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GENOTYPIC VARIATIONS IN SEED NUTRITIONAL QUALITY AND MICRONUTRIENT COMPOSITION OF COWPEA (*Vigna unguiculata* L.)

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ABSTRACT

Cowpea (*Vigna unguiculata* L.) is a nutritionally important pulse crop with strong potential to enhance dietary protein and mineral intake. A pot experiment was conducted at CCS Haryana Agricultural University, Hisar (India) during the summer season of 2023-24 to assess genotypic variability in quality traits and nutrient composition across ten cowpea genotypes. The study was arranged in a completely randomized design with three replications. Grain samples collected at harvest were analysed for protein, carbohydrate, nitrogen, potassium, and micronutrients (Zn, Fe, Mn, Cu), along with Zn concentration in pods and the overall index of nutritional quality (OINQ), using standard procedures. Significant ($p \leq 0.05$) differences were observed among genotypes for all parameters. Protein content was in the range of 18.0–23.5%, while seed Zn and Fe concentrations varied between 28.12 and 41.66 ppm, and 40.2 and 59.2 ppm, respectively. Correlation analysis revealed strong positive associations among protein, nitrogen, Zn, Fe, and Cu, suggesting coordinated accumulation of these traits, whereas carbohydrate content showed strong negative correlations, indicating a dilution effect. Principal component analysis (PCA) explained 90.26% of total variance within the first three components, with PC1 representing a nutritional quality axis dominated by protein and micronutrients. Genotypes 'Kashi Kanchan' and 'Cowpea-263' consistently exhibited superior nutritional profiles and higher OINQ values. The study highlights the utility of multivariate approaches in identifying nutritionally superior cowpea genotypes and provide promising candidates for biofortification-oriented breeding under semi-arid Indian conditions.

Keywords: Correlation, genotypic variation, micronutrient accumulation, nutrient composition, protein content

INTRODUCTION

Cowpea (*Vigna unguiculata* L.) is an extensively cultivated legume crop having considerable nutritional and economic significance, particularly in tropical and subtropical regions of Asia and Africa. It is an important source of dietary protein, carbohydrates, essential minerals, and micronutrients, contributing to food security and nutritional security in low-income populations (Nimbal and Ameen, 2024). Besides its nutritional value, cowpea improves soil fertility through symbiotic nitrogen fixation and is well adapted to semi-arid environments, making it a critical element of sustainable cropping systems (Shukla *et al*., 2024). Despite its nutritional potential, micronutrient deficiency commonly known as "hidden hunger" remains a persistent global challenge, affecting billions of people who depend on plant-based diets deficient in essential micronutrients like Zn, Fe, Mn and Cu. These deficiencies can lead to impaired immune function, stunted growth, and adverse health outcomes, particularly in vulnerable populations (Abebe *et al*., 2022). Therefore, improving the amount and bioavailability of micronutrients in staple crops has become a major concern in both agricultural studies and public wellbeing programmes.

Biofortification through classical breeding, agronomic practices, and advance biotechnology, represents a sustainable strategy for enhancing the micronutrient composition of food crops. Among these strategies, agronomic biofortification reportedly enhances micronutrient contents and grain quality in cowpea through foliar nutrient applications and improved nutrient management (Valenca, *et al.*, 2017). Recent studies have confirmed significant genotypic differences in cowpea for nutritional quality and responsiveness to micronutrient augmentation, thereby highlighting the potential of selecting and breeding genotypes with superior nutrient accumulation and grain quality traits (Padhi *et al.*, 2022; Araujo *et al.*, 2022). For instance, profiling of proximate and mineral composition across diverse cowpea genotypes revealed significant differences in protein and micronutrient concentrations, representing valuable genetic resources for the development of cultivars with enhanced grain nutritional attributes (Reddy *et al*., 2016).

