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Cytogenetic characterization and karyotype diversity in improved cowpea (*Vigna unguiculata* Walp.) varieties for breeding applications

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ABSTRACT: Understanding chromosomal variation among improved cowpea (*Vigna unguiculata* Walp.) varieties is crucial for advancing breeding strategies that aim to enhance yield stability and adaptability. This study employed cytogenetic analyses to characterise three improved cowpea varieties and evaluate their agronomic and breeding relevance. All varieties possessed a conserved diploid chromosome number (2n = 22; n = 11), confirming species identity. However, they differed in karyotype formulae: one variety exhibited 5 metacentric + 6 submetacentric (5M + 6SM) chromosomes, while another had 7M + 4SM. Notable variations were recorded in key karyological indices, including the coefficient of variation (CV), total form percentage (TF%), disparity index (DI), and intra- and inter-chromosomal asymmetry (A1 and A2). SAMPEA-19 showed the highest CV (14.59%), and SAMPEA-17 and SAMPEA-119 displayed the highest DI values (44.28%), indicating pronounced chromosomal asymmetry. SAMPEA-18 was cytogenetically distinct in its TF, divergence pattern, and karyotype structure. Cluster analysis grouped the varieties into two sub-varietal clusters, with SAMPEA-18 exhibiting the highest genetic divergence (similarity index = 60.59%). These findings underscore the utility of cytological data as a cost-effective tool for revealing intraspecific variation and informing varietal improvement in cowpea breeding programmes.

Keywords: Cowpea, Chromosome, FUAMPEA varieties, Variability, karyological properties

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a nutritionally and economically important legume, widely cultivated across sub-Saharan Africa and parts of Asia. As a multipurpose crop, cowpea contributes to food security, income generation, and sustainable agriculture, particularly in resource-constrained regions (Adetumbi *et al*., 2017). Its protein-rich grains serve as a vital dietary supplement, offering an inexpensive alternative to animal protein sources such as meat, fish, and eggs (Xiong *et al*., 2016). Furthermore, cowpea residues are a valued source of livestock fodder post-harvest, reinforcing their role in integrated crop-livestock systems (Badr *et al*., 2014). Beyond its nutritional value, cowpea is adapted to marginal environments. It exhibits early maturity, drought tolerance, and the ability to fix atmospheric nitrogen through root nodules, enabling cultivation in low-fertility soils and under erratic rainfall conditions. Nigeria and the Niger Republic account for over 60% of global production, although countries such as Brazil, India, and Sri Lanka also contribute substantially (Ibukun *et al*., 2013). The crop has a wide morphological diversity, including erect, prostrate, and climbing growth forms, which reflects its ecological versatility and genetic breadth.

Despite these agronomic and ecological advantages, the cytogenetic basis of varietal diversity in cowpea remains largely unexplored. Nonetheless,

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the lack of detailed cytogenetic profiling of these varieties limits understanding of their genetic differentiation, evolutionary relationships, and potential application in breeding programmes. Cytogenetic analyses—focusing on chromosome number, structure, and karyotype asymmetry—can reveal fundamental insights into genome organisation, evolutionary divergence, and potential cross-compatibility, all of which are critical for informed breeding decisions.

At Joseph Sarwuan Tarka University, Makurdi, several improved cowpea varieties have been developed and adopted by farmers across Benue State and neighbouring regions. Nonetheless, the lack of detailed cytogenetic profiling of these varieties limits the understanding of their genetic differentiation, evolutionary relationships, and suitability for use in breeding programmes. This gap in knowledge presents a barrier to the effective utilisation, conservation, and systematic classification of cowpea germplasm.

The present study addresses this gap by conducting a comprehensive cytological analysis of selected improved cowpea varieties. Specifically, the study aims to evaluate chromosome number, morphology, and karyotype parameters to determine intraspecific diversity, identify evolutionary patterns, and assess their breeding and systematic value. By integrating karyological data into cowpea genetic research, this study contributes to the development of robust, science-driven breeding strategies for sustainable legume improvement in sub-Saharan Africa.

