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RESEARCH ARTICLE

Diversity analysis of proximate principles, macro and micro nutritional traits in fodder cowpea

J S Suvadra[1], Swarnalata Das[2*], Niranjan Panda[3], Digbijaya Swain[1], Bandita Jena[4], Arbinda Dhal[2], Subhashree Das[5], Binayak Dash[2] & Sourav Ranjan Nanda[1]

[1]Department of Genetics and Plant Breeding, Odisha University of Agriculture & Technology, Bhubaneswar 751 003, Odisha, India
[2] All India Coordinated Research Project on Forage Crops & Utilisation, Odisha University of Agriculture & Technology, Bhubaneswar 751 003, Odisha, India
[3]Department of Animal Nutrition, Odisha University of Agriculture & Technology, Bhubaneswar,751 003, Odisha, India
[4] All India Coordinated Research Project on Micro Secondary Nutrients, Odisha University of Agriculture & Technology, Bhubaneswar 751 003, Odisha, India
[5]All India Coordinated Research Project on Cotton, Regional Research & Technology Transfer Station, Bhawanipatna, Odisha University of Agriculture & Technology, Kalahandi 766 001, Odisha, India

*Correspondence email - swarnalata@ouat.ac.in

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Abstract

Assessing nutritional diversity in fodder cowpea (*Vigna unguiculata* L. Walp.) is essential for genetic improvement of fodder quality. Nutritional diversity analysis helps to identify superior genotypes that enhance livestock nutrition and overall productivity. This study evaluated twenty-two fodder cowpea genotypes to quantify variation in proximate principles and mineral nutrient composition. Results revealed significant variation among the genotypes for different nutritional traits. Proximate traits such as dry matter, crude protein and crude fibre content ranged from10.99 - 24.43 %, 8.26 - 23.54 % and 23.67 - 35.79 %, indicating considerable nutritional diversity. Macro- (Calcium, Magnesium, Phosphorus, Potassium) and micro-nutrient (Zinc, Iron, Copper) concentrations also varied widely among genotypes. Principal component analysis for proximate principles revealed that PC1 & PC2 together contributed 54.83 % of total variability whereas for macro and micronutrients PC1 & PC2 components together contributed 56.66 % of total variability. Exploiting nutritional diversity through systematic breeding and selection strategies could contribute to the development of superior fodder cowpea cultivars with improved feed value, thereby supporting sustainable livestock production.

Keywords: diversity; fodder cowpea; macro and micronutrients; nutritional quality; PCA; proximate principles

Introduction

Diversity of nutritional traits in fodder cowpea (*Vigna unguiculata* L. Walp.) is crucial as it reveals significant genetic variation in traits like crude protein, fiber content, fat content and mineral composition. Nutritional characterization of diverse germplasm is essential for crop improvement programmes aimed at identifying superior genotypes that can enhance livestock nutrition, feed efficiency and overall productivity. Nutritional profiling of diverse accessions supports breeding programs aimed at developing high-yield, nutrient-rich varieties adapted to different agro-climatic conditions. The area under cultivated fodder has remained constant on 8.3 million ha (Mha) at individual crop basis (1). As per record, by 2025, the demand for green and dry fodder will show a deficit of 64.87 % and 24.92 % respectively (2). Cowpea has been considered as a potential legume crop for meeting the requirement of high-quality fodder. Fodder cowpea tends to have crude protein upto 26 % (3) making them a valuable protein source. In addition, cowpea fodder provides essential macro-nutrients (Ca, P, K) and micro-nutrients (Fe, Zn, Mn, Cu), which play key roles in maintaining animal health and productivity (4). Its capacity to fix atmospheric nitrogen further enhances its value, particularly for farmers adopting sustainable and low-input production systems.

Therefore, the present study aimed to assess the nutritional diversity of fodder cowpea genotypes based on proximate composition and mineral nutrient profiles to support future breeding programmes.

Materials and Methods

Plant materials

The experimental materials of the present study comprised of twenty-two fodder cowpea genotypes of four different states like Karnatak, Uttarakhand, Tamil Nadu and Telangana (list is furnished in Table 1).

Table 1. List of cowpea genotypes considered for nutritional profiling

Sl. No.	Genotype	Source	Sl. No.	Genotype	Source
1	MFC-08-14	Mandya, Karnataka	12	UPC-9202	Pantanagar, Uttarakhand
2	MFC-09-3	Mandya, Karnataka	13	UPC-801	Pantanagar, Uttarakhand
3	MFC-18-2	Mandya, Karnataka	14	UPC-802	Pantanagar, Uttarakhand
4	MFC-18-8	Mandya, Karnataka	15	UPC-804	Pantanagar, Uttarakhand
5	MFC-18-10	Mandya, Karnataka	16	UPC-2001	Pantanagar, Uttarakhand
6	EC-107120	Mandya, Karnataka	17	UPC-2002	Pantanagar, Uttarakhand
7	MFC-09-1	Mandya, Karnataka	18	N-311	Coimbatore, Tamil Nadu
8	NBC-40	Mandya, Karnataka	19	IFC-9304	Coimbatore, Tamil Nadu
9	NBC-43	Mandya, Karnataka	20	TNFC-926	Coimbatore, Tamil Nadu
10	UPC-4200	Pantanagar, Uttarakhand	21	Sweta	Hyderabad, Telangana
11	UPC-5287	Pantanagar, Uttarakhand	22	UPC 625	Pantanagar, Uttarakhand

