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Original Research Paper

Unveiling the genetic diversity in curry leaf (*Murraya koenigii* L. Spreng) genetic resources for nutritional traits

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ABSTRACT

Curry leaf (*Murraya koenigii* L. Spreng), a widely used spice and medicinal crop, rich in essential oils, bioactive compounds, and nutrients. The present study aimed to evaluate 80 curry leaf germplasm *ex situ*, to explore genetic variability in mineral nutrient content and their interrelationships. The observed ranges of these nutrients on a dry weight basis were, calcium (0.30–5.97%), phosphorus (0.11–0.77%), potassium (0.09–3.52%), magnesium (0.10–0.78%), sulphur (0.06–0.41%), iron (51.7–439.16 ppm), zinc (4.55–42.60 ppm), boron (26.02–143.38 ppm), copper (2.26–47.41 ppm), and manganese (4.12–92.47 ppm). Correlation analysis revealed a positive association of calcium with manganese, copper, and magnesium. Manganese and copper also exhibited a positive correlation with iron, while, zinc was positively correlated with phosphorus and magnesium. Both phenotypic and genotypic coefficients of variation were high for all traits studied. Furthermore, high heritability, coupled with substantial genetic advance, was observed, indicating the potential for improving these traits through breeding interventions. Principal component analysis demonstrated considerable population divergence in mineral nutrient composition, with calcium, magnesium, phosphorus, sulphur, and potassium contributing most to the overall variability. Hierarchical cluster analysis further revealed a significant regional pattern in mineral nutrient concentrations. The highest levels of calcium and iron were found in accessions collected from Karnataka, followed by those from Odisha and Himachal Pradesh.

Keywords: *Ex-situ* conservation, field genebank, food-to-food fortification, *Murraya*, variability

INTRODUCTION

Hidden hunger, or chronic micronutrient deficiency, remains a major global public health challenge, affecting over two billion people worldwide (WHO, 2021). Deficiencies in essential nutrients such as calcium, iron, zinc, vitamin A, and iodine are particularly prevalent across all age groups and socioeconomic strata. Among the various approaches to mitigate hidden hunger, food-to-food fortification, the strategic enrichment of diets using naturally nutrient-dense, low-calorie crops, has gained considerable attention (Kruger, 2020). Leafy vegetables, in particular, offer a sustainable and culturally acceptable avenue for micronutrient supplementation. For instance, *Moringa oleifera* leaf powder has been effectively used to enhance the nutritional status of women and children in West Africa and Uganda (Zongo et al., 2013).

Curry leaf (*Murraya koenigii* L. Spreng), a perennial aromatic species native to the Indian subcontinent, is traditionally valued for its culinary and medicinal properties. Though historically cultivated in home gardens, it has increasingly been adopted as a commercial crop in southern India due to its year-round market demand, low-input requirements, and adaptability to varied agro-climatic conditions (Raghu et al., 2020). Nutritionally, curry leaf is a rich source of essential mineral nutrients such as calcium (2–2.4%), potassium (1.3%), magnesium (0.56%), iron (12.0 mg/100 g), and zinc (2.4 mg/100 g), alongside bioactive compounds including β-carotene, carbazole alkaloids, and flavonoids (Raghu, 2020). Given this nutrient density and its widespread culinary use, curry leaf presents significant potential for integration into food-to-food fortification strategies aimed at addressing micronutrient malnutrition (Odoh et al., 2023).

Despite its nutritional promise, systematic efforts to collect, conserve, and characterize curry leaf germplasm for mineral nutrient traits remain limited. A comprehensive understanding of genotypic variation in mineral nutrient content is essential for its effective utilization in crop improvement programs and public health nutrition. Accordingly, the present study was undertaken to assess genotypic variability for key mineral nutrients among diverse curry leaf accessions collected across India, and to elucidate the interrelationships among these traits under *ex situ* conditions.

MATERIAL AND METHODS

Plant material

A total of 80 curry leaf accessions collected from diverse agro-ecological regions across India (Fig. 1) were used in this study and are conserved in a field gene bank at the ICAR-Indian Institute of Horticultural Research, Bengaluru, India (11° 07' N, 77° 29' E; 709 m amsl). The accessions were planted in an augmented block design with a spacing of 1.52 × 1.52 meters. Standard agronomic practices were followed throughout the study period, and all plants were subjected to quarterly pruning to ensure uniformity. Nutritional data were recorded over two consecutive years to capture genotypic variation under *ex situ* conditions.

