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CYTOGENETIC VARIATION AMONG IMPROVED COWPEA (*VIGNA UNGUICULATA* [L.] WALP) VARIETIES

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ABSTRACT: Cytogenetic variability is essential for improving agronomic performance and informing effective breeding strategies in cowpea (*Vigna unguiculata* [L.] Walp). This study carried out detailed karyological analyses of four improved cowpea varieties to assess chromosomal differences and their relevance to genetic enhancement. All varieties exhibited a diploid chromosome number of 2n = 22; however, significant variation was observed in key karyological parameters, including arm ratio, centromeric index, total form percentage, disparity index, and symmetry indices. FUAMPEA-1 had the shortest total chromosome length (15.05 µm) and lowest R-value (0.61), yet recorded the highest arm ratio (1.63) and centromeric index (2.04). FUAMPEA-2 displayed the highest coefficient of variation (14.78%), total form percentage (52.55%), and disparity index (49.43%), indicating greater structural asymmetry. FUAMPEA-1, FUAMPEA-2, and FUAMPEA-3 shared a consistent karyotype formula of 6M + 5SM (six metacentric and five submetacentric chromosomes), while FUAMPEA-4 was distinct with a unique formula of 7M + 4SM. The chromosomal diversity observed among the varieties reflects a wide genetic base and offers valuable cytogenetic markers for selection and hybridisation in cowpea improvement programmes.

K**eywords:** Breeding, Cowpea, Chromosome, SAMPEA varieties, Variability.

INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp) is a vital multipurpose crop cultivated extensively in sub-Saharan Africa and parts of Asia, where it contributes significantly to food security and household income (Adetumbi et al., 2017). It provides one of the most affordable sources of dietary protein, especially when compared to animal-based proteins such as fish, meat, and eggs (Xiong et al., 2016). In addition to its grain, cowpea residue serves as valuable fodder for livestock (Badr et al., 2014). Nigeria and the Republic of Niger are the leading producers globally, with other contributing countries including Brazil, India, Sri Lanka, and Australia (Ibukun et al., 2013). The crop displays a range of growth habits erect, prostrate, climbing, and glabrous forms and is well-adapted to drought-prone, high-temperature regions where other legumes often fail.

Over the years, several improved cowpea varieties have been developed through targeted breeding programmes aimed at addressing challenges faced by

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local farmers. However, information on the genetic diversity and evolutionary relationships among these varieties remains limited (Olasan et al., 2023). Such knowledge is essential for guiding further genetic improvement, conservation, and utilisation of the cowpea gene pool. While morphological markers have traditionally been used to assess diversity, they are often unreliable due to their susceptibility to environmental influences and confounding genetic interactions such as pleiotropy and epistasis (Omoigui et al., 2015). Although molecular techniques offer high precision in detecting genetic variation, their complexity, cost, and technical demands limit their widespread application in many breeding programmes.

Cytogenetic analysis, particularly karyological characterisation, provides a simpler, cost-effective, and informative approach to assess genetic variability and infer phylogenetic relationships (Mirzaei, 2021). Karyotype analysis, which examines chromosome number, size, and morphology, serves as a useful tool in understanding genome organisation and diversity within and among plant taxa (Kirian, 2018). Therefore, this study aimed to evaluate the cytogenetic diversity and karyotypic characteristics of four improved FUAMPEA-based cowpea varieties—FUAMPEA-1, FUAMPEA-2, FUAMPEA-3, and FUAMPEA-4—with a view to understanding their chromosomal architecture and potential implications for breeding and genetic enhancement.