Materials and Methods

Seed germination and root tip collection

Seeds of the cowpea varieties were pre-germinated in Petri dishes lined with moistened cotton wool in the New Biology Laboratory, College of Biological Sciences, Joseph Sarwuan Tarka University, Makurdi. Ten seeds per variety were used, following the protocols described by Alam et al. (2018) and Osuagwu et al. (2022). Young, healthy roots approximately 15 mm in length were harvested at two-hour intervals between 07:00 and 09:00 a.m. for cytological and karyotyping studies.

Pre-treatment and fixation

The root tips were rinsed twice in distilled water and pre-treated in a 0.002 M solution of 8-hydroxyquinoline (0.058 g in 200 mL distilled water) for four hours at room temperature. They were then rinsed again and fixed in freshly prepared Farmer's fixative (1 part glacial acetic acid to 3 parts ethanol) for 24 hours at room temperature. After fixation, samples were stored in 70% ethanol until further analysis.

Hydrolysis, staining, and slide preparation

Fixed root tips were hydrolysed in 1 N HCl at 60 °C for 6 minutes (Leliveld, 1965), then rinsed twice with distilled water. Each tip was placed on a grease-free microscope slide, and the apical 1 mm section (meristematic zone) was excised. One to two drops of 1% aceto-orcein stain were added, and the tissue was gently macerated. A cover slip was applied, and the slide was wrapped in filter paper. Firm thumb pressure was used to flatten the tissue and remove excess stain. Gentle tapping with the blunt end of a biro ensured even chromosome spreading. The edges of the cover slips were sealed with clear nail varnish. Slides were observed under a compound microscope, and photomicrographs were taken at ×1000 magnification using oil immersion.

Cytogenetic analysis

Five well-spread metaphase plates per variety were selected for karyotyping. Chromosomal measurements included: short arm length (S), long arm length (L), total chromosome length (TL), arm ratio (AR), centromeric index (CI), relative value (RV), total form percentage (TF%), and karyotype formula (KF), following Saponetti and Pignone (1996).

Chromosome image capture and analysis

Photomicrographs were taken using an Amscope Digital Camera (1000MA) connected to a microscope and laptop. Measurements were performed using IS Capture and Image-Pro Plus™ software. Homologous chromosomes were paired, and morphology was described using standard nomenclature (Fukui, 1986).

Calculation of karyological indices

The following formulae were applied:

i. Total length (TL) = L + S
ii. Arm ratio (AR) = L / S
iii. Relative value (RV) = S / L
iv. Total form percentage (%TF) = (ΣS / ΣTL) × 100
v. Centromeric index (CI) = S / (L + S)

Asymmetry indices (Romero-zarco, 1986):

- Intrachromosomal index (A1) = Σ(SX / LX) / N
- Interchromosomal index (A2) = SD / X

Where:

SX = short arm length of the chromosome
LX = long arm length
SD = standard deviation of TL
X = mean chromosome length
N = number of homologues (n = 11)

Statistical Analysis

Multivariate analyses including principal component analysis (PCA) and hierarchical cluster analysis were conducted. One-way analysis of variance (ANOVA) was performed, and means were separated using the least significant difference (LSD) test at $p < 0.05$.

Results

Plate 1 presents the metaphase chromosomes of the SAMPEA-17 variety, while Figure 1 depicts the corresponding karyotype structure. A diploid chromosome number of 2n = 22 was observed. The haploid set (n = 11) comprised five metacentric and six submetacentric chromosomes, yielding a karyotype formula of 5M + 6SM. As detailed in Table 1, the chromosomes exhibited long arm (LA) lengths ranging from 0.68 to 1.23 µm, short arm (SA) lengths between 0.42 and 0.85 µm, and total lengths (TL) spanning 1.12 to 1.86 µm. The arm ratio (AR) varied from 1.10 (chromosome 5) to 2.18 (chromosome 2), while relative values (RV) ranged from 0.46 (chromosomes 1 and 2) to 0.91 (chromosome 5). Mean values for these parameters were recorded as follows: LA = 0.92 µm, SA = 0.63 µm, TL = 1.56 µm, AR = 1.50, and RV = 0.70.