Sample collection and preparation

Leaf samples were harvested from 3-month-old shoots in June 2022 and 2023. Healthy, mature leaves were selected from each accession, and the petioles were removed to retain only the leaflets. The samples were thoroughly washed, oven-dried at 60°C for 48 hours,

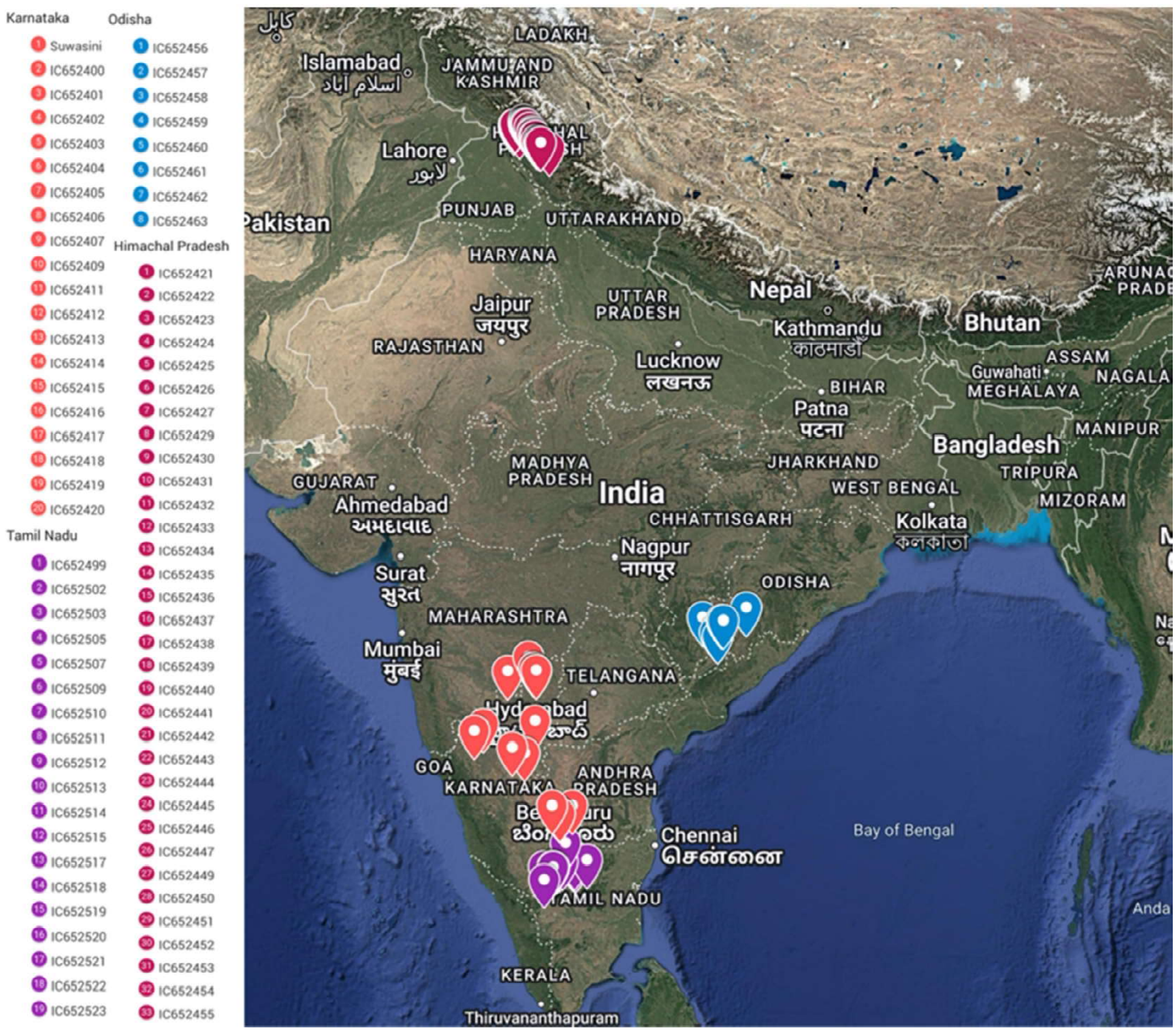

Fig. 1 : Collection sites of curry leaf germplasm across diverse agro-climatic regions of India

and then finely ground using a mechanical grinder. The powdered samples were stored in airtight containers for subsequent nutrient analysis.