METHODOLOGY

To ensure accessibility to young, actively growing root tips, cowpea seeds were pre-germinated in Petri dishes. This was carried out in the New Biology Laboratory, College of Biological Sciences, JOSTUM. Ten seeds of each variety (FUAMPEA-1 to FUAMPEA-4) were germinated under ambient laboratory conditions following protocols described by Alam et al. (2018) and Osuagwu et al. (2022). Root tips approximately 15 mm in length were harvested at two-hour intervals between 07:00 and 09:00 a.m. and used for cytological and karyological analysis. Harvested root tips were rinsed twice in distilled water and pre-treated in a 0.002 M solution of 8-hydroxyquinoline (prepared by dissolving 0.058 g in 200 mL of distilled water) for four hours at room temperature. After pre-treatment, the root tips were again rinsed in distilled water and fixed in freshly prepared Carnoy's solution (glacial acetic acid:ethanol, 1:3) for 24 hours. The fixed root tips were stored in 70% ethanol at 4°C until further use.

For hydrolysis, the root tips were rinsed twice in distilled water and treated in a water bath at 60°C for six minutes. The apical 1 mm portion of each root tip, identified by its white and dense appearance, was excised and placed on a clean, grease-free microscope slide. Excess moisture was removed with a sterile blade, and one to two drops of 1% aceto-orcein stain were applied. The root tips were then macerated thoroughly, covered with a coverslip, and pressed gently between folded filter paper using thumb pressure. The coverslip was gently tapped using a blunt object until the chromosomes were well spread. The corners of the coverslip were sealed with nail varnish to prevent drying.

Prepared slides were examined under a light microscope at various magnifications, and photomicrographs were taken at ×1000 magnification using an Amscope Digital Camera (Model 1000MA) mounted on the microscope. The best metaphase plates were selected for karyotyping based on clarity. Cytological parameters measured included short arm length (S), long arm length (L), total chromosome length (TL), relative value (RV), arm ratio (AR), centromeric index (CI), total form percentage (TF), and karyotype formula, following standard procedures (Osuagwu et al., 2022). Chromosome measurements and pairing were carried out using IS Capture and Image-Pro Plus™ (IPP) software (Fukui, 1986). Chromosomal morphology was described following the classification system proposed by Fukui (1986), ensuring consistency in nomenclature and comparative karyotype evaluation.

RESULTS

Karyological data were analysed using multivariate statistical tools, including principal component analysis (PCA) and hierarchical cluster analysis. One-way analysis of variance (ANOVA) was performed, and mean separation was conducted using the least significant difference (LSD) method. Plate 1 shows the chromosomes of the FUAMPEA-1 variety at the metaphase stage of mitosis, while Figure 1 presents the corresponding karyotype. The variety exhibited a diploid chromosome number of 2n = 22, consisting of 11 haploid chromosomes (n = 11), with a karyotype formula of 6 metacentric (M) and 5 submetacentric (SM) chromosomes (6M + 5SM) As presented in Table 1, the long arm (LA) lengths of the chromosomes ranged from 0.58 to 1.12 µm, and the

short arm (SA) lengths ranged from 0.39 to 0.70 µm. Total chromosome lengths (TL) varied between 1.21 and 1.92 µm. The arm ratio (AR) ranged from 1.05 in chromosome 11 to 1.79 in chromosome 1, while relative values (RV) ranged from 0.46 (chromosome 4) to 0.83 (chromosome 10). The average values across all chromosomes were as follows: LA = 0.84 µm, SA = 0.51 µm, TL = 1.64 µm, AR = 1.37, and RV = 0.63.