Genotypic differences in grain nutritional quality traits and nutrient indices are critically important for the selection of nutrient-dense genotypes in biofortification programmes (Nalawade *et al.*, 2021). Evaluating this variation under controlled conditions provides an opportunity for precise assessment of inherent genetic differences in nutrient uptake and accumulation, while minimizing the confounding field variability. Such information is essential for breeders to develop the cultivars with enhanced nutritional status and for agronomists to optimize nutrient management strategies that improve grain nutrient density (Nguyen *et al.*, 2019). Recent studies have profiled nutritional variability in cowpea germplasm globally. Padhi *et al.* (2022) evaluated 120 cowpea genotypes for nutritional quality of seed and pod at NBPGR, New Delhi and reported seed protein content ranging from 19.4 to 27.9%, total starch from 27.5 to 42.7 g 100 g^{-1}, amylose from 9.65 to 21.7 g 100 g^{-1}, total dietary fiber from 13.7 to 21.1 g 100 g^{-1}, and total soluble sugars from 1.30 to 8.73 g 100 g^{-1}. Massey *et al*. (2020) at GBPUAT, Pantnagar (India) observed variations in nutritional profile of cowpea genotypes with seed protein content ranging from 22.51 to 29.60%, iron content from 5.10 to 9.68 mg 100 g^{-1}, zinc content from 2.54 to 5.59 mg 100 g^{-1}, and manganese from 1.10 to 1.30 mg 100 g^{-1}. Nimbal and Ameen (2022) profiled 14 cowpea accessions and reported protein in the range of 17.52 to 21.12% while iron content ranged from 47.1 to 99.1 ppm, zinc from 26.2 to 56.3 and manganese from 3.3 to 9.8 ppm. However, none of these studies simultaneously integrated nutritional profiling, Pearson correlation analysis, PCA and overall index of nutritional quality (OINQ). The present research was aimed to assess genotypic variations in grain quality parameters, nutrient composition, and nutrient density among the selected cowpea genotypes under subtropical semi-arid conditions of Haryana (India) so as to identify nutritionally superior cowpea genotypes which may support biofortification-oriented breeding programmes. The novelty lies in the integrated use of nutritional profiling, Pearson's correlation analysis, PCA, and OINQ as a composite multi-trait evaluation framework to identify nutritionally superior cowpea genotypes for biofortification-oriented breeding under subtropical semi-arid conditions of Haryana (India).

MATERIALS AND METHODS

The current study was conducted as pot experiment in summer, 2023-2024 in Research Farm of the Department of Vegetable Science, CCSHAU, Hisar (India). Located at a latitude of 29°10′ N and longitude of 75°46′ E, the experimental site lies 215 m masl in subtropical semi-arid climate zone. The region experiences temperature between -1 (in winter) to 45°C (in summer). Earthen pots of 30 cm dia, filled with 17 kg processed sandy loam soil, were used to raise the crop. Prior to pot filling, representative soil samples were drawn and used to assess the mechanical and physicochemical properties using standard analytical methods. The soil had 0.25% organic carbon, 132 kg ha^{-1} available nitrogen, 13.8 kg ha^{-1} phosphorus, and 238 kg ha^{-1} potassium. The cowpea varieties evaluated were procured from different SAU's and ICAR institutes and comprised of 'Arka Garima' [from IIHR, Bangalore], 'C-152' [from UAS, Bagalkot], 'Kashi Kanchan' & 'Cowpea-263' [from CCSHAU, Hisar], 'Kashi Nidhi', 'Kashi Shyamal' & 'Kashi Gauri' [from IIVR, Varanasi], 'Pusa Komal' [from IARI, New Delhi], and 'Konkan Safed' & 'Konkan Sadabhar' [from Dr. BSKKV, Dapoli]. The experiment was laid out in a completely randomized design with ten treatments (genotypes) and each replicated three times. Five seeds of genotype (as per treatment) were planted per pot at 3-4 cm depth. All the recommended agronomic practices for cowpea were as recommended for the region were followed consistently across all the treatments (CCSHAU, 2019). Adequate care was taken to maintain uniform growth conditions in all pots to minimize environmental variability and experimental error (Ceritoglu *et al.*, 2021).

The plants were harvested at physiological maturity stage, and seed samples from each replicate collected, cleaned, oven-dried at 65-70°C until they reached a consistent weight, and then finely pulverized in a stainless-steel grinder. The powdered seed samples were stored in airtight containers until further use. Observations were recorded replication-wise, and mean values computed for statistical analysis. Zn, Fe, Mn, Cu, and K contents in cowpea seeds were determined after appropriate acid digestion using standard laboratory analytical procedures. Nitrogen content was estimated by Kjeldahl method (AOAC, 2000). The carbohydrate content in seeds was estimated as per Merrill and Watts (1973). All nutritional parameters were estimated on dry weight basis.

Nitrogen and protein estimation

Finely pulverized seed sample (0.5 g) was digested with conc. H_2SO_4 in presence of a digestion catalyst mixture (K_2SO_4: $CuSO_4$, 10:1 ratio w/w) until digest became clear and colourless. The digested sample was distilled with excess sodium hydroxide and the boric acid solution was used for absorption of liberated ammonia. The titration of absorbed ammonia was done against standard sulphuric acid and nitrogen content determined. The protein content was calculated by multiplying the nitrogen content by a conversion factor of 6.25 (AOAC, 2000).

Potassium estimation

Potassium content in seed sample was determined by flame photometric method after wet digestion (Jackson, 1973). For this, 0.5 g finely ground seed were digested with di-acid mixture (nitric acid and perchloric acid in 9:4 ratio) on a hot plate until digest became clear and colourless. Then digest was diluted with distilled water to make 50 mL volume. The solution was filtered through Whatman No. 42 filter paper and potassium content in filtrate estimated by flame photometer (model CL 378, ELICO, India) after calibration with standard potassium solutions (0-100 ppm).

