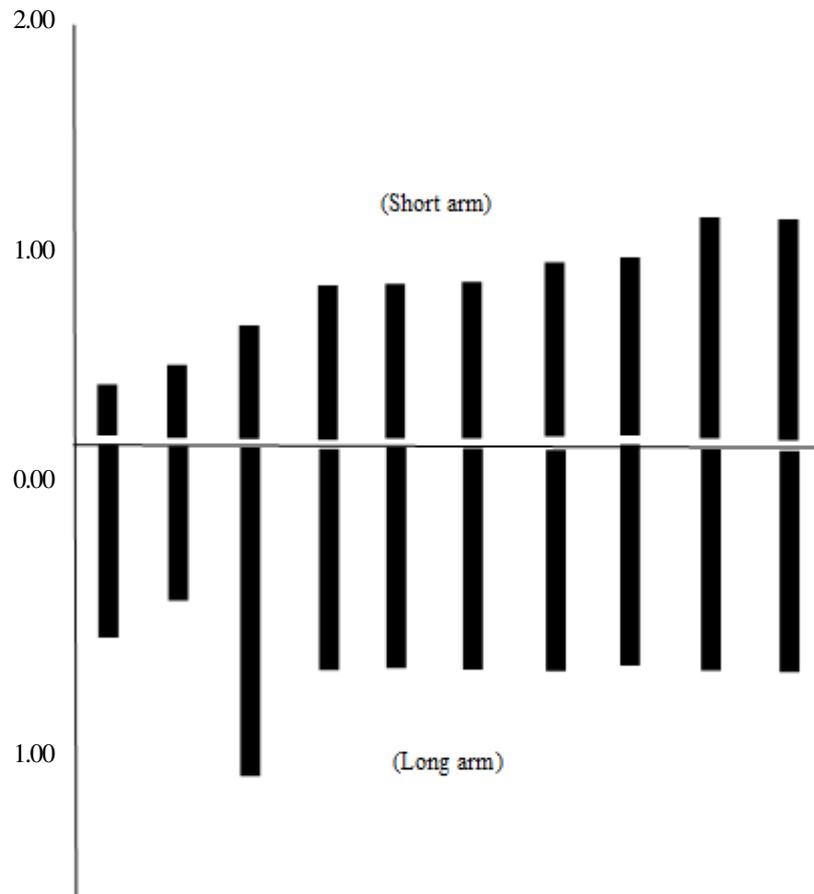


Figure 4. The ideogram of Tkg 8 representing diagrammatically its chromosomes with the aim of showing their relative sizes. The line separating the short and long arms represent the centromere.

chromosomes of genotypes of a species, the occurrence of uniform chromosome number indicates that such genotypes may have evolved from a common ancestor. Archana *et al.* (2013) reported that possession of same chromosome number and different karyotypic features are indications of ongoing evolutionary processes at microlevel. The uniform chromosome number observed among the accessions of *M. geocarpa* suggest the possibility of evolution from a common ancestor. This fact further gives the assurance of possible successful hybridisations among them since they would share a number of components of chromosomal morphology.

Analysis of the karyotype which was done for the first time in *M. geocarpa* revealed some

interesting features that are similar to some related species (Tables 1 and 2). The total chromosome lengths ranged from 0.85 - 2.00 μm and this is quite similar to the findings of Halder *et al.* (2012) in *M. uniflorum* who recorded a range of 1.26 - 2.41 μm . These chromosomal lengths are quite close to those of *Phaseolus* spp, which had the average range of 0.70 to 1.60 μm as recorded by Mercado-Ruaro and Alfonso (2009). All these ranges, however, are quite small when compared to the range of chromosomes of the most related species such as Bambara groundnut reported with a range of 1.5 to 4.0 μm . These facts authenticate Uguru *et al.*'s (2002) earlier assertion that both the chromosome number and chromosome morphology should be taken