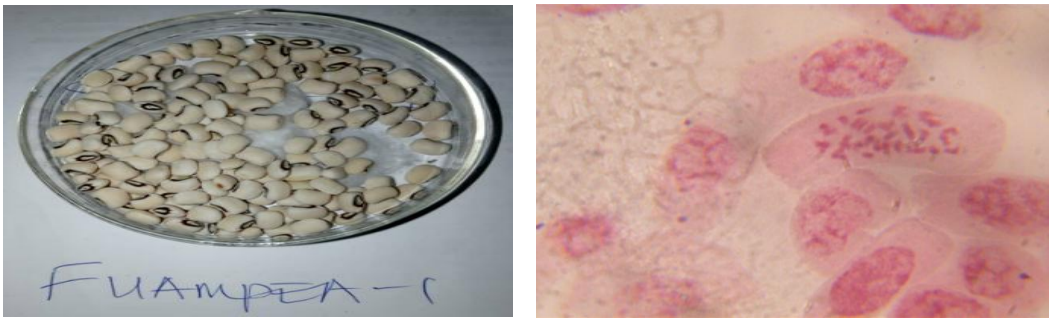

Plate 1: Chromosomes of FUAMPEA-1 Variety at Metaphase Stage of Mitosis

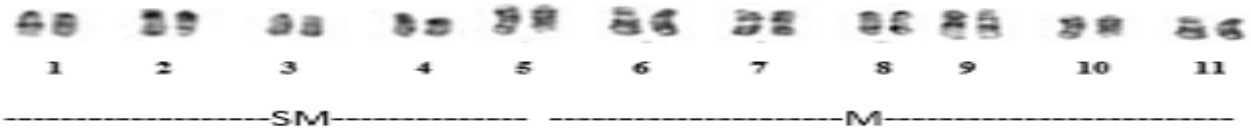

Figure 1: Karyotype composition and Formula in FUAMPEA-1 (n = 11, 2n = 22; KF= 6M + 5SM)

Table 1: Description of Chromosomes in FUAMPEA-1 Variety

FUAMPEA-1 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	1.12	0.62	1.80	1.79	0.55	M
Chromosome 2	0.96	0.70	1.37	1.66	0.73	M
Chromosome 3	1.00	0.52	1.92	1.62	0.52	M
Chromosome 4	1.07	0.49	2.18	1.56	0.46	SM
Chromosome 5	0.83	0.57	1.46	1.40	0.68	SM
Chromosome 6	0.89	0.47	1.89	1.36	0.53	SM
Chromosome 7	0.71	0.50	1.42	1.21	0.70	M
Chromosome 8	0.77	0.42	1.83	1.19	0.55	SM
Chromosome 9	0.65	0.50	1.30	1.15	0.77	M
Chromosome 10	0.58	0.48	1.21	1.06	0.83	M
Chromosome 11	0.66	0.39	1.69	1.05	0.59	SM
Mean	0.84	0.51	1.64	1.37	0.63	

Figure 2 presents the karyotype composition of the FUAMPEA-2 variety, while Plate 2 shows its chromosomes at the metaphase stage of mitosis. The karyotype consists of 22 diploid chromosomes (2n = 22), comprising 11 haploid chromosomes (n = 11), with 6 metacentric and 5 submetacentric types, giving a karyotype formula of 6M + 5SM. According to Table 2, the long arm (LA) lengths ranged from 0.80–

1.21 µm, short arm (SA) lengths from 0.62–0.83 µm, and total chromosome lengths (TL) from 1.42–2.00 µm. Arm ratios (AR) varied between 1.21 (chromosome 3) and 1.63 (chromosome 2), while relative values (RV) ranged from 0.61 (chromosome 2) to 0.85 (chromosome 5). The average values recorded were LA: 0.97 µm, SA: 0.72 µm, TL: 1.69 µm, AR: 1.35, and RV: 0.75.

Plate 2: Chromosomes of FUAMPEA-2 Variety at Metaphase Stage of Mitosis

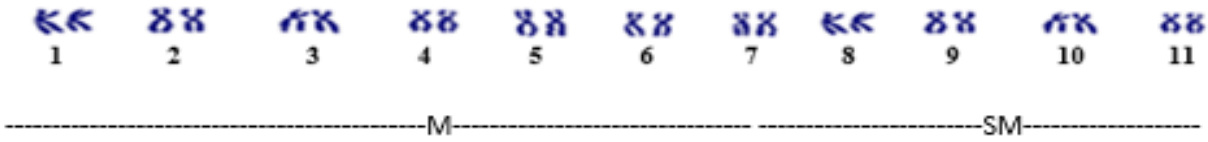

Figure 2: Karyotype composition and Formula in FAUMPEA -2 (n = 11, 2n = 22; KF= 6M + 5SM)