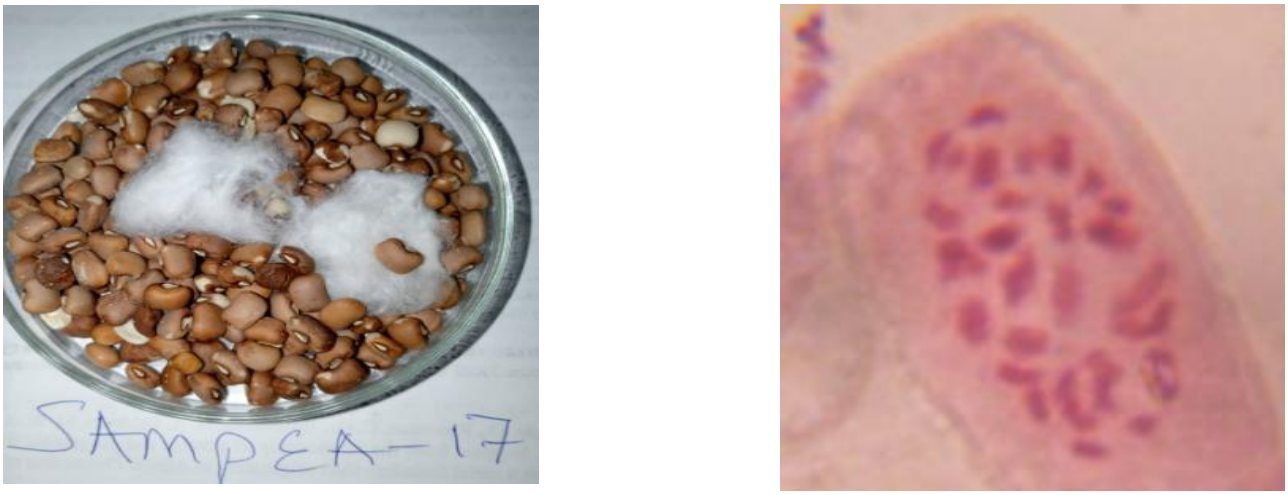

Plate 1: Chromosomes of SAMPEA-17 Variety at Metaphase Stage of Mitosis

Figure 1: Karyotype composition and Formula in SAMPEA-17 (n = 11, 2n = 22; KF= 5M + 6SM)

Table 1: Description of Chromosomes in SAMPEA-17 Variety

SAMPEA-17 Chromosome No.	Long Arm (µm) (LA)	Short Arm (µm) (SA)	Total Length (µm) (TL = LA+SA)	Arm Ratio (LA/SA)	Relative Value (SA/LA)	Chromosome type
Chromosome 1	1.23	0.57	1.80	2.15	0.46	M
Chromosome 2	1.20	0.55	1.75	2.18	0.46	SM
Chromosome 3	1.07	0.79	1.86	1.35	0.74	SM
Chromosome 4	1.07	0.73	1.80	1.47	0.68	SM
Chromosome 5	0.93	0.85	1.78	1.10	0.91	M
Chromosome 6	0.83	0.69	1.52	1.21	0.83	M
Chromosome 7	0.81	0.67	1.48	1.20	0.83	M
Chromosome 8	0.75	0.63	1.38	1.19	0.84	M
Chromosome 9	0.82	0.52	1.34	1.58	0.63	SM
Chromosome 10	0.75	0.53	1.28	1.41	0.71	SM
Chromosome 11	0.68	0.42	1.12	1.61	0.62	SM
Mean	0.92	0.63	1.56	1.50	0.70	

Plate 2 illustrates the metaphase chromosomes of the SAMPEA-18 variety, while Figure 2 presents the corresponding karyotype composition. The chromosome count revealed a diploid number of 2n = 22. The haploid complement (n = 11) comprised seven metacentric and four submetacentric chromosomes, resulting in a karyotype formula of 7M + 4SM. As summarised in Table 2, the haploid chromosomes displayed long arm (LA) lengths ranging from 0.60 to 1.33 µm, short arm (SA) lengths between 0.42 and 0.85 µm, and total lengths (TL) from 1.02 to 1.89 µm. The arm ratio (AR) ranged from 1.10 (chromosome 5) to 2.31 (chromosome 1), while the relative value (RV) varied from 0.43 (chromosome 1) to 0.91 (chromosome 5). The mean values for these chromosomal parameters were: LA = 0.94 µm, SA = 0.63 µm, TL = 1.58 µm, AR = 1.50, and RV = 0.70.

