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Exploring grain amaranth germplasm potential as a source of breeding nutritious leafy greens: insights from nutritional profiling, genetic and multivariate analysis

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Abstract

Background Grain amaranth (*Amaranthus hypochondriacus*) is a nutri-dense pseudocereal with dual-purpose potential as both grain and leafy vegetable. The exploration of grain amaranth types as leafy green could be useful in diversifying food systems, cropping systems and enhancing amaranth germplasm resources. The systematic nutritional profiling and multivariate analysis of grain amaranth leaves is limited, constraining targeted breeding efforts to improve nutritional quality. To establish its breeding potential for nutritious leafy greens, underscoring the compositional diversity of mineral nutrients and heavy metals accumulation in grain amaranth germplasm is vital.

Results Plants were grown in an augmented randomized block design, and nutrient profiling was performed using digestion-based methods and microwave plasma atomic emission spectroscopy (MP-AES). Significant genetic variations were observed for essential mineral nutrients and heavy metals concentrations. Exotic accession EC519523 exhibited superior N and P content, while indigenous collections IC42356 and IC47434 showed promise for K-related nutritional quality. Vegetable checks, Arka Neelachal Bainishi and Arka Neelachal Ruchitha, along with grain type accessions, displayed potential for Fe and Mn biofortification. Notably, the comparative analysis of heavy metals with corresponding FAO/WHO permissible limits depicted significant genotypic variations where several accessions remained within limits, while the mean Cd and Pb values exceeded the referenced permissible thresholds. These findings indicated that suitability for consumption should therefore be considered genotype-dependent rather than universally safe. Multivariate analyses revealed five principal components explaining > 80% of the total variation, while cluster analysis grouped accessions into five nutritional clusters. These results enabled the scope of selecting ideal

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parents from diverse clusters for escalating breeding for nutritional and food security. Strong correlations between macro- and micronutrients indicated shared uptake pathways.

Conclusions These findings highlighted the scope of developing *Amaranthus hypochondriacus* cultivars with dual-purpose value and offered the opportunities for diet diversification, biofortification, and sustainable food security. The results necessitated the need of further multi-location experiments to fetch a better understanding of breeding nutritious cultivars having level of heavy metals within permissible limits, and underscoring the trade-offs between leaf and grain yield in grain amaranths.

Keywords Amaranthus hypochondriacus, Mineral nutrients, Heavy metals, Genetic variation, Multivariate analysis, Safe food, Breeding

Background

In face of climate change, ensuring food and nutritional security of billions, is a daunting challenge for crop breeders, geneticists, horticulturist, and agronomists [1]. A combination of factors like growing population, which is expected to reach a mark of 10 billion by 2050, land degradation and rampant urbanisation, are further exerting a huge pressure on global food supply [2–4]. Globally, 'the big four'- wheat, rice, maize and potato, are staple foods as source of energy, but these may lack essential micronutrients, vitamins and other vital bioactive compounds [5]. In line with this, diversifying the food systems with underutilized nutrient-rich crops, tapping the nutritional wealth of such underutilized crops, and accelerating breeding for climate-resilient cultivars, offers a promising strategy to address global hunger and malnutrition [1, 6–9]. Vegetable crops, comprising a vast diversity of root and tuber crops, stem vegetables, fruit vegetables, legume and leafy vegetables, are considered as a vital component of diversified food systems and a healthy diet [10–13]. Among the variety of vegetable crops, the green leafy vegetables (GLVs) are considered as treasure trove of nutrition [14]. One of the promising vegetables in this category is amaranth, which is available in both leafy and grain types [5]. Amaranth, belonging to the family Amaranthaceae, is a versatile crop with nutritional abundance, climate-resilience, and tolerance to various biotic stresses [5, 8, 15–17].