Table 2: Description of Chromosomes in FUAMPEA-2 Variety

FUAMPEA-2 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	1.21	0.79	2.0	1.53	0.65	M
Chromosome 2	1.18	0.72	1.90	1.63	0.61	M
Chromosome 3	1.01	0.83	1.84	1.21	0.82	SM
Chromosome 4	1.07	0.74	1.81	1.44	0.69	M
Chromosome 5	0.95	0.81	1.76	1.17	0.85	M
Chromosome 6	0.95	0.72	1.67	1.31	0.76	M
Chromosome 7	0.90	0.72	1.62	1.25	0.80	SM
Chromosome 8	0.95	0.64	1.59	1.49	0.67	M
Chromosome 9	0.86	0.68	1.54	1.26	0.79	SM
Chromosome 10	0.81	0.66	1.47	1.22	0.81	SM
Chromosome 11	0.80	0.62	1.42	1.29	0.78	SM
Mean	0.97	0.72	1.69	1.35	0.75	

Plate 3 presents the metaphase chromosomes of the FUAMPEA-3 variety, while Figure 3 depicts the corresponding karyotype. The variety exhibited 22 diploid chromosomes (2n = 22), with the haploid set (n = 11) comprising six metacentric and five submetacentric chromosomes, yielding a karyotype formula of 6M + 5SM. As shown in Table 3, the chromosomes had long arm (LA) lengths ranging from 0.60 to 1.23 µm, short arm (SA) lengths from 0.42 to 0.85 µm, and total lengths (TL) between 1.02 and 1.92 µm. Arm ratios (AR) varied from 1.20 (chromosome 6) to 1.78 (chromosome 1), while relative values (RV) ranged from 0.56 (chromosome 1) to 0.93 (chromosome 5). The mean values for LA, SA, TL, AR, and RV were 0.91 µm, 0.65 µm, 1.56 µm, 1.42, and 0.72, respectively.

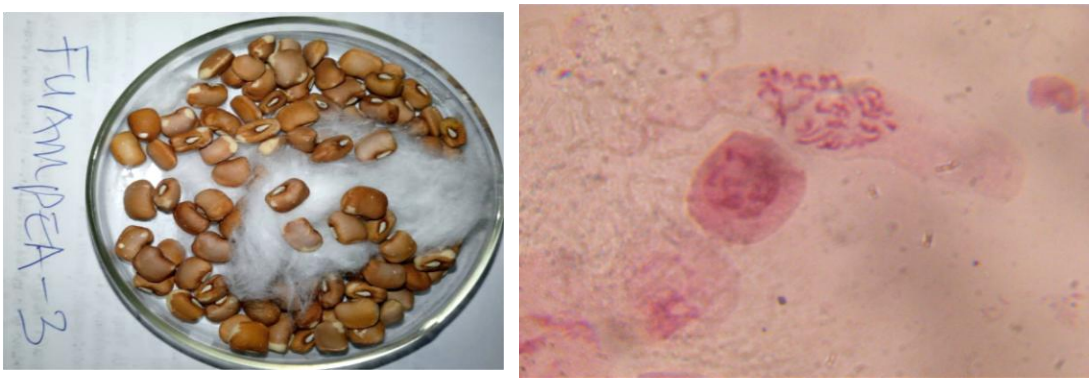

Plate 3: Chromosomes of FUAMPEA-3 Variety at Metaphase Stage of Mitosis

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

Plate 4 presents the metaphase chromosomes of the FUAMPEA-4 variety, while Figure 4 displays its corresponding karyotype composition. The diploid chromosome number was 22 (2n = 22), with the haploid complement (n = 11) comprising 7 metacentric and 4 submetacentric chromosomes, yielding a karyotype formula of 7M + 4SM. As detailed in Table 4, the chromosomes exhibited long arm (LA) lengths ranging from 0.82 to 1.26 µm, short arm (SA) lengths from 0.62 to 0.84 µm, and total lengths (TL) between 1.44 and 2.10 µm. The arm ratio (AR) spanned 1.07 (chromosome 3) to 1.51 (chromosome 2), while the relative values (RV) ranged from 0.66 (chromosome 2) to 0.85 (chromosome 5). The mean values recorded were: LA = 0.99 µm, SA = 0.72 µm, TL = 1.71 µm, AR = 1.32, and RV = 0.74.