Micronutrients estimation

Di-acid digested extract, obtained as above for K estimation, was used for Zn, Fe, Cu and Mn estimation by atomic absorption spectrophotometer [AAS] (model novAA800, Analytik Jena, Germany). AAS was operated in flame mode with air-acetylene flame. The air and acetylene flow rates were maintained at 10-15 L min^{-1} and 1.5-2.0 L min^{-1}. The current was adjusted to 5 mA for Zn, Cu and Mn, and 7 mA for Fe. The instrument was calibrated using standard stock solutions of 1000 ppm of each element (Zn, Fe, Cu and Mn). The working standards of Zn (0.25, 0.50 ,0.75 & 1.00 ppm), Fe, Mn (2, 4, 6, 8, & 10 ppm) and Cu (1, 2, 3, 4, & 5 ppm) were prepared for generating standard curve. The digested seed extracts were aspirated into AAS and absorbance noted at their respective wavelengths (213.9 nm for Zn, 248.3 nm for Fe, 279.5 nm for Mn and 324.8 nm for Cu). Each micronutrient content was estimated by interpolating the sample reading on standard curve and expressed in ppm on dry weight basis (Analytik Jena GmbH+Co.KG, 2023).

Carbohydrate content estimation

Total carbohydrate content in seed samples was determined by Merrill and Watts (1973) method. For this, 100 mg finely pulverized seed samples were hydrolysed with 5 mL (2.5 N) HCl in a boiling water bath for 3 h, then contents cooled to room temperature and carefully neutralized with sodium carbonate. The final volume was adjusted to 100 mL with distilled water and filtered through

Whatman No. 42 filter paper. Subsequently, to I mL filtrate in a test tube 4 mL freshly prepared anthrone reagent was added, then reaction mixture heated in a boiling water bath for 10 min and immediately cooled rapidly. The absorbance of green-coloured complex was measured at 620 nm spectrophotometerically (Spectronic 200, Thermo Scientific, USA). Total carbohydrate was determined by interpolating the sample reading on standard curve and expressed in percentage on dry weight basis.

Estimation of overall index of nutritional quality (INQ)

The overall index of nutritional quality (OINQ) is a composite index that quantifies the nutritional density of a food or crop genotype by comparing the amount of key nutrients (protein, Fe, and Zn) available per 1,000 kcal energy to the corresponding recommended dietary allowances (RDA) per 1,000 kcal (Drewnowski, 2005). The INQ framework has widely been adopted in biofortification studies to provide an integrated, energy-adjusted measure of nutritional value that accounts for both macro- and micro-nutrient content simultaneously (Freitas *et al*., 2022). RDA values adopted for this study were: protein = 50 g day^{-1}, Fe = 18 mg day^{-1} and Zn = 11 mg day^{-1}, as per ICMR (2020) guidelines for adult reference intakes. A reference daily energy intake of 2,000 kcal was assumed for scaling purposes, such that the reference RDA per 1,000 kcal equals half the daily RDA. Higher overall INQ scores indicate that a genotype delivers superior protein, Fe, and Zn contents relative to its carbohydrate-derived energy load, and therefore represents a nutritionally preferable candidate for biofortification programmes.

$$\text{INQ} = \frac{\text{Amount of nutient per 1000 kcal in a variety}}{\text{RDA for nutrient per 1000 kcal}}$$

Wherein, OINQ = ($\text{INQ}\textit{protein} + \text{INQ}\textit{Fe} + \text{INQ}\textit{Zn}$) ÷ 3; and

$\text{INQ}i$ = Nutrient*i* per 1000 kcal in genotype ÷ RDA*i* per 1000 kcal

Metabolizable energy was estimated on dry weight basis from the carbohydrate and protein contents of each genotype using Atwater conversion factors, while assuming that the contribution of fat was negligible due to the characteristically low lipid content of legume seeds. Accordingly, the energy value was computed as the sum of energy derived from carbohydrates and proteins, expressed as (carbohydrate content, g per 100 g × 4) + (protein content, g per 100 g × 4) and reported as kcal 100 g^{-1} seed sample. Thereafter, nutrient density on an energy-adjusted basis was determined by calculating the amount of each nutrient per 1,000 kcal, using the formula (mg or g nutrient per 100 g × 1000) ÷ (kcal per 100 g). INQ for each nutrient was subsequently derived by dividing the nutrient content per 1000 kcal by the corresponding RDA normalized to a 1000 kcal basis. For standardization, a reference daily energy intake of 2000 kcal was assumed. The daily RDA for Zn (11 mg day^{-1}) was converted to 5.5 mg per 1000 kcal. Finally, the OINQ for each genotype was calculated as the arithmetic mean of individual INQ values for protein, Fe, and Zn, thereby providing a comprehensive measure of nutrient density relative to energy content and enabling the identification of nutritionally superior genotypes for biofortification and crop improvement programmes.