Experimental details

The genotypes were evaluated in a randomised block design with three replications at AICRP on forage crops & utilisation, OUAT, Bhubaneswar during the year 2022 & 2023. Each experimental plot consisted of 6 rows of 4 m length with row to row spacing of 30 cm. Five plants were selected randomly at 50 % flowering stage to take observations on shoot fresh weight (green fodder yield) per plant and required quantity of plant samples were collected at this stage for the estimation of proximate principles (dry matter, ash, crude fat, crude fibre and crude protein), macro and micronutrient nutrients (P, K, Ca, Mg, Fe, Zn & Cu).

Estimation of proximate principles

Plant samples were sun dried and then oven dried for estimation of dry matter content (5). The ash content is derived from dried sample placed in the muffle furnace until the sample turned into white or light-grey ash (indicating that all organic matter was combusted). The weight of ash was recorded for determination of ash percentage (6). Samples of ground material were analysed for percentage nitrogen using a micro-Kjeldahl procedure. Percentage of crude protein was then calculated by multiplying the Kjeldahl nitrogen content with a factor of 6.25 (7). Formula for estimating of crude protein is mentioned below.

$$\text{Percent N content in sample} = \frac{\text{Sampletitrate - Blank titate)} \times \text{Normality of HCL} \times 14 \times \text{Volume by digestion} \times 100}{\text{Aliquot of digestion taken} \times \text{Sample weight (gm)} \times 1000}$$

Protein content of plant sample had been determined by estimating the nitrogen content as per the modified Kjeldahl's method and multiplying the nitrogen content with a factor 6.25 and expressed on percentage basis for each genotype.

$$\text{Protein \%} = \text{Nitrogen \%} \times 6.25$$

For the estimation of crude fat percentage, 2-5 g of dried sample was weighed and placed in an extraction thimble suspended in a round bottom flask assembled with the Soxhlet extractor and 250 mL of petroleum ether was added to the flask followed by subsequent distillation and condensation of the solvent. By the end of the cycle the flask was weighed with extracted fat to determine the fat percentage. For crude fibre estimation the fat processed sample was placed in the muffle furnace and weighed for loss of weight (8). The proximate principles were analyzed using AOAC, 2005.

Estimation of macro & micronutrient components

For estimation of macro & micronutrient tri–acid digestion method (9) (nitric acid + sulphuric acid + perchloric acid) at5: 1: 2 ratios were used. 0.5 g dried and powdered plant sample was weighed in a 100 mL conical flask. Then 10 mL concentrated HNO_3 was added and samples were left overnight for pre-digestion by adding 1 ml concentrated H_2SO_4 and 2-3 mL of $HClO_4$. The samples were heated to about 100 °C for first 1 hr and then temperature was raised to about 200 °C. Digestion continued until the contents become colorless and white dense fumes appeared. The acid contents were reduced to about 2-3 mL by continuing heating at the same temperature. Flasks were removed from hotplate, cooled and 30 mL of distilled water was added. Solutions were filtered through Whatman No. 42 filter paper into 100 mL volumetric flask and volume was made up to 50 mL. The estimation of macro nutrient P, K, Ca, Mg (%) and micronutrient Fe, Zn and Cu content (ppm) were carried out using ICP - OES (Inductively coupled plasma - optical emission spectroscopy).

Statistical analysis

Statistical analysis (ANOVA with CRD) for all the nutritional traits was done following SAS software, version, 9.3. Diversity study for the nutritional traits under study was done through principal component analysis (PCA) using GRAPES software (10) and cluster analysis was done using PAST software (11).

Results and Discussion

Variation in nutritional traits of fodder cowpea genotypes

Substantial variation was observed among the 22 genotypes for proximate composition (Fig. 1). Dry matter, crude protein and crude fibre exhibited the widest ranges, indicating strong genetic divergence. Genotype MFC-18-10 recorded the highest dry matter content (24.43 %), while MFC-09-3 was superior for crude protein (23.54 %). Total ash (TA) content varied from 6.15 % (MFC-08-14) to 11.92 % (N-311). Crude fat (CFA) ranged from 1.14 % (NBC-40) to 3.78 (UPC-802. Crude fibre (CF) was found to be the maximum in EC-107120 (35.79 %). The current study displayed significant variation among the genotypes for its nutritional traits. Such variability aligns with earlier reports on fodder legumes (12), highlighting opportunities for targeted quality improvement.