Table 3: Description of Chromosomes in FUAMPEA-3 Variety

FUAMPEA-3 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	0.69	1.92	1.78	0.56	M
Chromosome 2	1.18	0.68	1.86	1.73	0.58	SM
Chromosome 3	1.07	0.79	1.86	1.35	0.74	M
Chromosome 4	1.07	0.73	1.81	1.47	0.68	M
Chromosome 5	0.91	0.85	1.76	1.07	0.93	M
Chromosome 6	0.83	0.69	1.52	1.20	0.83	SM
Chromosome 7	0.92	0.6	1.52	1.53	0.65	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	0.60	0.42	1.02	1.43	0.70	M
Mean	0.91	0.65	1.56	1.42	0.72	

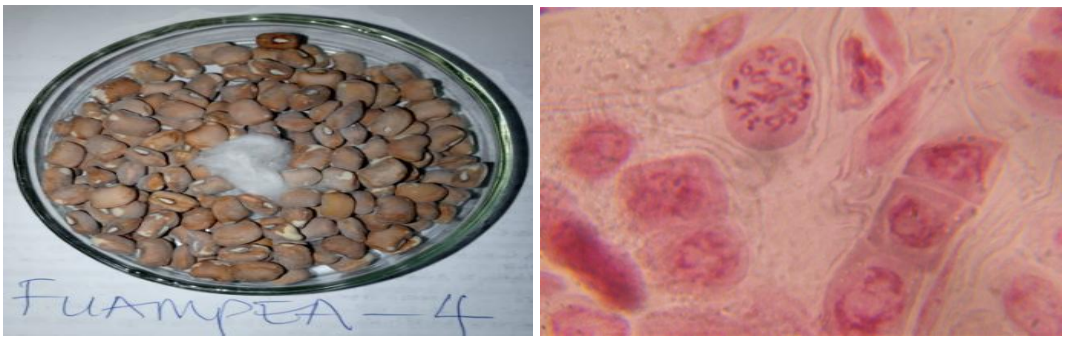

Plate 4: Chromosomes of FUAMPEA-4 Variety at Metaphase Stage of Mitosis

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

Table 4: Description of Chromosomes in FUAMPEA-4 Variety

FUAMPEA-4 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	1.26	0.84	2.10	1.50	0.67	M
Chromosome 2	1.24	0.82	2.06	1.51	0.66	M
Chromosome 3	1.01	0.94	1.95	1.07	0.93	M
Chromosome 4	1.07	0.74	1.81	1.44	0.69	SM
Chromosome 5	0.95	0.81	1.76	1.17	0.85	M
Chromosome 6	0.95	0.72	1.67	1.31	0.76	M
Chromosome 7	0.90	0.72	1.62	1.25	0.80	SM
Chromosome 8	0.95	0.64	1.59	1.48	0.67	M
Chromosome 9	0.86	0.68	1.54	1.26	0.79	M
Chromosome 10	0.84	0.66	1.50	1.28	0.79	SM
Chromosome 11	0.82	0.62	1.44	1.32	0.76	SM
Mean	0.99	0.74	1.73	1.33	0.76	