Estimation of mineral and trace element content

The plant samples were analysed for 10 mineral nutrients, including five macronutrients (phosphorus, potassium, sulphur, magnesium, and calcium) and five micronutrients (iron, zinc, boron, manganese, and copper). The oven-dried, pulverised plant samples were decomposed in a diacid mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 9:4 ratio (Jackson, 1959). From the diacid extracts of the plant samples, total P and S contents were determined using a UV-VIS spectrophotometer by the vanado-molybdate and turbidometric methods, respectively. Total K was estimated using a flame photometer, while B content was determined by the Azomethine-H method using a UV-VIS spectrophotometer (Gross et al., 2008). The estimation of Ca, Mg, Fe, Cu, Mn, and Zn was carried out using atomic absorption spectroscopy (AAS) (PerkinElmer, Model Analyst 200) (Singh et al., 2005). The macronutrients were reported as percentages, while the micronutrients were expressed in parts per million (ppm) on a dry weight basis.

Statistical analysis

Nutrient data collected over two years were first transformed using the arc sine square root transformation to stabilize variances. Statistical analyses were conducted using R software (version 4.2.2; R Core Team, 2022). Analysis of variance (ANOVA), correlation, principal component analysis (PCA), and hierarchical clustering were carried out to assess variability and trait interrelationships. Phenotypic and genotypic coefficients of variation (PCV and GCV) were calculated following the method described by Burton & DeVane (1953). Broad-sense heritability (h^2) was estimated as the ratio of genotypic variance to phenotypic variance, expressed as a percentage, as per Allard (1960). Genetic advance as a percentage of the mean (GAM), assuming selection of the top 5% of accessions, was computed using the procedure of Johnson et al. (1955).

RESULTS AND DISCUSSION

Genotypic variation and high-performing accessions

Analysis of variance revealed significant genotypic differences for all evaluated macro- and micro-nutrients, indicating substantial genetic variability within the curry leaf germplasm (Table 1). The nutrients calcium, phosphorus, potassium, iron, copper, manganese, and boron exhibited highly significant variation ($p \leq 0.01$), while magnesium, sulphur, and zinc showed moderately significant variation ($p \leq 0.05$). Nutrient distribution among germplasm are depicted in heatmap (Fig. 2).

Among macro-nutrients, calcium was the most abundant (2.43%) ranged from 0.30% to 5.97% (Table 1 & Supplementary Table 1), followed by potassium (1.76%) ranged from 0.09% to 3.52%. Notably, 30 accessions exceeded 2.3% calcium, and five acessions namely IC652403, IC652404, IC652419, IC652462 and IC652409 recorded values above 5.0%, surpassing those reported in earlier studies (Shanthala & Prakash, 2005; Uraku & Nwankwo, 2015; Parnami & Verma, 2019; Rajendran et al., 2020). These accessions represent valuable resources for calcium biofortification efforts.

Iron was the most prevalent micronutrient, with a mean of 207.94 ppm and values ranging from 51.71 to 439.16 ppm (Table 1). Approximately 39% of accessions surpassed 200 ppm, with IC652517 and IC652515 exceeding 400 ppm. Similar results were reported by Kumar et al. (2020), highlighting the nutritional potential of curry leaves as an iron-rich leafy vegetable. Zinc ranged from 4.55 to 42.60 ppm, with a mean of 23.82 ppm, two accessions IC652461 and IC652458 recorded values above 40 ppm. While, the zinc content fell within a moderate range, it surpassed earlier reports from most studies (Parnami & Verma, 2019; Odoh et al., 2023), indicating scope for further genetic enhancement through broader germplasm exploration.

The concentrations of phosphorus, magnesium, manganese, and copper also exceeded those reported in previous studies (Uraku & Nwankwo, 2015). Phosphorus ranged from 0.11% to 0.77%, magnesium from 0.10% to 0.78%, manganese from 4.12 to 92.47 ppm, and copper from 2.26 to 47.41 ppm. These elevated values reflect the presence of underutilized mineral-rich genotypes with potential economic and nutritional significance.