The maximum, minimum and mean values of different macro and micronutrients are shown in Fig. 2 & 3. Calcium content ranged from 0.301 % (UPC 625) to 0.670 % (MFC-18-8) with the mean of 0.448. Magnesium content ranged from 0.250 % (MFC-18-10) to 0.568 % (MFC-08-14) with mean of 0.362 %. Phosphorus and potassium content was the highest in UPC-4200 (0.202 %) and in

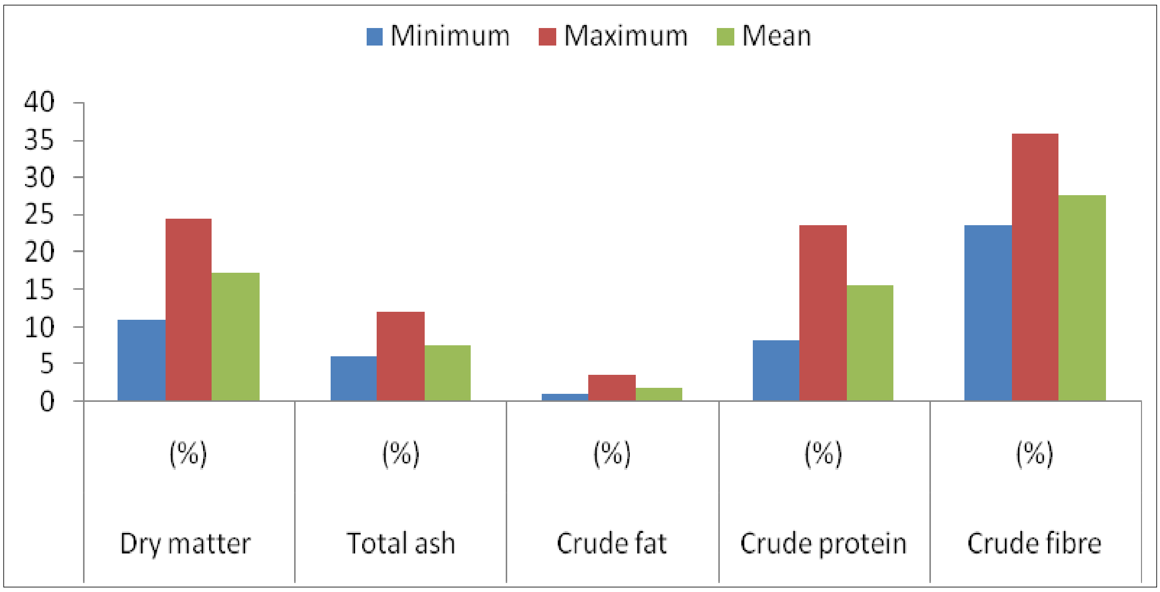

Fig. 1. Variation in proximate principles of fodder cowpea genotypes.

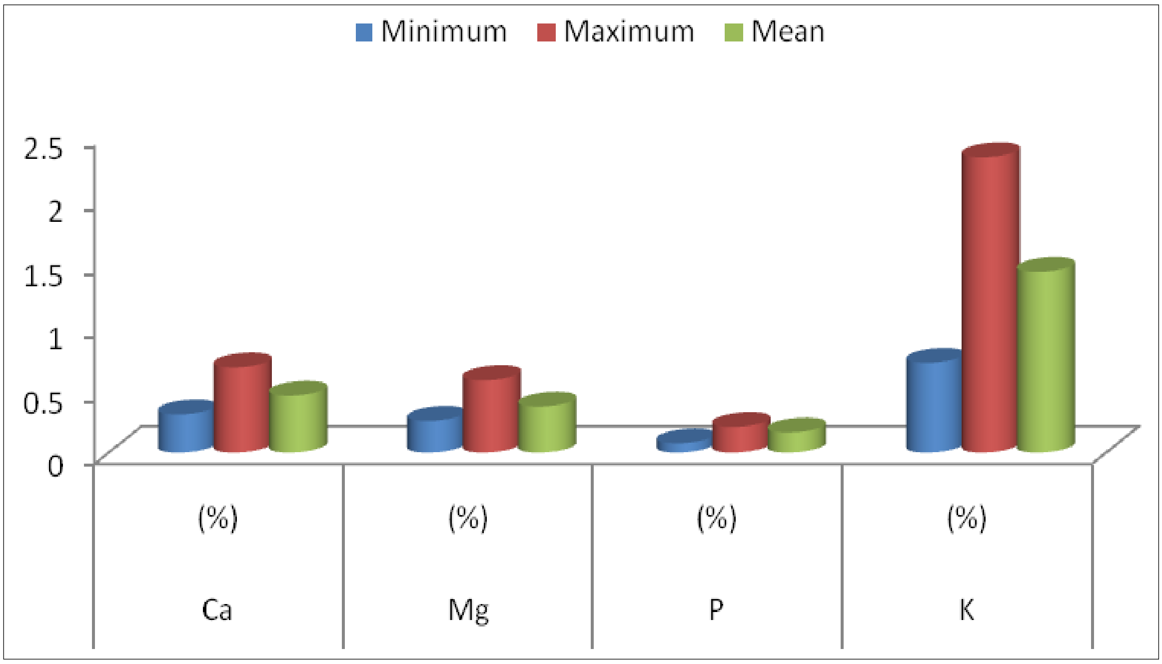

Fig. 2. Variation in macro nutrients of fodder cowpea genotypes.