Plate 4 presents the chromosomes of the FUAMPEA-4 variety at the metaphase stage of mitosis, while Figure 4 shows the corresponding karyotype. The variety exhibited 22 diploid chromosomes (2n = 22), comprising 11 haploid chromosomes, with a karyotype formula (KF) of 7 metacentric and 4 sub-metacentric types (7M + 4SM). As shown in Table 4, the chromosomes were characterized by long arm (LA) lengths ranging from 0.82 to 1.26 µm, short arm (SA) lengths of 0.62 to 0.84 µm, and total lengths (TL) of 1.44 to 2.10 µm. The arm ratio (AR) ranged from 1.07 (chromosome 3) to 1.51 (chromosome 2), while the relative value (RV) varied from 0.66 (chromosome 2) to 0.85 (chromosome 5). The mean values recorded for these parameters were LA (0.99 µm), SA (0.71 µm), TL (1.70 µm), AR (1.33), and RV (0.75).

Table 5: 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
FUAMPEA-1	5.66± 0.88	9.24±0.15	15.05± 1.43	1.63± 0.13	0.61± 0.03	2.04± 0.01
FUAMPEA-2	7.93± 0.66	10.69±0.67	18.62± 0.23	1.34± 0.05	0.74± 0.03	1.72± 0.02
FUAMPEA-3	7.13± 0.57	10.04±0.21	17.18± 0.03	1.41± 0.03	0.71± 0.02	1.78± 0.01
FUAMPEA-4	8.19± 0.37	10.85±0.24	19.04± 1.33	1.32± 0.12	0.75± 0.01	1.22± 0.01
P-value	P<0.05	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05

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

Table 6: Variability in Karyological Indices of Cowpea Varieties

Cowpea accession	CV %	Total form (%)	DI (%)	A_1	A_2	KF	CN (2n)
FUAMPEA-1	14.16	51.67	44.28	0.024	0.058	6M+5Sm	22
FUAMPEA-2	14.78	52.55	49.43	0.021	0.039	6M+5Sm	22
FUAMPEA-3	14.59	51.67	44.28	0.024	0.058	6M+5Sm	22
FUAMPEA-4	14.59	51.67	44.28	0.024	0.058	7M+4Sm	22