For the first time, sulphur and boron concentrations in curry leaves were quantified. Sulphur ranged from 0.06% to 0.40%, with a mean of 0.17%. Boron was the second most abundant micronutrient after iron,

Table 1 : Analysis of variance, mean performance and estimates of genetic parameters for mineral nutrients content in the leaves of curry leaf

Trait	ANOVA			Mean performance					Genetic parameter			
	Block (df=5)	Genotype (df=79)	Residuals (df=5)	Mean (adjusted)	Range	CD (*p*=.05)	CV	Skewness	PCV	GCV	h^2b (%)	GA (% of mean)
Calcium (%)	0.06	2.46**	0.15	2.43	0.30-5.97	1.74	14.54	0.60	54.43	52.00	91.26	102.49
Phosphorous (%)	0.01	0.01**	0.01	0.22	0.11-0.77	0.14	14.76	3.20	44.44	42.02	89.41	81.98
Potassium (%)	0.01	1.75**	0.01	1.76	0.09-3.52	0.32	4.46	0.12	70.51	70.39	99.66	144.96
Magnesium (%)	0.01	0.02*	0.01	0.44	0.10-0.78	0.26	13.14	0.06	32.99	30.07	83.07	56.54
Sulphur (%)	0.01	0.01*	0.01	0.17	0.06-0.41	0.11	13.75	0.36	31.57	28.08	79.14	51.55
Iron (ppm)	493.00	7544.00**	428.00	207.94	51.71-439.16	92.06	9.69	0.35	41.41	40.20	94.23	80.51
Zinc (ppm)	21.40	63.15*	8.42	23.82	4.55-42.60	12.91	12.28	0.36	32.92	30.58	86.31	58.62
Boron (ppm)	4.00	349.00**	7.00	53.22	26.02-143.38	12.01	4.92	1.87	30.73	30.31	97.27	61.68
Copper (ppm)	16.00	80.50**	6.60	18.01	2.26-47.41	11.42	13.41	0.88	44.56	42.22	89.77	82.54
Manganese (ppm)	13.40	269.90**	4.00	31.79	4.12-92.47	8.93	6.14	1.24	50.87	50.47	98.46	103.33

Block: mean sum of squares for block, Genotype: mean sum of squares for genotype, Residuals: residuals mean sum of squares, df: degrees of freedom, *significant at *p*: 0.05, **significant at *p*: 0.01, h^2b: heritability-broad sense, GA: genetic advance

ranging from 26.02 to 143.38 ppm, with an average of 53.22 ppm. The accession IC652440 showed the highest boron content (143.38 ppm), followed by IC652415 (93.98 ppm). These levels far exceed typical values in common vegetables (Nielsen, 2016), positioning curry leaves as a rich source of dietary boron.

Both PCV and GCV were high (>20%) across all traits, reflecting substantial inherent variability. High heritability (>70%) coupled with high genetic advance as a percentage of the mean (>20%) suggests that most traits are governed by additive gene effects, and thus, genetic gain through selection is feasible (Johnson et al., 1955; Deshmukh et al., 1986). Nutrient