Statistical analysis

The experimental data was analysed by analysis of variance (ANOVA) and critical difference (CD at 5%) were computed using OPSTAT statistical software (Sheoran *et al*., 1998). Pearson's correlation analysis and principal component analysis (PCA) were performed using KAU Grapes v.2.0 (Kerala Agricultural University, India) statistical software. Eigen values, percentage variance explained, factor loadings, cos^2 values, and contribution scores were extracted. A PCA biplot (PC1 × PC2) and a variable correlation circle were constructed to visualize genotype-trait associations.

RESULTS AND DISCUSSION

The success of a crop improvement programme relies not only on the variability in yield and other important economic traits but also on the interrelationships among these traits within the population.

Means performance of cowpea genotypes

Pulse breeders worldwide have been exploiting available genetic material to develop varieties that meet the evolving nutritional needs of society. To establish cowpea as an ideal vegetable crop for sustainable agriculture, both producers and consumers must prioritize superior nutritional quality. In cowpea improvement programmes, certain nutritional traits deserve special emphasis, including protein content and micronutrients such as Fe, Zn, Cu, and Mn. Hence, cowpea germplasm must be systematically screened for these traits to identify suitable accessions for breeding programmes. In present study, several promising varieties were assessed under Haryana weather conditions to identify a suitable and superior variety for the region. Significant variation was noticed among the cowpea genotypes for seed quality traits including protein, micronutrients (Zn, Fe, Mn, Cu), K, N, carbohydrate, and OINQ, indicating considerable genetic diversity for nutritional improvement. The overall response of these varieties was assessed by studying different parameters (Table 1).

Protein content in the tested cowpea genotypes varied from 18.0 to 23.5% revealing differences in genetic constitution, nitrogen uptake efficiency and nitrogen fixation ability. ‘Kashi Kanchan’ had significantly highest protein content, though at par with ‘Cowpea-263’, due to higher nitrogen assimilation efficiency and efficient source sink relationship. Genotype ‘Konkan Safed’ was significantly lowest among all the genotypes, while cv. ‘C-152’, ‘Kashi Nidhi’, and ‘Konkan Sadabhar’ did not differ significantly among themselves. The variability observed in present study is consistent with Padhi *et al.* (2022) who reported similar results for broad genetic diversity of seed protein content (19.4- 27.9%) in cowpea. Gondwe *et al.* (2019) also observed variability in cowpea seed protein content. High-protein genotypes *viz.*, ‘Cowpea-263’ and ‘Kashi Kanchan’ are nutritionally rich, as cowpea is a major protein source in pulse-based diets. A similar trend was observed for nitrogen content, corroborating the well-established relationship between nitrogen assimilation and protein synthesis in legumes (Taiz and Zeiger, 2015), with values ranging from 2.90 to 3.76%. Genotypes ‘Kashi Kanchan’ (3.76%) and ‘Cowpea-263’ (3.65%) were markedly superior to all other genotypes, while ‘Konkan Safed’ recorded the lowest nitrogen content.

Table 1: Means performance of cowpea genotypes for seed protein, Zn, Fe, Mn, Cu, K, N, carbohydrate content, pod Zn, and OINQ evaluated under pot culture conditions

Genotypes	Protein (%)	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	K (%)	Zn(pod) (ppm)	N (%)	Carbohydrate (%)	OINQ
Kashi Kanchan	23.50	41.66	59.20	21.30	10.10	1.76	7.96	3.76	54.2	2.56
Arka Garima	22.10	38.05	53.10	19.50	10.60	1.82	7.21	3.54	54.6	2.35
Cowpea-263	22.80	39.87	56.20	22.90	12.20	1.70	7.81	3.65	53.8	2.48
Kashi Nidhi	20.30	40.20	52.50	18.10	10.90	1.94	7.89	3.25	55.4	2.33
Kashi Shyamal	20.85	35.94	50.45	17.80	8.60	1.85	6.75	3.34	55.1	2.24
C-152	21.90	33.14	53.20	16.80	7.40	1.74	6.24	3.50	54.8	2.26
Kashi Gauri	19.40	34.61	44.50	20.40	8.10	1.69	6.48	3.10	56.2	2.08
Pusa Komal	20.60	32.78	47.20	18.90	7.80	1.88	6.18	3.30	55.3	2.12
Konkan Sadabhar	18.90	30.80	41.50	21.60	6.60	1.95	5.46	3.02	56.4	1.95
Konkan Safed	18.00	28.12	40.20	19.10	7.30	1.91	5.05	2.90	56.8	1.84
$CD_{0.05}$	0.859	1.712	2.304	0.932	0.749	0.04	0.421	0.219	0.876	0.06