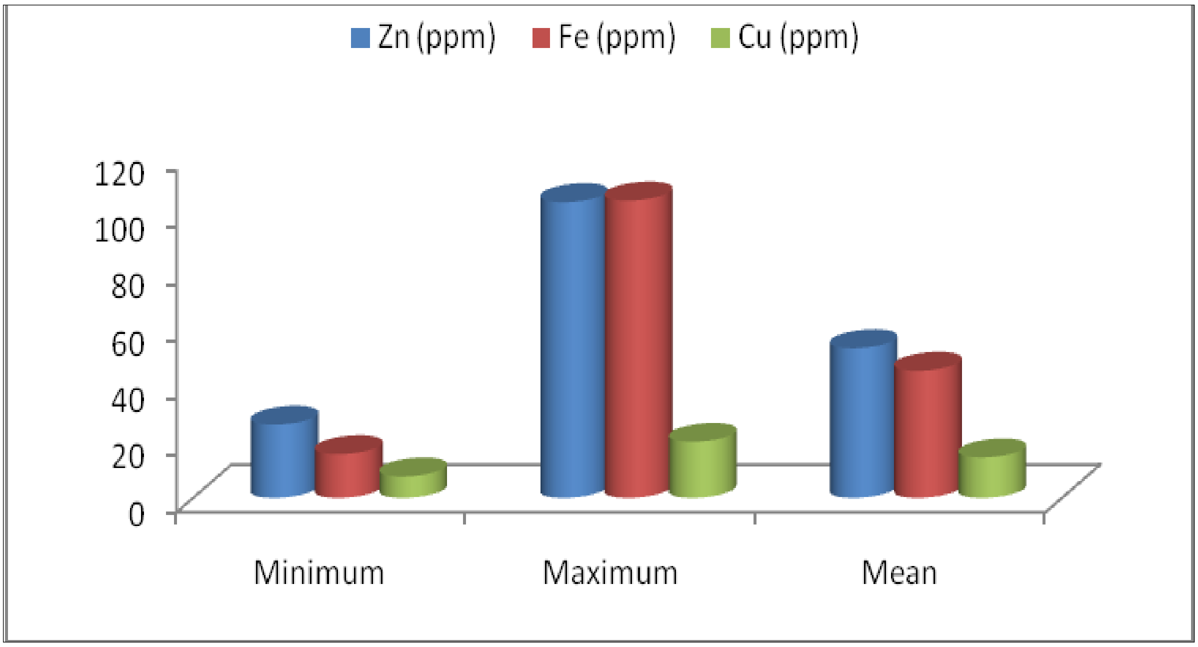

Fig .3. Variation in micro nutrient composition of fodder cowpea genotypes.

NBC-40 (2.325 %). Zinc (Zn) content ranged from 25.74 ppm in SWETA to 103.99 ppm in UPC-4200; Iron (Fe) content ranged from 15.38 ppm (UPC-802) to 104.56 ppm (UPC-2001); Copper (Cu) content of the genotypes ranged from 7.52 % (N-311) to 19.71 % (UPC-2001). Coefficient of variation was the maximum in case of iron (45.25 %) followed by zinc (42.15 %).

Variation in micronutrient composition such as zinc (25.74-103.99 ppm) and iron (15.38 - 104.56 ppm) may reveal that UPC-4200 and UPC-2001 could be used for biofortification. Similarly, previous researchers (13) studied variation in Zn and Fe content of wheat to identify bio-fortified genotypes. Difference in macronutrient composition, including Ca, Mg, P and K indicated a scope for metabolic and stress regulation. NBC-40 having the highest potassium content could be useful in stress management for improved nutrient uptake and drought tolerance (14). Crude fat content exhibited high variation with CV of 32.75 % suggesting lipid content being highly genotype dependent (15). In previous researchers found substantial variation in Cu, Fe and Zn content of cowpea leaves(16). This study indicated a wide range of variation that could be utilised for genetic improvement in mineral composition of fodder quality.

Divergence analysis of genotypes based on proximate principles

Divergence analysis in fodder cowpea for proximate principles, macro and micronutrient was assessed following principal component and cluster analysis. The principal component analysis (PCA) performed on the proximate principles revealed that PC1, PC2 and PC3 had the eigen value above 1 (1.765, 1.525 and 1.037) and these three components contributed 29.41 %, 25.42 % and 17.29 % of total variability respectively (Table 2). PC1 was positively influenced by dry matter content (0.508), crude fibre content (0.497) and shoot fresh weight (0.399) whereas PC2 was positively influenced by crude protein and crude fat content (Table 2). Both the PC1 & PC2 were negatively affected by total ash content (-0.451, -0.326). From PC1 and PC2 biplots (Fig. 4) it was observed that the 22 genotypes were scattered in four different quadrants forming four groups and this indicated a high degree of diversity among the genotypes. The proximate principles were also classified into four distinct groups. Dry matter content formed one group, crude fat and crude protein formed second group, total ash content was in third group and crude fibre content was in the fourth group. Genotype MFC-18-10 present in 4th quadrant showed high dry matter content followed by MFC-08-14. Genotype MFC-08-14 has the highest shoot fresh weight (green fodder yield/plant). MFC-09-3 is best suited for crude protein content. The present study summarised that crude protein and crude fibre, widely contributed towards variability of PC1 components. The same finding was supported by (17, 18). This identification of traits contributing towards variability helps facilitate efficient selection for breeding programs of fodder (19, 20).