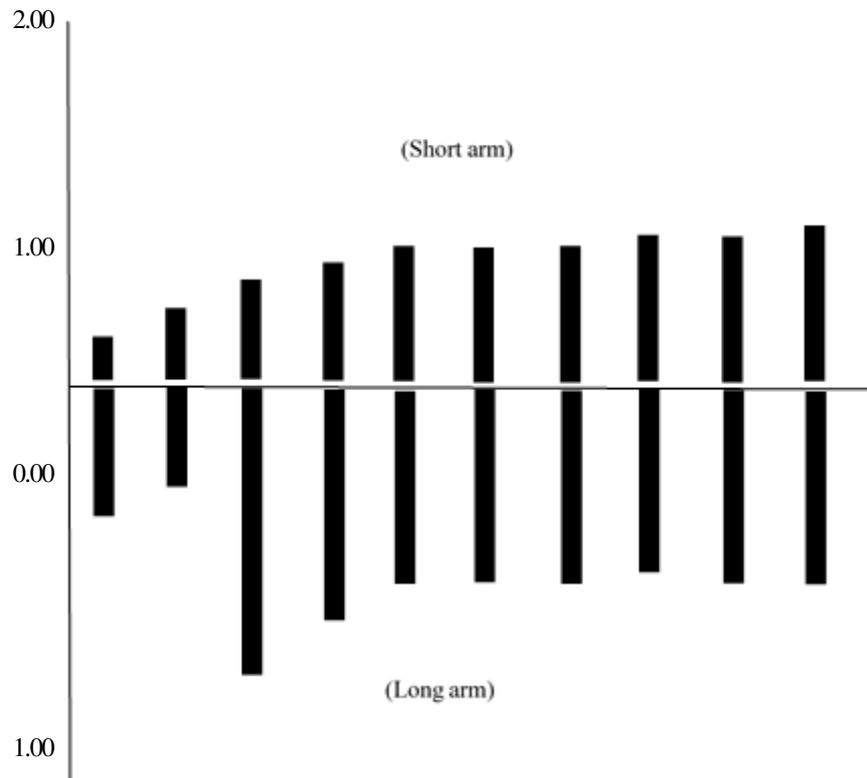


Figure 5. The ideogram of Tkg 9 representing diagrammatically its chromosomes with the aim of showing their relative sizes. The line separating the short and long arms represent the centromere.

into consideration in making breeding decisions. Significant variations in chromosome lengths could pose chromosomal cross over barriers among these species especially among Bambara groundnut (*Vigna subterranean*) and *M. geocarpa*, *M. uniflorum* and *Phaseolus* species.

The highest total chromosome lengths were observed in Tkg1 and Tkg 8; while the least total lengths were observed in Tkg 12. Kiran (2018) noted that chromosome length is an indirect indicator of the total DNA amount. Thus, longer chromosomes like those of Tkg 1 and Tkg 8 could have more DNA contents than the smaller chromosomes of Tkg 12. Uguru *et al.* (2002) citing Stebbins (1971) reported that longer chromosomes are primitive; while shorter chromosomes are more advanced. Chromosomal size differences among species can arise due to differences in

the repetitive DNA sequences (Schmidt *et al.* 1998 cited in Agbo and Ukwu, 2010). It appears that in the course of evolution, Tkg 12 may have lost some chromosomal segments and ended up having the smallest total chromosome length among the Kersting's groundnut accessions. This view is in tandem with that of Weiss-Schneeweiss and Schneeweiss (2013); who listed factors that lead to changes in karyotype asymmetry to include modifications in chromosome size and morphology. According to them, these modifications can arise as a result of DNA sequence expansions or deletions or due to centric fusions/fissions.