Seed Zn and Fe contents in test genotypes varied from 28.12 to 41.66 and 40.20 to 59.20 ppm, respectively (Table 1). The large variation may be attributed to the differences in root absorption capacity, translocation efficiency and genetic potential for nutrient accumulation. Significantly highest concentration for Zn and Fe was recorded in cv. ‘Kashi Kanchan’ closely followed by ‘Cowpea-263’ whereas ‘Konkan Safed’ had lowest contents. Genotypes possessing efficient nutrient transport systems and higher root absorption capacity tend to accumulate greater amounts of Zn and Fe in seeds and such variability reveals the potential for genetic enhancement of Zn and Fe concentration in cowpea. Comparable ranges of seed Zn and Fe contents have been reported previously by Dakora *et al*. (2019) who reported Zn and Fe contents in the range of 33.9 to 69.2 and 45.1 to 67.0 µg g^{-1}, respectively. Massey *et al*. (2020) reported Zn and Fe contents in the range of 2.54-5.59 and 5.10-9.68 mg 100 g^{-1} emphasizing the relevance of cowpea in micronutrient biofortification programmes.

Mn content in test cowpea genotypes varied from 16.8 to 22.9 ppm with cv. 'Cowpea-263' proving significantly superior over other genotypes. Genotypes 'Kashi Kanchan' and 'Konkan Sadabhar' were statistically at par, while cv. 'C-152' proved inferior in Mn content (Table 1). Genotypic differences in Mn content are attributed to genetic regulation of mineral uptake and partitioning (Gerrano *et al*., 2015); Pawar *et al*., 2016). Cu content in genotypes varied from 6.6 to 12.2 ppm with 'Cowpea-263' and 'Kashi Kanchan' significantly superior to all genotypes. Kashi Nidhi and Arka Garima were statistically at par, while 'C-152' and 'Konkan Safed' were inferior to all. Gonçalves *et al*. (2016) and Verma *et al*. (2019) also reported comparable genotypic variation in cu content in cowpea and other grain legumes.

Potassium content in cowpea genotypes ranged from 1.69 to 1.95% with highest content in 'Konkan Sadabhar' which was at par with cv. 'Konkan Safed'. Genotype 'C-152' was lowest in Cu content (Table 1). Genotypic variation in K uptake efficiency in legumes has previously been reported by Otitoju *et al*. (2015) and Osunbitan *et al.* (2016). Carbohydrate content ranged from 53.8 to 56.8% and the differences among most genotypes were non-significant. 'Konkan Safed' and 'Konkan Sadabhar' were at par and recorded comparatively higher carbohydrate content. The narrow variation observed suggests that carbohydrate is relatively conserved across the genotypes, a pattern commonly observed in legume species and is attributed to competition between starch and protein biosynthesis pathways during seed development (Khairnar *et al.*, 2017). This inverse relationship between carbohydrate accumulation and protein-micronutrient content has extensively been documented in legumes (Hamid *et al*., 2016; Gaikwad and More, 2018).

Overall INQ in cowpea genotypes varied from 1.84 to 2.56 with 'Kashi Kanchan' proving significantly superior to all genotypes, followed by 'Cowpea-263' and 'Arka Garima' (Table 1). Higher INQ values reflect a balanced contribution of protein and essential micronutrients relative to carbohydrate-derived energy, thereby validating the effectiveness of INQ as an integrated composite nutritional indicator (Hansen, 1973). From a physiological perspective, nutrient accumulation in seeds is influenced by several processes including root absorption of nutrients, translocation through xylem and phloem, and remobilization of nutrients during seed development. Genotypes possessing efficient nutrient uptake systems and higher metabolic activity are capable of accumulating greater quantities of essential nutrients in seeds, so are promising candidates for nutritional improvement and biofortification breeding programmes in cowpea (Freitas *et al.*, 2022).

Correlation analysis among quality traits

Pearson correlation analysis was performed to elucidate the inter-relationships among major seed nutritional traits of cowpea, including protein, carbohydrate, N, K, and micronutrients (Table 2, Fig. 1). The analysis revealed an intricate network of strong, moderate, and weak associations that indicated both coordinated and independent nutrient accumulation patterns (Mbuma *et al*., 2021).