Taxonomically, Amaranthaceae family consists of two groups, section *Amaranthus* section and Blitopsis dumort [18]. The section *Amaranthus* is considered dibasic in nature with x = 16 and x = 17, except for one tetraploid species, *Amaranthus dubius* (2n = 4x = 64) [5]. Broadly, there are 70–80 species in the genus *Amaranthus*, of which most of the species have their origin in America [19]. These species are utilized as pseudocereal, leafy vegetables, weedy types, animal feed purpose, and as ornamental types [20]. Amaranth fits well in cropping systems due to short crop cycle, and C_4 pathway makes it to outperforms the staple crops like wheat and maize, under marginal soils [5]. The nutritional store house capacity of vegetable types is more than common leafy vegetables like spinach and lettuce [21, 22]. The common leafy types are, *Amaranthus tricolor*, *Amaranthus spinosus*, *Amaranthus viridis*, *Amaranthus lividus*, *Amaranthus dubius* and *Amaranthus blitum* [5]. The leaves and flowers are more nutritious with high antioxidant activity as compared to other plant parts in amaranth. The wide variation is present in *Amaranthus* species regarding their utility across the regions. In West Africa, commonly grown species are, *viz. A. blitum*, *A. hybridus*, *A. cruentus*, and *A. dubius*, while in India and China, *A. tricolor* is mostly cultivated species [23]. In South America, *A. caudatus* is popular as a cereal crop. *A. hybridus* is becoming popular as grain or leafy type in Nepal, Philippines, Indonesia, Thailand and Malaysia [23]. *A. caudatus* is has become popular both as leafy and grain type in Ethiopia [24]. Thus, the grain types are also consumed as leafy vegetables at their young stage [5].

The major species useful as grain amaranth are under the *Amaranthus hybridus* complex, such as *Amaranthus caudatus*, *Amaranthus cruentus*, and *Amaranthus hypochondriacus* [5, 25]. These grain types come under the category of 'pseudocereals', that serves as a valuable food source for smallholder farmers, particularly in regions with marginal climatic conditions [8]. The grain amaranth seed protein contains balanced amount of essential amino acids (lysine, methionine) and is gluten-free, hence a viable option for gluten intolerant consumers [5, 26]. The seed protein content in the grain types is in the range of 13–19%, where promising protein type is globulin [27]. Further, the starch and lipids content in grain types varies from, 48–69% and 1.9–9.7%, respectively [5, 26]. The macro and micro nutrients like Fe, Zn, P, Mn, Ca, and Cu content is comparatively higher in grain type amaranth than other cereals [28]. Although, wide variation is present in different cultivars of different species of amaranth. For instance, Nicodemas and Rwabona [29] reported 75.89 mg/100 g of Fe in 'Nafaka' cultivar of *Amaranthus hypochondriacus*, and 3.284 mg/100 g of Zn in 'Lishe Njano' cultivar. Further, the food supplementation with amaranth oil helps in management of blood pressure and lipid metabolism. The oil from *A. cruentus* has demonstrated hypolipidemic activities and decreases

level of blood cholesterol [30]. The leaves are generally more nutritious than grains in amaranth [5, 24]. In a recent study, the leaves of *A. caudatus* demonstrated high content of K, Ca and Zn, while there were not any traces of heavy metal, cadmium (Cd) [24]. The aim of accomplishing United Nation's SDGs (sustainable development goals) of zero hunger and malnutrition, and potential of amaranth as a super food, has spurred growing research interest in amaranth, globally [16]. The most research efforts have targeted the grain yield and stress tolerance potential of grain types. Despite having dual-use properties, the breeding and research efforts are limited on exploring the nutritional value of edible leaves in grain types. Thus, the leafy vegetable potential of grain types has remained unexplored. The global demand of nutri-dense climate resilient crops accentuates the significance of tapping the underexploited genetic resources of grain types for their leafy vegetable potential. The past reports on underscoring leafy nutrition in amaranth germplasm have largely focused on leafy type species such as *tricolor*, *viridis*, *dubius* [5, 21, 22, 31], while grain types have been primarily explored for grain yield and seed quality [15, 18, 27, 30]. It is well evident that leaves of grain amaranth are also traditionally consumed at the early vegetative stage [18, 24, 32, 33], their nutritional potential has not been systematically quantified across diverse germplasm, nor their heavy metals (Cr, Ni, Pb, Co, Cd) accumulation variation have been critically assessed. In this context, the present investigation was undertaken to systematically characterize the leaf mineral nutrition and toxic element profiles in grain amaranth germplasm, using multivariate chemometric approaches to identify nutritionally superior and safe food genotypes. Leaves of green leafy vegetables including amaranth accumulate higher concentrations of toxic metals like Cd, Pb, As, and Ni than grains due to direct translocation from roots. These heavy metals pose acute and chronic health risks. The comparative analysis of heavy metal accumulation in the leafy vegetables with corresponding FAO/WHO safe limits is the key to identify safe germplasm accessions, which has been analysed in the present investigation. By directly comparing genotypic diversity in essential nutrients and heavy metals, this work establishes a scientific basis for the dual-purpose utilization of grain amaranth as both a grain and leafy vegetable crop, thereby expanding its functional and nutritional relevance. The nutritional profiling and exploration of leafy vegetable potential of grain type amaranths may enhance germplasm strength for diversifying food systems, cropping systems and further ensuring food and nutritional security to accomplish United Nations Sustainable Development Goals (UN-SDGs). Hence, the present investigation was prudently undertaken to explore the nutritional profiling of edible leaves of grain type amaranth based on macro and micronutrients composition along with heavy metals assessment. The study also aimed to characterize and analyse the genetic variation among the grain types amaranth germplasm collections using multivariate techniques like principal component analysis (PCA) and cluster analysis.