Loureiro *et al.* (2010) further reported that inter- and intra-specific genome size variation can reveal taxonomic heterogeneity and incipient speciation. The significant differences between Tkg 12 and the other accessions with

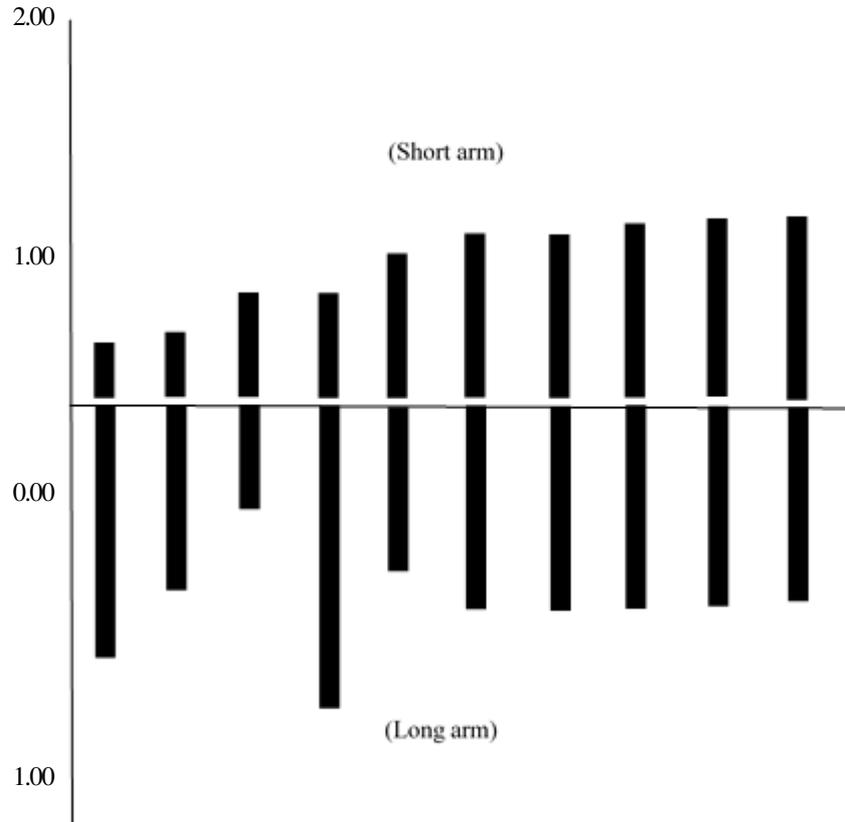


Figure 6. The ideogram of Tkg 11 representing diagrammatically its chromosomes with the aim of showing their relative sizes. The line separating the short and long arms represent the centromere.

respect to short arm length, long arm length and total chromosome length is a pointer to some evolutionary processes going on at microlevel in Tkg 12, as earlier observed by Archana *et al.* (2013). The resolution of this fact awaits the employment of more advanced cytological techniques in the study of the chromosomes of this species.

Yang *et al.* (2015) from earlier literature, revealed that karyotype evolution proceeds from symmetry to asymmetry in higher plants and based on this fact, karyotypes were classified into 12 types which depicted increasing asymmetry *viz.*: 1A - 4A, 1B - 4B, and 1C - 4C. The authors further noted that symmetric karyotypes are characteristic of primitive plants while asymmetric karyotypes are found in more evolutionarily advanced taxa. Five of the accessions studied in this

work were grouped into karyotype 2A; while one, Tkg 9 was grouped into karyotype 2B (Table 2). Yang *et al.* (2015) and Liu and Luo (2019) observed that karyotypes of 2A have higher intrachromosomal symmetry (more primitive) than those of 2B. Therefore, Tkg 9 has higher chromosomal asymmetry than the other accessions and is perhaps advancing evolutionarily faster than them. There appears to be an association between asymmetrical karyotype of Tkg 9 and its shorter total chromosome length as was observed by Mousavi *et al.* (2013) on Iranian lettuce. This tends to justify Uguru *et al.* (2002) statement that shorter chromosomes are more advanced; thus providing further evidence of Tkg 9 being more advanced than the other accessions.