Table 2: Phenotypic correlation matrix among nine seed nutritional traits of ten cowpea genotypes

Nutrients	Protein (%)	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	K (%)	Zn(pod) (ppm)	N (%)	Carbohydrates (%)
Protein (%)	1	0.638*	0.723**	0.301	0.434	-0.406	0.651*	1	-0.875
Zinc (ppm)	0.638*	1	0.625*	0.242	0.397	-0.242	0.997	0.638*	-0.782**
Iron (ppm)	0.723**	0.625*	1	0.170	0.352	-0.474	0.690	0.740**	-0.847**
Manganese (ppm)	0.301	0.242	0.170	1	0.317	-0.262	0.197	0.298	-0.157
Copper (ppm)	0.434	0.397	0.352	0.317	1	-0.235	0.411	0.436	-0.716*
Potassium (%)	-0.406	-0.242	-0.474	-0.262	-0.235	1	-0.268	-0.401	0.521
Zn(pod) (ppm)	0.651*	0.997**	0.690*	0.197	0.411	-0.268	1	0.753**	-0.799**
Nitrogen (%)	1	0.638*	0.740**	0.298	0.436	-0.401	0.753**	1	-0.876**
Carbohydrates (%)	-0.875**	-0.782**	-0.847**	-0.157	-0.716*	0.521	-0.799**	-0.876**	1

** Significant at $p \leq 0.01$ level (two tailed); * Significant at $p \leq 0.05$ level (two tailed)

Protein showed a perfect positive association with nitrogen (r = 1.00), strong positive relation with Fe (r = 0.723), Zn (r = 0.638), and Zn in pod (r = 0.651), and a moderate positive association with Cu (r = 0.434) [Table 2]. These correlations reveal that cowpea seeds accumulate micronutrients and protein in a complementary way. N metabolism likely facilitates the synthesis of metal-binding proteins and organic chelators, thereby promotes the translocation and deposition of micronutrients in seeds. This coordinated accumulation of protein and micronutrients has extensively been documented in grain legume biofortification (Araujo *et al*., 2022; Padhi *et al*., 2022).

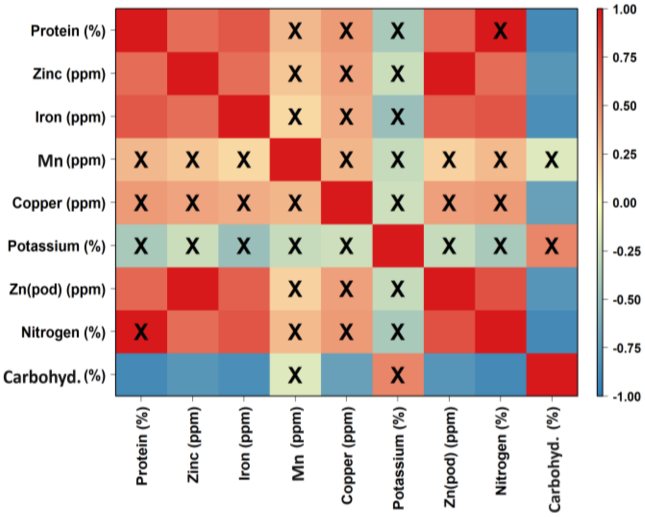

Fig. 1: Shaded phenotypic correlogram depicting Pearson's correlation coefficients among nine seed nutritional traits (protein, Zn, Fe, Mn, Cu, K, pod-Zn, N, carbohydrate) of ten cowpea genotypes. Colour intensity indicates correlation strength; green = positive, purple = negative and yellow = perfect correlation, and X = non-significant

Zn content in seed revealed a near-perfect positive correlation with Zn in pod (r = 0.997), indicating consistent genotypic reliability in Zn accumulation across plant organs. This result suggests that selection for elevated Zn concentration in seeds would simultaneously improve pod Zn content, which is advantageous for both vegetable-type and grain-type cowpea improvement programmes (Padhi *et al*., 2022). Zn also showed strong positive correlations with Fe (r = 0.625), protein (r = 0.638), and N (r = 0.638). Similarly, Fe exhibited strong positive associations with N (r = 0.740), protein (r = 0.723), Zn in pod (r = 0.690), and Zn in seed (r = 0.625) [Table 2]. These results suggest that Zn and Fe share common physiological and molecular pathways governing their uptake, translocation, and storage in seeds (Gonçalves *et al*., 2016). Several studies by Massey *et al.*(2020) and Dakora *et al*. (2019) have reported similar co-localization and co-regulation of Zn and Fe in seeds, supporting the feasibility of simultaneously improving both micronutrients through targeted breeding.

Cu showed moderate positive correlations with protein (r = 0.434), N (r = 0.436), Zn in pod (r = 0.411), and Fe (r = 0.352) [Table 2]. These moderate associations suggest that Cu accumulation is partly linked to protein and micronutrient metabolism, though the associations are weaker than those observed for Zn and Fe. Cu is familiar for its role in various enzymatic processes associated with N metabolism, which explains these moderate correlations. In contrast, Mn showed weak positive correlations with most traits, including protein (r = 0.301) and Cu (r = 0.317), indicating an independent accumulation pattern. This weak association may be due to the relatively low phloem mobility of Mn and its distinct uptake and transport mechanisms compared to other micro-nutrients.