Materials and methods

Description of experimental location

The planting of plant material was executed at the research farm of ICAR–Central Horticultural Experimental Station (CHES), Aiginia, Bhubaneswar, Odisha, India during the cropping season of 2024-25. The study area is located at 20°15′ N latitude and 85°40′ E longitude, at above mean sea level of 35 m. The climatic conditions of the study location are warm and humid, where an average annual rainfall of about 1500 mm is recorded with an average humidity of 75–80%. The experimental location is represented by hot summers during March to June, a long rainy season prevails from mid-June to September, and a mild winter from November to January. The soil type of experimental farm is sandy loam with an acidic pH of 4.8 and have low organic carbon content of 0.2%.

Plant materials and experimental design

A set of 32 amaranth genotypes were used in the current study for their nutritional profiling analysis (Table 1). This plant material comprised of 30 indigenous and exotic collections of grain amaranth procured from ICAR-NBPGR, Regional Station, Shimla, Himachal Pradesh. Two vegetable types amaranth cultivars were used as check (Arka Neelachal Bainishi and Arka Neelachal Ruchitha) in the current study (Table 1). The field experiment was laid out in augment randomized block design with 5 blocks and 8 entries per block, where two checks were replicated 5 times in each block. Seed sowing was done in a row length of 3.0 m, with a row-to-row spacing of 30 cm and plant-to-plant spacing of 10 cm. Seeds were sown by line sowing, and thinning was carried out soon after germination to maintain a uniform plant population. The experimental soil was lateritic in nature, highly acidic, with a pH ranging from 4.8 to 5.2, and contained low organic carbon (0.39–0.42%). The soil exhibited low available nitrogen (< 200 kg ha^{-1}), medium available phosphorus (10–15 kg ha^{-1}), and medium available potassium (150–200 kg ha^{-1}) [34]. A fertilizer dose of 70 kg N, 34 kg P_2O_5, and 26 kg K_2O per hectare was applied following the recommended package of practices for amaranth. Fertilizers were applied in two splits, with the basal dose applied at sowing and the remaining nitrogen applied as top dressing at three weeks after planting. Weed management was carried out manually using a hoe; the first weeding was done at two weeks after sowing (WAS), followed by a second weeding at four weeks after sowing. Irrigation was

Table 1 Indigenous and exotic germplasm collections of grain amaranth under study