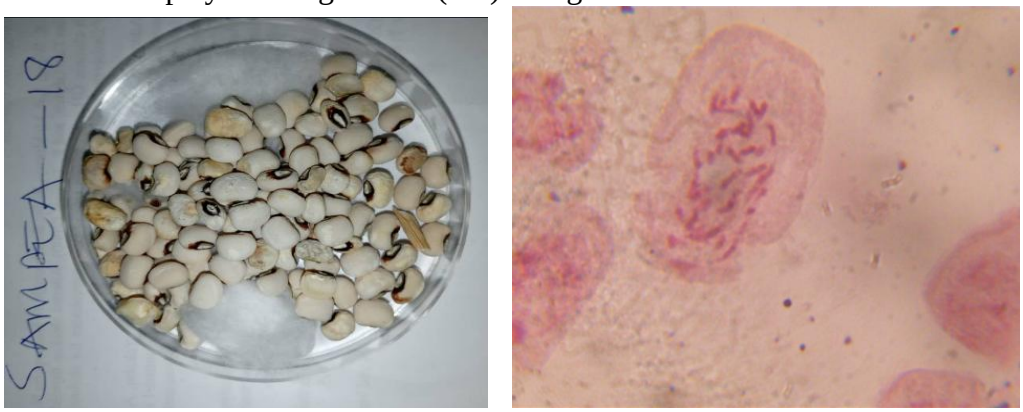

Plate 2: Chromosomes of SAMPEA-18 Variety at Metaphase Stage of Mitosis

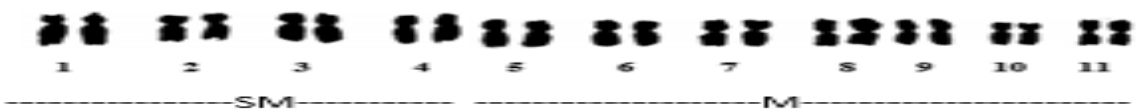

Figure 2: Karyotype composition and Formula in SAMPEA -18 (n = 11, 2n = 22; KF= 7M + 4SM)

Table 2: Description of Chromosomes in SAMPEA-18 Variety

SAMPEA-18 Chromosome No.	Long Arm (µm) (LA)	Short Arm (µm) (SA)	Total Length (µm) (TL = LA+SA)	Arm Ratio (LA/SA)	Relative Value (SA/LA)	Chromosome type
Chromosome 1	1.32	0.57	1.89	2.31	0.43	M
Chromosome 2	1.33	0.64	1.87	2.07	0.48	M
Chromosome 3	1.07	0.79	1.86	1.35	0.74	SM
Chromosome 4	1.07	0.73	1.80	1.47	0.68	SM
Chromosome 5	0.93	0.85	1.78	1.10	0.91	M
Chromosome 6	0.83	0.69	1.52	1.21	0.83	M
Chromosome 7	0.92	0.60	1.52	1.54	0.65	SM
Chromosome 8	0.75	0.63	1.48	1.19	0.84	M
Chromosome 9	0.82	0.52	1.34	1.58	0.63	M
Chromosome 10	0.66	0.53	1.29	1.25	0.80	M
Chromosome 11	0.60	0.42	1.02	1.43	0.70	SM
Mean	0.94	0.63	1.58	1.50	0.70	

Plate 3 presents the metaphase chromosomes of the SAMPEA-19 variety, while Figure 3 depicts the corresponding karyotype configuration. The diploid chromosome number was 2n = 22. The haploid complement (n = 11) comprised five metacentric and six submetacentric chromosomes, yielding a karyotype formula of 5M + 6SM. According to Table 3, the haploid chromosomes exhibited long arm (LA) lengths ranging from 0.63 to 1.08 µm, short arm (SA) lengths from 0.42 to 0.63 µm, and total lengths (TL) between 1.05 and 1.60 µm. The arm ratio (AR) varied from 1.25 (chromosome 10) to 2.32 (chromosome 4), while the relative value (RV) ranged from 0.43 (chromosome 4) to 0.84 (chromosome 8). The mean values across chromosomes were: LA = 0.89 µm, SA = 0.51 µm, TL = 1.41 µm, AR = 1.76, and RV = 0.60.