Potassium showed negative correlations with protein (r = −0.406), N (r = −0.401), Fe (r = −0.474), Zn (r = −0.242), and Cu (r = −0.235), while exhibiting a moderate positive correlation with carbohydrate content (r = 0.521) [Fig. 1]. This pattern suggests that K plays a more prominent role in carbohydrate synthesis, translocation, and storage than protein or micronutrient accumulation (Silva *et al*., 2021). Physiologically, K is known to regulate photosynthate translocation, enzyme activation, and starch metabolism, which explains its positive association with carbohydrate content and negative relationships with protein- and micronutrient-dense seeds (Romheld and Kirkby, 2010).

Carbohydrate exhibited strong negative correlations with protein (r = −0.875), N (r = −0.876), Fe (r = −0.847), Zn (r = −0.782), Zn in pod (r = −0.799), and Cu (r = −0.716) [Table 2]. The strong inverse relationship confirms a dilution or resource allocation effect, whereby increased carbohydrate deposition reduces the relative concentration of protein and micronutrients in seed.

Such correlations between carbohydrate accumulation and nutritional quality parameters have been well documented in seed composition studies (Vaggar *et al*., 2022). Collectively, the strong positive correlations among protein, N, Zn, Fe, and Cu indicate that simultaneous improvement in nutritionally important traits is achievable through targeted selection. Genotypes with lower carbohydrate but higher protein and micronutrient concentrations represent desirable ideotypes for nutritional intensification and biofortification (Reddy *et al*., 2025).

Principal component analysis (PCA)

PCA was used to analyse the magnitude and pattern of variability across cowpea genotypes with respect to seed nutritional parameters, as well as to explain interrelationships between these traits. The Eigen value analysis demonstrated that the first three main components accounted 90.26% of total variance, implying that the multidimensional nutritional dataset could be effectively summarized with minimal loss of information (Suganthi *et al.*, 2007; Abdi and Williams, 2010). The first principal component (PC1) had highest Eigen value (5.154) and accounted for 57.19% of total variance, indicating it as a single dominant component for genotypic variability (Table 3). With an Eigen value of 1.916, the second principal component (PC2) accounted an extra 21.10% of variance, increasing the total variance explained by the first two components to 78.29% (Reddy *et al*., 2021). Multivariate studies on nutritional characteristics of legume crop have shown similar patterns of variance concentration in the first two to three principal components, indicating strong correlations across the seed composition factors (Aramendiz *et al*., 2018).

Table 3: Eigen values, percentage of variance explained, and cumulative variance for principal components (PC1–PC9) derived from PCA of nine nutritional traits across ten cowpea genotypes

Principal components	Eigen values	Variance (%)	Cumulative variance (%)
PC1	5.154	57.19	57.19
PC2	1.916	21.10	78.29
PC3	1.082	11.97	90.26
PC4	0.704	7.83	98.09
PC5	0.145	1.53	99.62
PC6	0.028	0.28	99.90
PC7	0.007	0.08	99.98
PC8	0.001	0.02	100
PC9	0	0	100

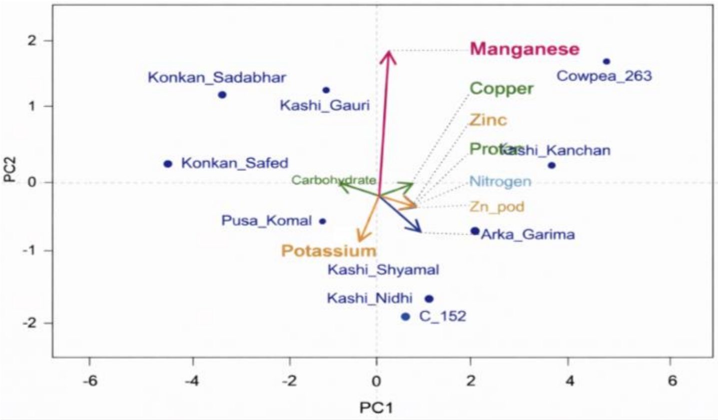

Fig. 2: PCA biplot (PC1 × PC2, explaining 78.29% of total variance) showing genotype scores (labelled points) and trait loading vectors for ten cowpea genotypes evaluated for seed nutritional quality.

The PCA biplot developed with PC1 (57.19%) and PC2 (21.10%) explained 78.29% of the total variance and offered a reliable two-dimensional illustration of relationships between genotype and trait (Fig. 2). Strong positive loadings for protein, N, Fe, Zn, and Cu were found in PC1, and they were all orientated positively along the horizontal axis. These characteristics had lengthy vectors and high squared cosine (cos²) values (>0.85), meaning that the first two PCs accounted for more than 85% of their total variation (Girgel, 2021). The acute angles (< 25-30°) among these vectors confirm strong positive inter-trait relationships. Biologically, this clustering is highly meaningful as nitrogen is the primary constituent of amino acids and seed storage proteins, so its close association with protein content is expected. Micronutrients such as Fe and Zn are often bound to storage proteins or enzymes involved in N metabolism and protein synthesis, leading to their coordinated accumulation in seeds (Cakmak *et al*., 2010). PC1 therefore represents a nutritional quality axis, integrating protein and micronutrient density.