Sr. No.	Genotype/Accession	Species	Source
1	EC 519,523	*A. hypochondriacus*	ICAR-NBPGR, Shimla
2	IC 95,247	*A. hypochondriacus*	ICAR-NBPGR, Shimla
3	IC 47,434	*A. hypochondriacus*	ICAR-NBPGR, Shimla
4	IC 42,402	*A. hypochondriacus*	ICAR-NBPGR, Shimla
5	IC 42,358	*A. hypochondriacus*	ICAR-NBPGR, Shimla
6	IC 42,353	*A. hypochondriacus*	ICAR-NBPGR, Shimla
7	IC 95,299	*A. hypochondriacus*	ICAR-NBPGR, Shimla
8	IC 95,293	*A. hypochondriacus*	ICAR-NBPGR, Shimla
9	IC 17,926	*A. hypochondriacus*	ICAR-NBPGR, Shimla
10	IC 95,301	*A. hypochondriacus*	ICAR-NBPGR, Shimla
11	IC 95,302	*A. hypochondriacus*	ICAR-NBPGR, Shimla
12	EC 519,512	*A. hypochondriacus*	ICAR-NBPGR, Shimla
13	IC 95,284	*A. hypochondriacus*	ICAR-NBPGR, Shimla
14	IC 42,356	*A. hypochondriacus*	ICAR-NBPGR, Shimla
15	EC 524,457	*A. hypochondriacus*	ICAR-NBPGR, Shimla
16	IC 107,144	*A. hypochondriacus*	ICAR-NBPGR, Shimla
17	IC 47,436	*A. hypochondriacus*	ICAR-NBPGR, Shimla
18	EC 146,546	*A. hypochondriacus*	ICAR-NBPGR, Shimla
19	IC 42,421	*A. hypochondriacus*	ICAR-NBPGR, Shimla
20	IC 42,352	*A. hypochondriacus*	ICAR-NBPGR, Shimla
21	IC 42346-6	*A. hypochondriacus*	ICAR-NBPGR, Shimla
22	EC 146,543	*A. hypochondriacus*	ICAR-NBPGR, Shimla
23	IC 94,661	*A. hypochondriacus*	ICAR-NBPGR, Shimla
24	IC 93,946	*A. hypochondriacus*	ICAR-NBPGR, Shimla
25	IC 95,315	*A. hypochondriacus*	ICAR-NBPGR, Shimla
26	IC 95,334	*A. hypochondriacus*	ICAR-NBPGR, Shimla
27	IC 82,625	*A. hypochondriacus*	ICAR-NBPGR, Shimla
28	IC 95,249	*A. hypochondriacus*	ICAR-NBPGR, Shimla
29	IC 95,250	*A. hypochondriacus*	ICAR-NBPGR, Shimla
30	IC 7836	*A. hypochondriacus*	ICAR-NBPGR, Shimla
31	Arka Neelachal Bainishi	*A. tricolor*	ICAR-IIHR, CHES, Bhubaneswar
32	Arka Neelachal Ruchitha	*A. blitum*	ICAR-IIHR, CHES, Bhubaneswar

provided at 6–7 days intervals, depending on soil moisture status and prevailing climatic conditions, to ensure uniform water availability across all treatments. Leaf samples for nutritional quality analysis were collected at 30 days after sowing (DAS), corresponding to the active vegetative growth stage, prior to stem elongation and reproductive development. For each genotype, healthy, fully expanded leaves were harvested from the upper to middle canopy of randomly selected plants within each plot and pooled to form a composite sample for subsequent nutritional analyses.

Nutritional composition analysis

The estimation of macro and micronutrients along with heavy metals assessment was performed at the Charles Renard Analytical Laboratory (CRAL), ICRISAT, Hyderabad, India. A total of 30 grain amaranth germplasm lines consisting of indigenous and exotic collections, and 2 leafy amaranth varieties as check were subjected to nutritional profiling. These germplasm lines were evaluated for the composition of macronutrients (N, P, K, Ca, Mg, S), micronutrients or trace elements (Fe, Mn, Cu, B, Zn), and heavy metals (As, Cd, Cr, Ni, Pb, Co). The samples were prepared from five random plants from each plot per block for each of the germplasm line under study.

Estimation of total N and P in amaranth leaves

For the estimation of total N and P content in the leaves of amaranth germplasm under study, the sulphuric acid (H_2SO_4)- selenium digestion based colorimetric method was used [35]. About, 0.150 g finely ground plant sample of each line was taken into a digestion tube of 75 ml. simultaneously, one blank and one standard sample was included in each set. Then, 3.5 ml of digestion mixture of sulphuric acid and selenium powder was added into digestion tube followed by heating on the digestion block for 2 h. Then let the digestion tube to cool down and final volume was made up to 75 ml with distilled water. The sample reading was taken for N and P on auto-analyzer at

a wavelength of 660 nm. Total N and P were calculated as per following equations:

$$\text{TotalNandP (ppm)} = \frac{(C - B) \times 75}{W} \quad (1)$$

$$\text{Total N and P (\%)} = \frac{Total\ N\ or P(ppm)}{10000} \quad (2)$$

Where, C is the concentration or reading of Total N (ppm) on auto-anlyzer, B is blank, W is weight of the sample (g) and 75 is the dilution factor.