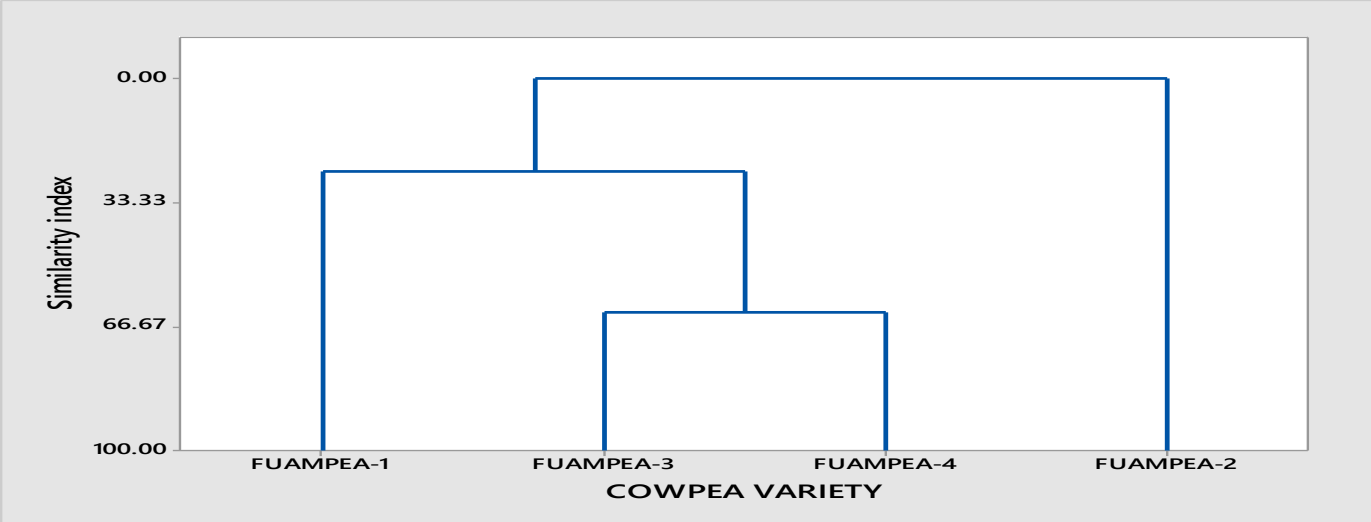

Figure 5: Dendrogram of the four cowpea varieties

DISCUSSION

The findings of this study suggest that evolutionary changes in cowpea are not solely influenced by chromosome number, but also by inherent variations in chromosome types, morphology, and behavior, as well as other karyological indices. These results corroborate previous karyological studies in mungbean and Vigna species (Aremu et al., 2016; Chan, 2021; Gupta, 2019; She et al., 2020; Singh & Gupta, 2020). Each cowpea variety investigated exhibited unique karyological features contributing to genetic diversity. FUAMPEA-1 possessed the shortest chromosomes, while FUAMPEA-2 showed the highest coefficient of variation and formed the widest clustering pattern, indicating notable disparity in chromosomal structure. Karyological features have been linked to physiological adaptations such as improved stomatal efficiency and evapotranspiration, enhancing plant productivity (Chan, 2021; Gupta, 2019). Hence, the chromosomal variability and karyotype indices observed among the ten improved cowpea varieties may enhance adaptation as expressions of underlying genetic diversity

These karyological parameters have previously been used to distinguish taxa across family, genus, and species levels in legumes, particularly in Vigna (Bhowmick & Jha, 2019; Osuagwu et al., 2022). The clustering patterns derived from cytogenetic data further support reports of high genetic variability among the varieties. The ten varieties grouped into five distinct sub-clusters, suggesting wide dissimilarity indices. Cytogenetic evidence has proven valuable for taxonomic classification, varietal identification, and establishing phylogenetic relationships (Osuagwu et al., 2022). Ogunkanmi et al. (2014) used SSR markers to confirm the high genetic divergence among cowpea genotypes. Similarly, Olasan et al. (2023) used molecular tools to assess phylogenetic relationships among four FUAMPEA varieties and found FUAMPEA-1 to be the most genetically distinct, followed by FUAMPEA-4.

Karyological analysis offers a cost-effective, rapid, and reliable method for assessing genetic variability in cowpea, especially in breeding programs where molecular tools may be inaccessible. This view aligns with Mirzaei (2021), who emphasized the use of genetic tools for phylogenetic studies in new varieties. Karyotype analysis, encompassing chromosome number and structure, remains critical for understanding genetic diversity (Alam et al., 2018; Osuagwu et al., 2022). Chromosomal traits are stable across generations and remain fundamental in genome organization studies (Alam et al., 2018).

CONCLUSION

Significant variation was observed in the coefficient of variation, total form, disparity index, and both intra- and inter-chromosomal symmetry, as well as the karyotype formula across the varieties. FUAMPEA-1 had the lowest coefficient of variation (14.16), indicating reduced chromosomal variability compared to FUAMPEA-2. Other parameters such as symmetry, total form, and disparity index were relatively consistent in FUAMPEA-1, FUAMPEA-3, and FUAMPEA-4, whereas FUAMPEA-2 showed marked deviations. These karyological findings indicate considerable genetic variability among the four varieties, which is valuable for breeding and agronomic improvements, as traits like chromosome size, arm ratio, and symmetry may correlate with yield potential, disease resistance, and adaptability. Despite all varieties having the same chromosome number, confirming their placement within the same species without evidence of evolutionary speciation, noticeable chromosomal differences suggest significant intra-species diversity. Breeders should exploit this chromosomal variability and related karyological traits to enhance cowpea improvement programs. FUAMPEA-2 was particularly notable for its high coefficient of variability and disparity index. Further karyological studies are recommended on cowpea landraces and other Fabaceae members to explore untapped genetic resources.

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