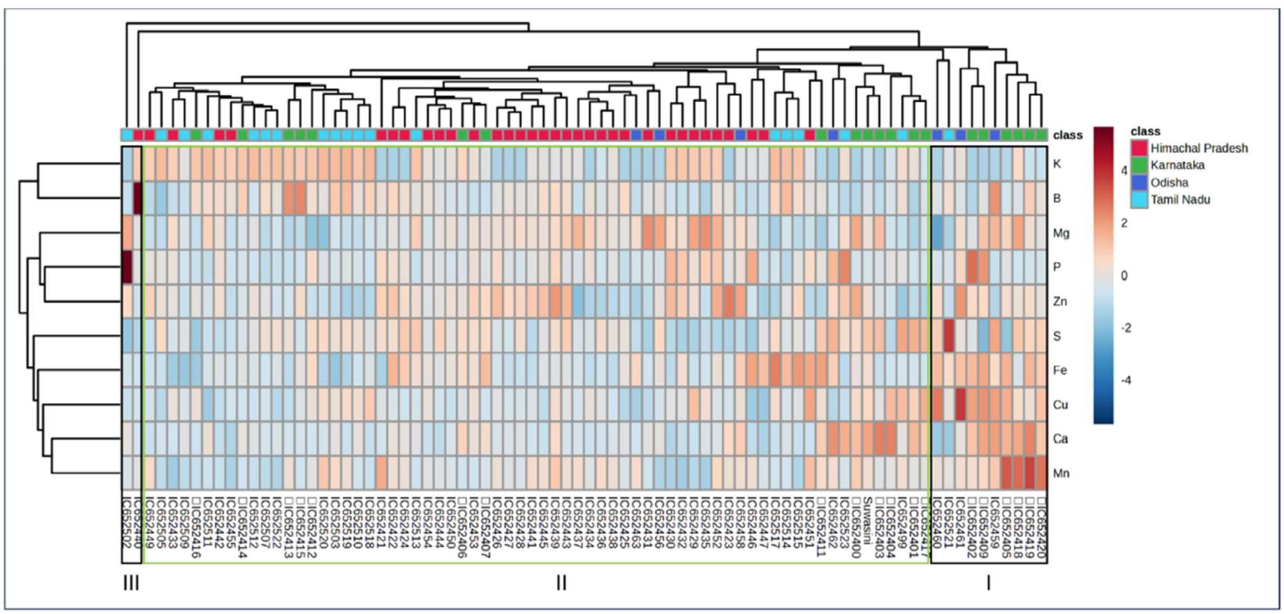

Fig. 2 : Heatmap diagram for mineral nutrient variation among curry leaf germplasm

Symbols indicated minerals, phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn) and boron (B). The degree of similarity in nutrient concentrations among different accessions is represented by a coloured bar in the legend, where positive values in deep red suggest high similarity and negative values in deep blue indicate significant dissimilarity. Additionally, a stacked bar chart illustrates the origins of the genotypes, which correspond to four different states in India

Table 2 : Curry leaf accessions (elite accessions) belong to the first two highest significant groups (significant at p = 0.05) in the Duncan's multiple comparison of adjusted means for macronutrients

Accession	P (%)			K (%)			Ca (%)			Mg (%)			S (%)		
	2022	2023	Mean$	2022	2023	Mean$	2022	2023	Mean$	2022	2023	Mean$	2022	2023	Mean$
IC652402	0.49	0.53	0.53*	0.07	0.13	0.09	3.83	4.09	4.12	0.43	0.37	0.41	0.12	0.20	0.16
IC652403	0.20	0.18	0.21	0.09	0.11	0.09	5.88	5.73	5.97**	0.57	0.67	0.63	0.21	0.29	0.25
IC652404	0.24	0.18	0.23	1.40	1.00	1.19	5.71	5.86	5.94**	0.38	0.34	0.37	0.14	0.10	0.12
IC652419	0.18	0.16	0.16	0.86	0.94	0.99	5.57	5.85	5.66*	0.41	0.55	0.48	0.25	0.19	0.23
IC652456	0.16	0.22	0.17	0.16	0.24	0.20	2.15	2.43	2.05	0.64	0.76	0.78**	0.24	0.18	0.19
IC652459	0.18	0.12	0.13	0.17	0.23	0.20	5.09	5.01	4.81	0.74	0.56	0.73	0.23	0.33	0.28*
IC652502	0.80	0.78	0.77**	0.08	0.12	0.10	2.25	2.39	2.08	0.60	0.77	0.77*	0.05	0.11	0.06
IC652518	0.18	0.14	0.18	3.35	3.64	3.42*	1.22	1.40	1.42	0.38	0.34	0.30	0.17	0.15	0.17
IC652519	0.22	0.18	0.22	3.47	3.72	3.52**	1.36	1.46	1.52	0.37	0.27	0.26	0.23	0.17	0.21
IC652520	0.16	0.22	0.21	3.46	3.53	3.42*	1.73	1.65	1.80	0.11	0.21	0.10	0.24	0.19	0.23
IC652521	0.15	0.23	0.21	1.42	1.58	1.42	0.14	0.24	0.30	0.28	0.20	0.18	0.45	0.34	0.41**

**Genotype belongs to the first significant group, * Genotype belongs to the second significant group, $Adjusted mean derived from combined analysis of means of two years, 2022 and 2023, phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S)

Table 3 : Curry leaf accessions (elite genotypes) belong to the first two highest significant groups (significant at p = 0.05) in the Duncan's multiple comparison of adjusted means for micronutrients