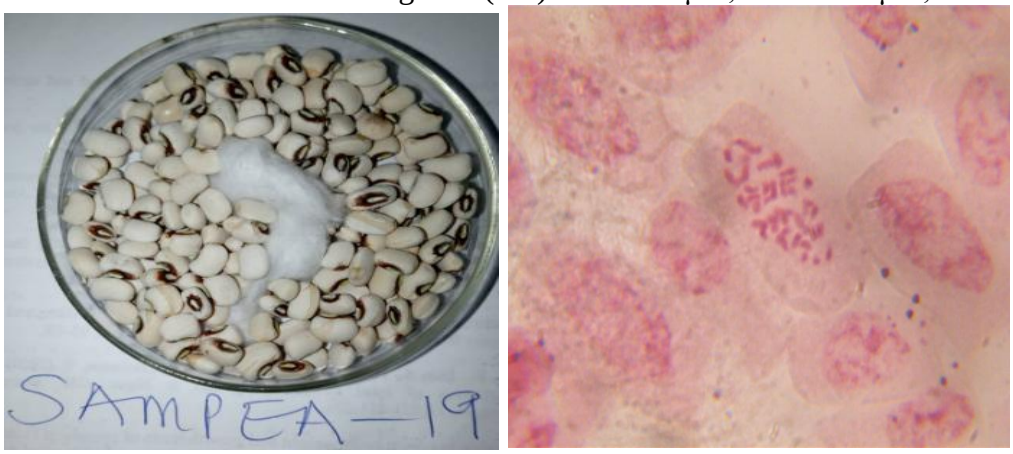

Plate 3: Chromosomes of SAMPEA-19 Variety at Metaphase Stage of Mitosis

Figure 3: Karyotype composition and Formula in SAMPEA -19 (n = 11, 2n = 22; KF= 5M + 6SM)

Table 3: Description of Chromosomes in SAMPEA-19 Variety

SAMPEA-19 Chromosome No.	Long Arm (µm) (LA)	Short Arm (µm) (SA)	Total Length (µm) (TL = LA+SA)	Arm Ratio (LA/SA)	Relative Value (SA/LA)	Chromosome type
Chromosome 1	1.08	0.52	1.60	2.07	0.48	M
Chromosome 2	1.07	0.50	1.57	2.14	0.47	SM
Chromosome 3	1.07	0.49	1.56	2.18	0.46	SM
Chromosome 4	1.07	0.46	1.53	2.32	0.43	M
Chromosome 5	0.99	0.50	1.49	1.98	0.51	SM
Chromosome 6	0.83	0.56	1.39	1.48	0.67	M
Chromosome 7	0.87	0.51	1.38	1.70	0.59	SM
Chromosome 8	0.75	0.63	1.38	1.19	0.84	SM
Chromosome 9	0.82	0.52	1.34	1.58	0.63	SM
Chromosome 10	0.66	0.53	1.19	1.25	0.80	M
Chromosome 11	0.63	0.42	1.05	1.50	0.67	M
Mean	0.89	0.51	1.41	1.76	0.60	

Table 4 compares the chromosomal morphologies among the three cowpea varieties. SAMPEA-19 exhibited the shortest total chromosome length (15.48 µm) and the lowest relative value (0.58), but recorded the highest arm ratio (1.74), indicating a predominance of asymmetrical chromosomes. SAMPEA-18 showed the longest short arm length (6.97 µm), highest total length (17.37 µm), greatest R-value (0.67), and the highest centromeric index (2.10), suggesting enhanced chromosomal symmetry. SAMPEA-17 possessed the longest long arm (10.44 µm). Significant variation was observed in the karyological attributes across the varieties. As shown in Table 5, all three varieties shared the same diploid chromosome number (2n = 22; n = 11), yet differed in coefficient of variation (CV), total form percentage (TF%), disparity index (DI), and chromosomal symmetry indices (intra- and interchromosomal). SAMPEA-19 had the highest CV (14.59%) and TF% (53.8%). Both SAMPEA-17 and SAMPEA-19 displayed identical disparity indices (44.28%), exceeding that of SAMPEA-18 (39.86%). A similar pattern was observed in the symmetry indices, where SAMPEA-18 was the only variety with a distinct symmetry profile. SAMPEA-17 and SAMPEA-19 shared the same karyotype formula (KF = 5M + 6SM), consisting of five metacentric and six submetacentric chromosomes, whereas SAMPEA-18 differed with a formula of 7M + 4SM. The dendrogram (Figure 4) revealed a maximum similarity index of 60.59%, clustering SAMPEA-17 and SAMPEA-19 together and distinguishing SAMPEA-18 as the most genetically divergent variety.