In spite of the differences in the ranges of the $F\%$ values, all the accessions of *M.*

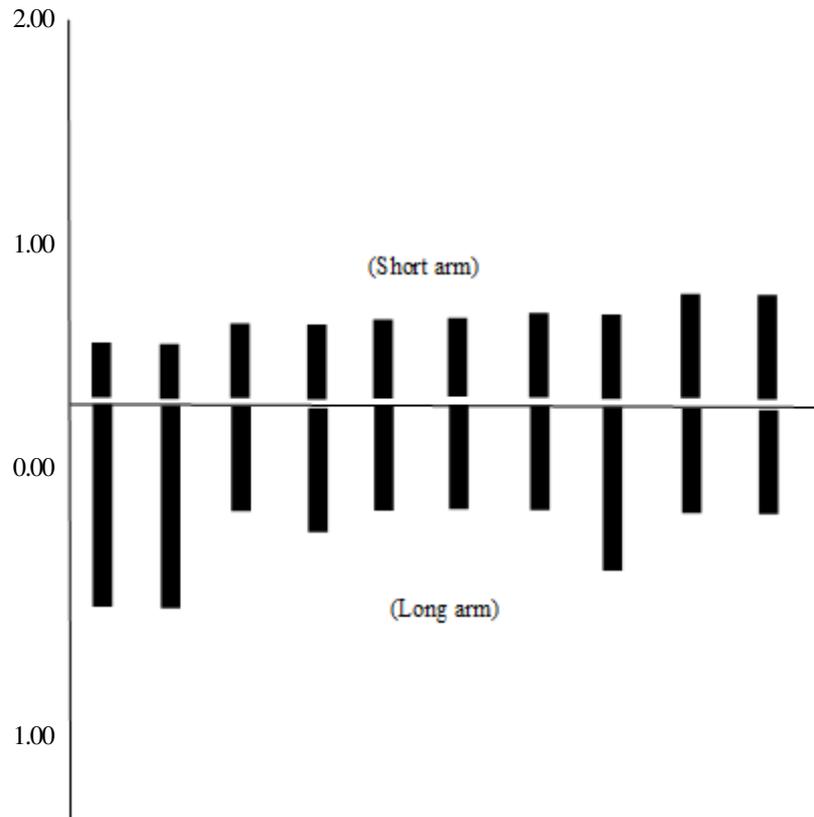


Figure 7. The ideogram of Tkg 12 representing diagrammatically its chromosomes with the aim of showing their relative sizes. The line separating the short and long arms represent the centromere.

geocarpa had metacentric, submetacentric and subtelocentric chromosomes (Table 2). However, there was a preponderance of metacentric chromosomes which was also observed by Halder *et al.* (2012) on *M. uniflorum*. Agbo and Ukwu (2010) suggested that the preponderance of metacentric and submetacentric chromosomes in *G. latifolia* indicated that the clones were still evolving and so by extension, *M. geocarpa* accessions can be presumed to be still evolving. The TF% values in this study were high and according to Archana *et al.* (2013), such high values represent a high symmetric karyotype which is a primitive condition. A symmetrical karyotype (Lakshmanan *et al.*, 2015) mainly possesses metacentric and submetacentric chromosomes of approximate equal sizes whereas asymmetrical karyotypes arise by

shifts in centromeric position towards the telomere. Kersting's groundnut accessions used in this study had symmetric karyotype, but with increasing traces of asymmetry as could be seen in the presence of subtelocentric chromosomes. This shows a gradual shift of the centromere towards the telomere.

The subtelocentrics could have arisen because of deletions and subsequent size reduction of one of the chromosome arms (Uguru *et al.*, 2002). The effect of this would be the shifting of the position of the centromere thus leading to a reduction in the absolute size of the chromosome. This conclusion was earlier drawn by Uguru *et al.* (2002) on Bambara groundnut and Nejatizadeh-Barandozi and Akbari (2013) on Aloe species. Other factors that could lead to increase in asymmetry are unequal translocations,