Accession	Cu (ppm)			Zn (ppm)			Fe (ppm)			Mn (ppm)			B (ppm)		
	2022	2023	Mean$	2022	2023	Mean$	2022	2023	Mean$	2022	2023	Mean$	2022	2023	Mean$
IC652405	34.24	27.76	31.11	29.23	25.77	29.40	348.70	330.10	348.86	89.10	77.10	79.97*	41.60	46.44	42.29
IC652415	16.77	13.83	13.06	25.87	28.53	22.95	188.40	210.00	181.01	19.60	22.80	24.27	94.38	89.72	93.98*
IC652418	18.96	23.64	19.06	24.56	27.44	21.75	185.20	165.40	157.11	73.20	83.80	81.57*	49.25	44.27	48.68
IC652419	18.45	19.55	16.76	28.83	26.37	23.35	324.10	343.50	315.61	86.50	92.30	92.47**	59.50	75.28	69.31
IC652440	11.34	13.86	18.06	16.98	20.42	14.55	146.20	158.40	164.46	26.00	31.00	26.42	136.72	148.20	43.38**
IC652458	21.12	16.08	18.21	42.60	34.80	40.60*	268.20	297.60	285.36	38.17	35.23	35.07	36.47	42.07	40.10
IC652459	36.97	29.83	33.01	15.76	13.44	16.50	243.20	267.80	257.96	49.45	47.75	46.97	81.55	94.50	88.68*
IC652460	36.11	41.29	38.31*	29.23	26.57	29.80	311.50	323.10	319.76	38.20	32.00	33.47	39.25	57.19	49.05
IC652461	45.34	50.26	47.41**	36.53	44.87	42.60**	304.50	318.70	314.06	35.16	40.64	36.27	56.60	37.00	47.62
IC652515	15.32	17.28	15.41	31.14	27.26	31.95	389.50	383.10	401.26**	13.85	10.75	14.17	53.50	65.58	57.81
IC652517	21.12	20.68	20.01	12.72	15.48	16.85	434.12	414.28	439.16**	24.60	21.40	24.87	61.50	67.80	62.92

**Genotype belongs to the first significant group, * Genotype belongs to the second significant group, $Adjusted mean derived from combined analysis of means of two years, 2022 and 2023, copper (Cu), zinc (Zn), iron (Fe), manganese (Mn) and boron (B)

distributions across traits were positively skewed (Table 1), indicating the presence of elite genotypes with exceptionally high concentrations. These high-performing accessions are listed in Tables 2 and 3.

Association among nutritional trait composition

Interrelationships among macro- and micronutrients reveal co-inheritance patterns and inform strategies for simultaneous nutritional enhancement. In this study, calcium showed positive correlations with manganese (r = 0.46), copper (r = 0.29), and magnesium (r = 0.28), but a strong negative correlation with potassium (r = –0.40). Zinc correlated positively with phosphorus (r = 0.34) and magnesium (r = 0.25), while iron was positively associated with manganese (r = 0.26) and copper (r = 0.23), but negatively with potassium (r = –0.25). Boron showed mostly non-significant associations, except a weak positive correlation with potassium (r = 0.22) (Fig. 3). These patterns align partially with findings of Qin et al. (2017) in spinach and Kumari et al. (2022) in Dolichos beans, where similar nutrient correlations were observed, indicating certain nutrient associations may be genetically or physiologically conserved.

Multivariate analysis of nutrient traits

PCA analysis

Principal component analysis was employed to dissect the multivariate structure of the nutrient data and identify key traits contributing to variation among accessions. Following the criterion of Rencher et al. (2002), the first five principal components accounted