Table 4: Comparative Description of Chromosomes among Improved Cowpea Varieties

Cowpea Variety	Short arm S (µm)	Long arm L (µm)	Total length TL (S+L) (µm)	Arm Ratio AR L/S (µm)	R-value S/L (µm)	Centrometric index (S/L + S) µm
SAMPEA-17	6.95± 0.24	10.44±0.01	17.17± 0.67	1.50± 0.01	0.66± 0.04	1.90± 0.03
SAMPEA-18	6.97± 0.12	10.30±0.33	17.37± 1.57	1.47± 0.12	0.67± 0.08	2.10± 0.08
SAMPEA-19	5.64± 0.43	9.84±0.13	15.48 ±0.51	1.74± 0.06	0.57± 0.04	1.98± 0.05
P-value	P<0.05	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05

Table 5: Variability in Karyological Indices of Cowpea Varieties

Cowpea accession	CV %	Total form (%)	DI (%)	A_1	A_2	KF	CN (2n)
SAMPEA-17	14.12	51.67	44.28	0.024	0.058	5M + 6Sm	22
SAMPEA-18	14.43	53.80	39.86	0.023	0.042	7M + 4Sm	22
SAMPEA-19	14.59	51.67	44.28	0.024	0.058	5M+6Sm	22

Legend:
CN = Chromosom number, 2n = diploid; M = metacentric chromosome and SM = Sub metacentric chromosomes; CV = Coefficient of variation; TF = Total form; DI = Disparity index; A1 = Intrachromosomal index 1; A2 = Interchromosomal index; KF = Karyotype formula XM + YSm

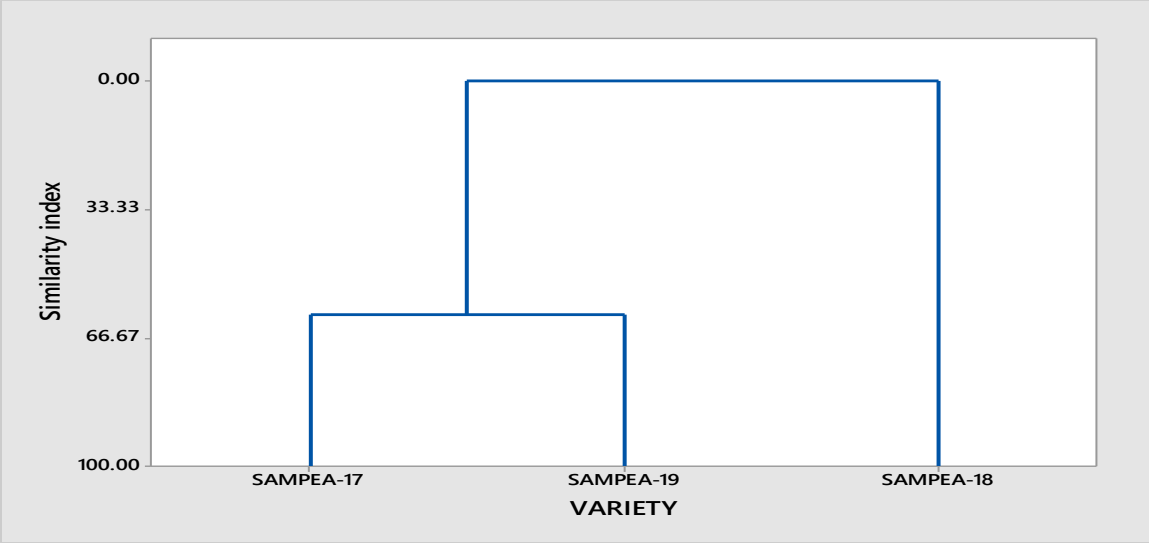

Figure 4: Dendrogram of the three varieties

Discussion

The cytogenetic analysis of the three cowpea varieties revealed substantial chromosomal variability, evidenced by significant differences in karyological parameters. These findings reflect the genetic improvement efforts that have led to the development of morphologically and genetically distinct varieties for various agronomic purposes. Chromosome size, a critical factor in cytogenetic studies, influences the DNA content and gene-coding capacity of each chromosome (Badiane *et al.*, 2012). The observed chromosome lengths in the present study were notably larger than those reported in unrelated taxa such as cucurbits (Bhowmick and Jha, 2019; Osuagwu *et al*., 2022), although all varieties shared a conserved diploid number of 2n = 22.