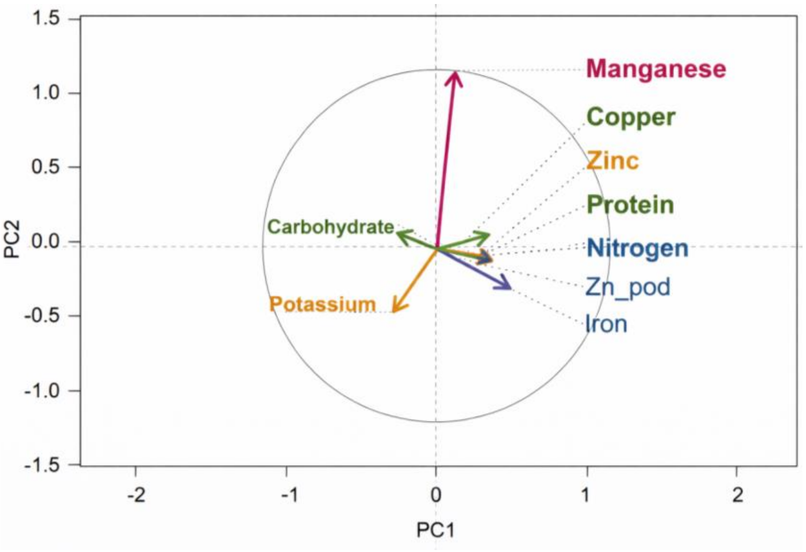

Fig. 3: PCA variable correlation circle showing contribution and quality of representation (cos²) of nine nutritional traits on PC1 and PC2 for ten cowpea genotypes

The genotypes 'Cowpea-263' and 'Kashi Kanchan' were positioned along the positive side of PC1 and showed close association with protein and micronutrient vectors, indicating superior nutritional quality and balanced nutrient composition. In contrast, cv. 'Konkan Safed' and 'Konkan Sadabhar' were located on the negative side of PC1 and were associated with higher carbohydrate content, reflecting relatively lower nutritional density. Genotypes positioned near the origin exhibited moderate values for most traits and contributed less to overall variability.

The second principal component (PC2) primarily distinguished genotypes based on K and Mn levels (Abreu *et al*., 2019). K loaded favourably along PC2, but Mn loaded negatively, indicating that these elements accumulate differently. K showed moderate cos² values (0.65-0.70), while Mn showed cos² values of 0.60, indicating significant but secondary contributions to overall variability. The near-perpendicular orientation (angles close to 90°) of K and protein vectors suggests a weak or negligible link between these attributes (Singh *et al.*, 2018). Mn is engaged in redox processes, photosystem II activity, and antioxidant defense, while K is crucial for osmotic regulation, enzyme activation, and assimilate translocation. Rather than being directly related to protein accumulation, their separation along PC2 indicates genotype-specific variations in mineral uptake efficiency, transport, and storage. Multivariate studies of legumes and cereal grains have revealed similar mineral-specific differentiation along secondary main components.

The PCA variable correlation circle further clarified that protein, N, Fe, Zn, and Cu were located close to the circumference of the correlation circle, confirming strong contributions to the first two PCs. In contrast, K and Mn were positioned closer to the interior of the circle, reflecting moderate contributions and partial representation by PC1 and PC2 (Fig. 3). This confirms that while protein and micronutrients dominate overall variability, certain minerals contribute additional, genotype-specific information that may be exploited for targeted improvement (Kamai, 2022).

Limitations and future directions

The study contributes important regional data on genotypic nutritional variability under semi-arid Haryana conditions, yet genetic analysis, molecular markers, bioavailability studies, or multi-location field validation would substantially strengthen the translational value of these results. Also, the present study is based on a single-season pot experiment so multi-season field validation is recommended before definitive cultivar recommendations.

Conclusions: The present study demonstrated the existence of substantial genotypic variation in protein, micronutrient content and the overall index of nutritional quality in cowpea genotypes. Strong positive correlations among protein, N, Zn, Fe, and Cu indicated a coordinated accumulation pattern, suggesting the feasibility of synchronized enhancement of these nutritionally important traits. PCA efficiently summarized the complex multivariate nutritional dataset and identified protein and micronutrient traits as the primary contributors to overall genotypic variability. Genotype 'Kashi Kanchan' and 'Cowpea-263' consistently outperformed other genotypes in terms of protein and micronutrient contents, PCA loadings, and overall INQ values, qualifying them as nutritionally elite candidates for biofortification-oriented breeding under semi-arid conditions.

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