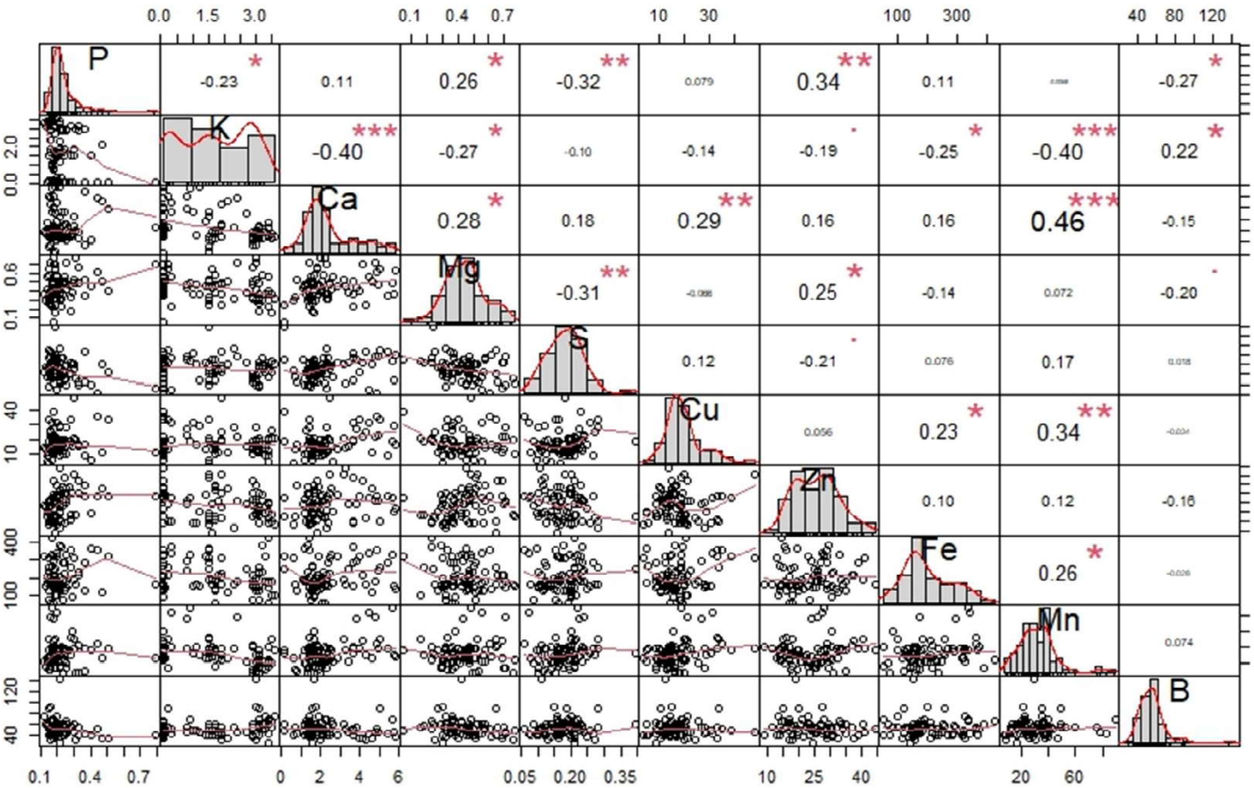

Fig. 3: Correlation plot for different mineral nutrients of the curry leaf accessions

Symbols indicated in the diagonal cells are minerals phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn) and boron (B). Histogram with red-line graph indicated distribution of each mineral in the studied population

for 73.04% of the total variance (Table 4), with the first two PCs explaining 43.97%. The biplot of PC1 vs. PC2 (Fig. 4) illustrates the relationships among traits and accessions. Based on factor loadings ≤ 0.5, calcium, manganese, phosphorus, sulphur, and potassium emerged as the most influential traits contributing to genotypic diversity. This finding parallels the results of Kumari et al. (2022), who identified phosphorus, iron, copper, and zinc, along with protein and phenol content, as major contributors to nutritional variability in Dolichos genotypes.

Varimax rotation further clarified trait associations: Factor 1 showed positive associations with potassium, manganese, and zinc, and a negative one with phosphorus, while, Factor 2 was positively linked with sulphur and negatively with calcium and zinc (Table 5). Accessions IC652405, IC652419, and IC652461 exhibited high factor loadings (>0.5), indicating their strong contribution to overall nutritional variability and highlighting their potential as nutrient-dense candidates. This approach parallels studies in amaranth (Sarkar et al., 2017) and Dolichos (Kumari et al., 2022).

Cluster analysis

Hierarchical cluster analysis using Euclidean distance and Ward's method grouped 80 curry leaf accessions into three distinct clusters based on 10 nutritional traits (Fig. 3). Cluster II was the largest, comprising 68 accessions, followed by Cluster I (10 accessions) and Cluster III (2 accessions), the latter distinctly separated from the main groups. Despite some admixture, regional grouping patterns emerged, suggesting the influence of local edaphic conditions and environmental pressures, along with evolutionary forces such as genetic drift, spontaneous mutation, and natural or artificial selection. Similar patterns have been reported in maize (Rezai & Frey, 1990) and oats (Alika et al., 1993), supporting the role of geographical and ecological differentiation in shaping nutrient-rich genotypes.