The chromosomal stability observed across all ten cowpea varieties aligns with previous cytogenetic findings in *Vigna unguiculata* and related legumes like *Phaseolus vulgaris* (Cimpeanu *et al.*, 2005). This stability suggests that chromosome number has remained largely conserved within Fabaceae, contributing to the genetic cohesion of the family. Such conservation may partly explain the reported slow evolutionary rate in *Vigna* and other legumes. In contrast, studies on cucurbits have shown variable chromosome numbers (14–26) across species (Osuagwu *et al*., 2022), highlighting interfamily differences in chromosomal evolution.

The present findings diverge from those of Adetula (2005), who reported a chromosome number of 23 in *V. unguiculata ssp. dekindtiana var.*

pubescens, and from Cimpeanu et al. (2005), who found only morphologically uniform submetacentric chromosomes in *Phaseolus vulgaris*. In the current study, the karyotypes comprised a combination of metacentric and submetacentric chromosomes, with variation in their numbers and arrangement. SAMPEA-17 and SAMPEA-19 shared a karyotype formula of 5M + 6SM, while SAMPEA-18 was distinct with 7M + 4SM. These differences contribute to intraspecific genetic diversity and suggest that chromosomal morphology—not just chromosome number—is a key determinant of evolutionary divergence.

The data also indicate that other karyological indices—such as total form percentage (TF%), arm ratio, coefficient of variation (CV), disparity index (DI), and chromosomal symmetry—contribute to varietal differentiation. For instance, SAMPEA-19 had the highest CV and TF%, while SAMPEA-18 demonstrated the greatest centromeric symmetry. These chromosomal features are consistent with findings in other legumes, where karyological characteristics have been associated with physiological adaptations such as improved stomatal efficiency and drought resilience (Kirian, 2018; Gupta, 2019; Chan, 2021).

Dendrogram analysis further confirmed the genetic divergence among the varieties, clustering SAMPEA-17 and SAMPEA-19 closely while placing SAMPEA-18 in a distinct group. This clustering is consistent with previous molecular studies employing SSR markers, which also found high genetic polymorphism in SAMPEA-17 and SAMPEA-18 (Olasan *et al*., 2023b; Ogunkanmi *et al.*, 2014). Thus, the cytogenetic data support molecular evidence of genetic diversity and offer additional resolution for identifying promising breeding lines.

Karyological analysis emerges as a cost-effective, efficient, and informative tool for studying genetic diversity, especially in low-resource settings where molecular techniques may be inaccessible. The present findings support the utility of chromosomal studies in phylogenetic analysis and cultivar differentiation, as also advocated by Alam et al. (2018) and Osuagwu *et al.* (2022). Despite uniform chromosome numbers across the varieties, cytogenetic variability facilitated classification into sub-varietal groups, underscoring significant intraspecific diversity.

Conclusion

This study demonstrated that, while the investigated cowpea varieties share a conserved diploid chromosome number (2n = 22), they exhibit substantial variability in chromosomal morphology and karyological indices. These differences are indicative of genetic divergence resulting from targeted breeding interventions. SAMPEA-18, in particular, was distinguished by its unique karyotype formula, chromosomal symmetry, and divergence pattern, making it a valuable candidate for future breeding efforts.

The application of cytogenetic data, including karyotype formulae, chromosomal symmetry, and statistical indices, has proven to be an effective approach for evaluating genetic diversity within cowpea. This methodology offers a practical alternative to molecular techniques, especially in resource-limited breeding programmes. The findings underscore the importance of chromosomal traits in understanding intraspecific variation and improving genetic resource management in *Vigna unguiculata*.

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