The correlation plot between proximate principles and principal components (Fig. 5) explained the extent to which each proximate principle correlated to each PC. Dry matter (DM %), crude fibre (CFI %) and shoot fresh weight (SHFW) exhibited

Table 2. Loading factors of proximate variables

Proximate principles	PC1 (Dim. 1)	PC2 (Dim. 2)	PC3 (Dim.3)
Dry matter %	0.508	0.266	-0.080
Total ash %	-0.451	-0.326	0.493
Crude fat %	-0.354	0.444	-0.564
Crude protein %	-0.082	0.658	0.323
Crude fibre %	0.497	-0.353	-0.237
Shoot fresh weight (g) (SHFW)	0.399	0.261	0.522
Eigenvalue	1.765	1.525	1.037
Variability %	29.411	25.415	17.29
Cumulative %	29.411	54.825	72.115

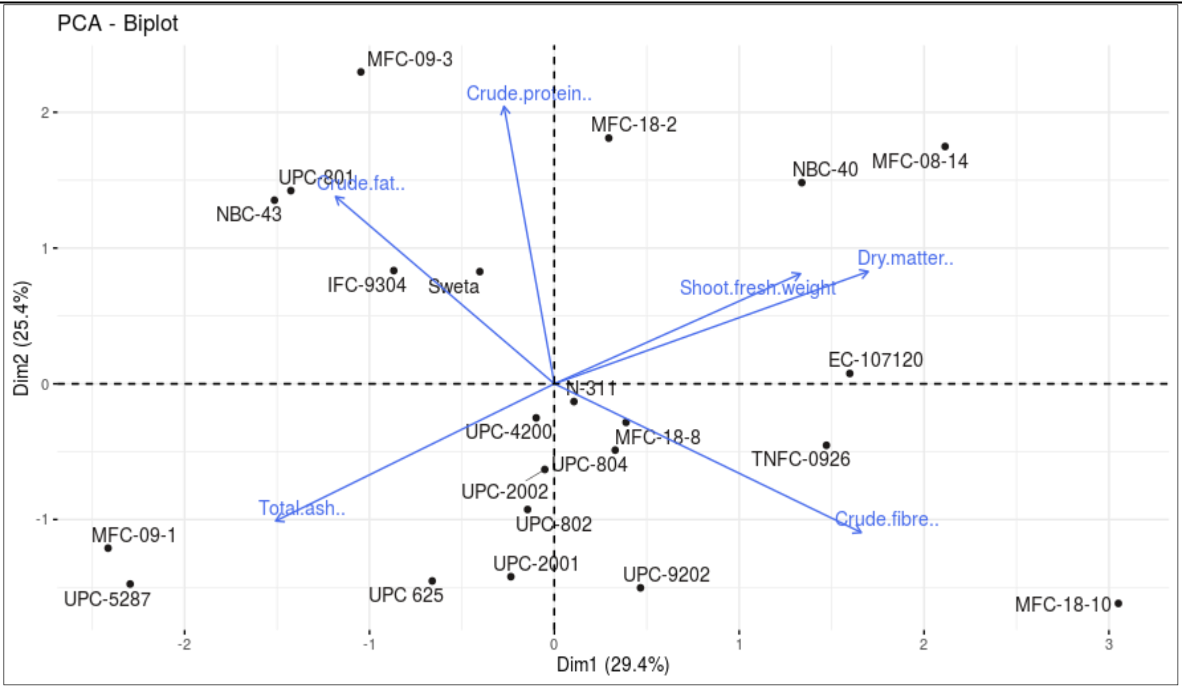

Fig. 4. PC1 & PC2 biplot showing divergence of proximate principles in fodder cowpea.