Cluster I exhibited higher average concentrations of multiple nutrients *viz.*, sulphur, iron, copper, calcium,

Table 4 : Principal components, eigen value and explained variance

PC	Eigen value	Proportion of variance (%)	Cumulative proportion
1	2.50	25.05	25.05
2	1.89	18.92	43.97
3	1.09	10.92	54.90
4	0.99	9.96	64.87
5	0.81	8.17	73.04
6	0.71	7.18	80.22
7	0.58	5.85	86.07
8	0.52	5.21	91.29
9	0.48	4.80	96.10
10	0.38	3.89	100.00

Table 5 : Factor loadings of variables

Variable	Factor				
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Calcium	0.28	**-0.39**	**0.33**	-0.12	0.08
Phosphorous	**-0.45**	-0.05	0.17	0.12	**0.41**
Potassium	**0.44**	0.16	**-0.32**	0.06	0.15
Magnesium	0.28	-0.39	**-0.43**	0.24	0.01
Sulphur	-0.003	**0.51**	-0.25	**-0.39**	-0.05
Iron	0.27	0.28	0.30	0.05	**0.74**
Zinc	**0.30**	-0.29	0.23	0.14	-0.06
Copper	0.25	0.23	**0.57**	-0.10	**-0.44**
Manganese	**0.38**	0.33	-0.06	**0.34**	-0.06
Boron	-0.22	0.23	0.10	**0.76**	-0.18

Bold values show a significant association between a nutrient and a specific factor. A positive value indicates a positive association, while a negative value indicates a negative association

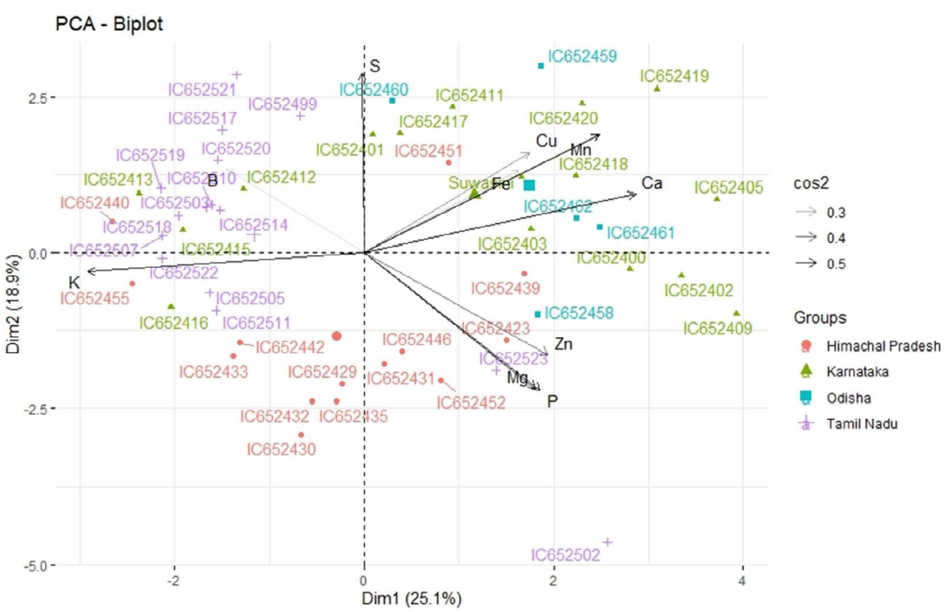

Fig. 4 : Biplot (PC_1 vs PC_2) displays curry leaf accessions relative to mineral nutrient components

Symbols indicated minerals phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn) and boron (B) and manganese, indicating a multi-nutrient enriched group. Accessions IC652419 had outstanding levels of calcium (5.71%), iron (333.80 ppm), manganese (92.47 ppm), and boron (67.39 ppm), while IC652461 showed elevated copper (47.41 ppm), zinc (42.60 ppm), iron (311.60 ppm), and calcium (2.44%). These nutrient-dense accessions are promising for biofortification programs. Clusters II and III contained accessions rich in specific nutrients but lacking broader nutrient profiles, reflecting considerable nutritional diversity. Such variation is consistent with findings in Dolichos bean (Kumari et al., 2022), common bean (Hacisalihoglu & Settles, 2013), and Moringa (Yadav et al., 2024), highlighting opportunities for selecting complementary accessions to enhance the nutritional quality of curry leaf cultivars.

CONCLUSION

This study revealed significant genetic variability in macro- and micronutrient content among 80 curry leaf accessions, with several accessions exhibiting exceptionally high levels of calcium, iron, zinc, and boron. High heritability and genetic advance indicate strong potential for nutritional trait improvement through selection. Multivariate analyses identified key nutrient-dense accessions, such as IC652419 and IC652461, suitable for biofortification and dietary diversification. These findings highlight curry leaf's untapped potential as a functional leafy vegetable to combat micronutrient malnutrition.

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