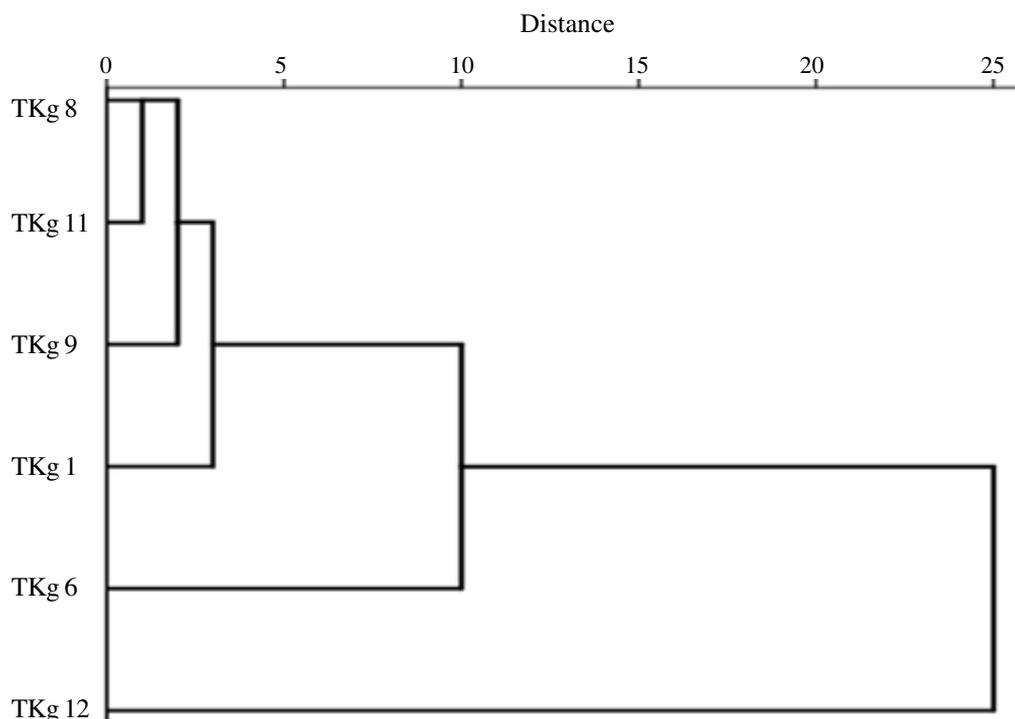


Figure 8. Dendrogram depicting relationships among the six accessions of *Macrotyloma geocarpa*.

inversions, polyploidisation with possible genome downsizing (Baltisberger and Horandl, 2016; Martin *et al.*, 2020). All these changes in the characteristics of a genome are often presumed to be associated with the evolution of advanced plants as was reported by Sirin *et al.* (2019). These may have been occurring in Kersting's groundnut accessions in spite of all of them having the same chromosome number. *Macrotyloma geocarpa* accessions can therefore, be said to be evolving gradually from symmetrical (primitive) condition to increasing asymmetry (advanced lines).

Clustering observed in the dendrogram (Fig. 8) could indicate close genetic proximity or relatedness. Accessions Tkg 1, Tkg 8, Tkg 9 and Tkg 11 in cluster 1 appear to be closely related. Relative closeness could be due to seed exchanges among farmers in different regions (Yi *et al.*, 2008). Accessions Tkg 6 and Tkg 12 are not closely related to the other accessions and could create wider variations when crossed with them. This fact had earlier

been noted by Maity *et al.* (2009), who reported that identification of parents for hybridisation programmes could be on the basis of distance among accessions in different clusters. Using such distantly related accessions could result in generation of wider variability which is a prerequisite for crop improvement programmes.

CONCLUSION

All the Kersting's groundnut accessions used in this study have the same chromosome number likely due to their origin from a common ancestor. Morphologically, metacentric, submetacentric and subtelocentric chromosomes are evident in all accessions although, there is a preponderance of metacentric chromosomes suggesting a symmetrical karyotype which is known to be a primitive condition. The presence of few subtelocentrics is an indication of evolutionary advancement towards asymmetry. Tkg 6 and

Tkg 12 are shown through hierarchical cluster analysis to be distantly related to the other accessions and so could serve as parents in hybridisation programmes to generate wider variability for the improvement of the species. Thus, this study has revealed that some chromosomal variability exist among the accessions of Kersting's groundnut.

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