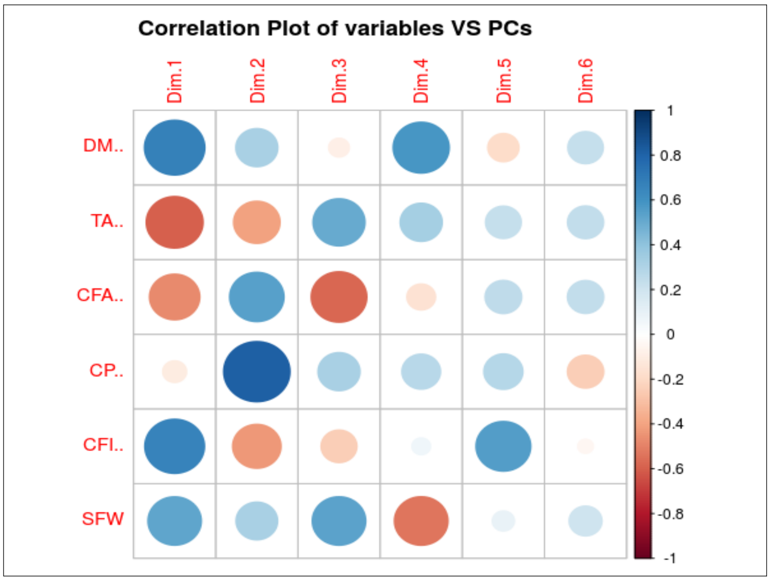

Fig. 5. Correlation plot of proximate principles and shoot fresh weight with PCs.

highest positive correlations with PC1. Total ash (TA) and crude fat (CFA) displayed highly negative correlations with PC1. Crude protein (CP) showed highest positive correlation with PC2. PC3 showed the strongest associations with CFA, SHFW and TA. Dry matter and shoot fresh weight per plant point in the same direction, indicating a strong positive correlation between the two (21, 22). On a contrary, vector for crude fibre and crude fat points in opposite direction, suggesting a negative correlation, similar result has been recorded in earlier studies (20). Crude fibre and crude fat were in opposite direction suggesting negative correlation. Similar result has been observed in maize by (23).

Cluster analysis was also performed following Euclidean distance method to study divergence among fodder cowpea genotypes for proximate principles. From Supplementary Fig.1, it was observed that similarity increases with the decrease in Euclidean distance. At higher distance similarity (close relationship) is low and at lower distance it is high. At dissimilarity coefficient of 10 all the genotypes appeared as a single unit. The genotypes were classified into four groups at dissimilarity coefficient of 7 (Euclidean distance is 7 units). The larger group consisted of twelve numbers of genotypes, second group comprised of eight genotypes and rest two groups contained single genotype each (MFC-09-1 & MFC -18-10). This study clearly indicated diversity among the genotypes based on proximate principles. Genotypes present in different clusters may be used in crossing programme for genetic improvement of fodder quality. Similar approach has been used previously (24) to categorize Malabar spinach samples.

Divergence analysis of genotypes based on macro- and micro-nutrients

The loading factor of the principal components for macro and micronutrients is presented in Table 3. The principal component analysis (PCA) performed on the macro and micronutrients showed that PC1, PC2 and PC3 had the eigen value greater than one (3.023, 1.510 and 1.149) and these three components contributed 37.79 %, 18.87 % and 14.36 % of total variability respectively (Table 3). PC1 and PC2 together contributed 56.66 % of total variability. All the macro (Ca, Mg, P, K) and micronutrients (Zn, Cu, Fe) had positive influence on PC1. Calcium contributed the maximum variability (0.508) towards PC1 followed by Potassium (0.420). High calcium content is essential for making bones stronger and potassium content improves muscle functions. PC2 was positively influenced by copper (0.408) and negatively influenced by phosphorus (-0.472) (Table 3). From PC1 and PC2 biplots (Fig. 6) it was observed that the 22 genotypes were dispersed in four different quadrants forming four groups and this indicated a high level of diversity among the genotypes. The macro and micronutrients were classified into two distinct groups. Genotype UPC- 4200 shows high zinc content. Genotype NBC-40 has the highest calcium content. MFC-08-14 is best suited for copper content.

The correlation plot of macro and micronutrients with principal components is depicted in Fig. 7. All the macro and micronutrients exhibited positive correlations with PC1 and the maximum was being observed with calcium. Copper had the highest positive correlation with PC2. Ca, K, Mg, P contributed most towards variation. Similar observations were recorded in pigeon pea (25).

Table 3. Loading factors of macro and micronutrients

Macro & micro nutrient	PC1 (Dim. 1)	PC2 (Dim. 2)	PC3 (Dim.3)
Zinc	0.302	-0.192	-0.335
Iron	0.201	0.368	-0.724
Copper	0.382	0.408	0.088
Calcium	0.502	0.004	0.058
Magnesium	0.403	0.197	0.060
Phosphorus	0.360	-0.472	0.255
Potassium	0.420	-0.137	0.204
SHFW	-0.035	-0.617	0.492
Eigenvalue	3.023	1.510	1.149
Variability %	37.79	18.87	14.36
Cumulative %	37.79	56.66	71.02

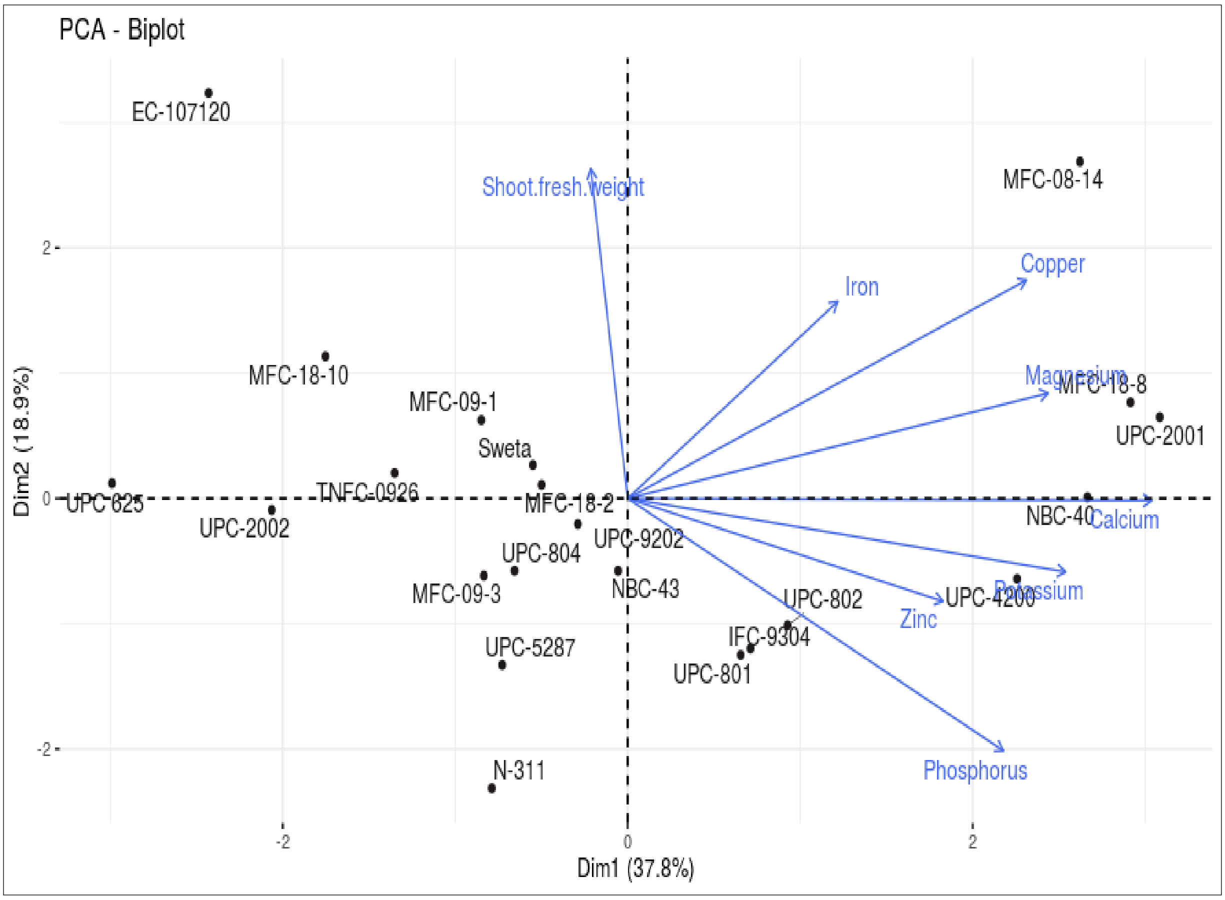

Fig. 6. PC1 & PC2 biplot showing divergence in macro and micro nutrients of fodder cowpea.

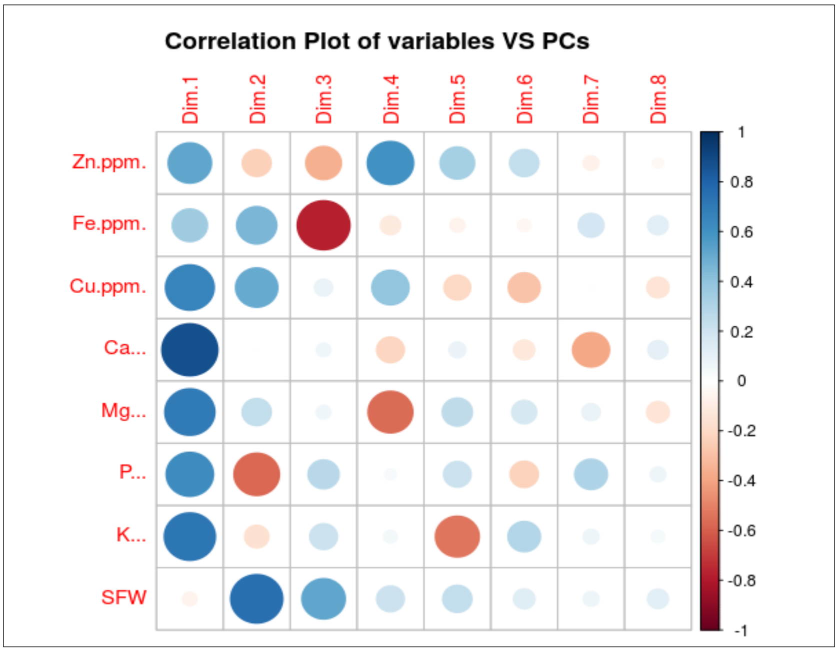

Fig. 7. Correlation plot of macro & micronutrients with principal components.

Cluster analysis in genotypes for macro and micronutrients is shown in Supplementary Fig. 2. At Euclidean distance of 30 units, the genotypes were classified into six groups. The first group comprised of eight numbers of genotypes; second group consisted of seven genotypes; third group contained four genotypes and rest three groups had single genotype each. Genotypes present within each cluster have similar fodder quality and selection of genotypes should be done from different clusters for hybridisation programme. This result indicates genotypes showed variation in their mineral composition and such variation could be further utilized for crop improvement.

Cluster analysis of 22 fodder cowpea genotypes based on all the nutritional parameter is shown in Fig. 8. It was observed that at Euclidean distance of 30 units the genotypes were classified into six groups. The first group comprised of eight numbers of genotypes; second group consisted of seven genotypes; third group contained four genotypes and rest three groups had single genotype each. Similar approach of clustering showing diversity in nutritional traits was performed by previous researchers in Turkish pea germplasm and in cluster bean (26, 27).

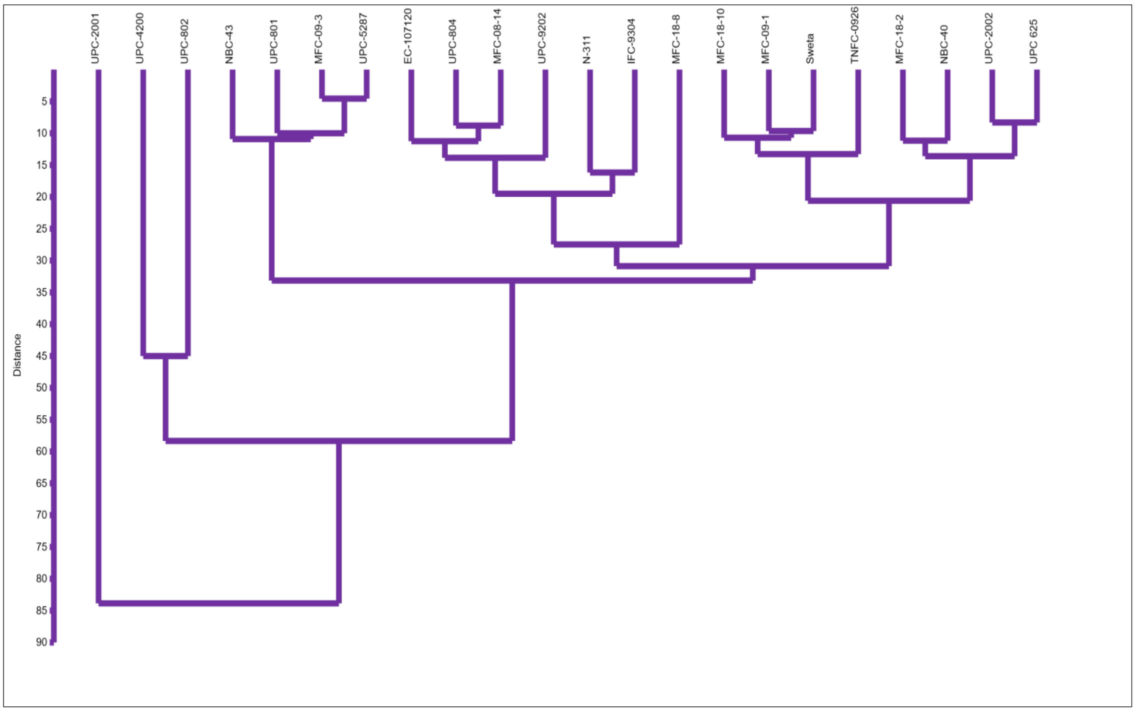

Fig. 8. Clustering of 22 fodder cowpea genotypes based on all nutritional traits.

Conclusion

The study demonstrated substantial variation among fodder cowpea genotypes in both proximate principles and macro- and micro-nutrient traits. The diversity identified in macro- (Calcium, Magnesium, Potassium and Phosphorus) and micronutrients (iron, zinc, copper) further highlights the importance of specific genotypes for addressing livestock nutritional requirements and combating mineral deficiencies. The variability in crude protein, fiber fractions and mineral composition highlights the potential for genetic improvement. This diversity study can be effectively utilized in breeding programs to develop nutritionally superior fodder cowpea cultivars for sustainable livestock production (28, 29).

Authors' contributions

JSS conducted lab experiments, field experiments and estimated all nutritional parameters. SD[1] manuscript drafting and experimental design formulation. NP provided lab facility for proximate analysis. DS prepared the graphs. BJ assisted in analysis of micro and macro nutrients. AD provided field facility. SD[2] helped in checking of the manuscript. BD helped in field work. SRN helped in data analysis. All authors read and approved the final manuscript [SD[1]- Swarnalata Das; SD[2]- Subhashree Das].

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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