Microwave plasma atomic emission spectroscopy (MP-AES) based estimation of mineral elements (Fe, Zn, Cu, Mn, Ca, K, Mg, Na, B, S) and heavy metals

To determine the composition of mineral nutrients and heavy metal analysis, Nitric acid- hydrogen peroxide digestion method was employed [36]. The finely ground 0.3 g of plant samples were taken into a labelled polypropylene tubes of 50 ml. Then 2 ml of nitric acid followed by 0.5 ml hydrogen peroxide (H_2O_2) were added into the tube. Tube's cap was hand tightened and vortexed to make the sample wetted in solution. There tubes were left overnight at room temperature of 20–22 °C. In the next morning, again the tubes were vortexed and then kept in a digestion block with a set programme of initial digestion at 80 °C for 30 min followed by digestion at 125 °C for 2 h. Then, after completion of digestion, the samples were allowed to cool at room temperature. After cooling, the final volume in each tube was made up to 25 ml by adding distilled water followed by mixing for 5 min at vortex. Samples were filtered and supernatant was taken for analysis by Microwave Plasma Atomic Emission Spectroscopy (MP-AES)-Agilent.

$$\text{Totalnutrients (ppm)} = \frac{(C - B) \times 25}{W} \quad (3)$$

Where, C is reading in ppm on autoanlyzer, B denotes the blank, W is weight of sample (g) and 25 is the dilution factor.

Statistical analysis

The data was subjected to statistical analysis for analysis of variance (ANOVA), mean performance, and genetic variability parameters using augmentedRCBD R package developed by Aravind et al. [37]. The estimation of σ^2g (genetic variance) and σ^2p (phenotypic variance) was performed for genetic analysis of traits. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) estimates were grouped into three classes as, low (0–10%), medium (10–20%) and high (> 20%) [38, 39].

$$\text{PCV (\%)} = \frac{\sqrt{\sigma^2 \text{p}}}{\overline{x}} \times 100 \quad (4)$$

$$\text{GCV (\%)} = \frac{\sqrt{\sigma^2 \text{g}}}{\overline{x}} \times 100 \quad (5)$$

Likewise, the estimates of heritability were categorized as low (0–30%), medium (30–60%) and high (> 60%) [40].

Multivariate analysis

The nutritional data obtained from all the germplasm collections was subjected to multivariate analysis to determine the extent of genetic variation. R studio is a statistical software with multiple packages for graphical representation and interpretation of results (R studio 2020, https://rstudio.com). The cluster analysis was perf ormed utilizing Mahalanobis distance (D^2) and Euclidian distance. The cluster analysis based on nutritional data for the 32 genotypes under study was done using silhouette technique and NbClust package extracted from R studio [41, 42]. The inter-cluster and intra-cluster distances, and cluster means were computed using 'clv' package of R studio. The principal component analysis (PCA) was performed using R statistical packages 'FactoMineR and 'factoextra'. The determination of eigenvalues, principal components, graphical visualization of PCA biplots, and clusters was carried out during PCA analysis [39]. The major principal components (PCs) contributing total variation were determined using eigenvalues, where PCs having eigenvalue more than 1 were taken into consideration [31]. The R statistical package 'corrplot' [43] was utilized to study the interaction analysis among various nutritional traits under study based on Pearson correlation coefficient.

Results

Analysis of variance based on nutritional traits and heavy metals composition

The analysis of variance conducted using indigenous and exotic collections of grain amaranth for mineral nutrients and heavy metals indicated that, for all the traits under study, the treatment effect was significant (Table 2). These results indicated that considerable variation is present among the grain amaranth germplasm under study, and also wide range of variation is present within the studied nutritional traits and heavy metals. In the current study, vegetable type amaranth genotypes were selected as check cultivars, and the variance analysis revealed significant variation for all traits in check cultivars (Arka Neelachal Bainishi and Arka Neelachal Ruchitha) except for nitrogen, potassium, and copper. Whereas in the test treatments, significant mean squares were observed for all the traits except for nitrogen and potassium content.

Table 2 Variance analysis based on nutritional traits in studied amaranth germplasm

Source		Treatment (ignoring Blocks)	Treatment: Check	Treatment: Test	Treatment: Test vs. Check	Block (eliminating Treatments)	Residuals
Df		31	1	29	1	4	4
Mean.Sq	N	0.29*	0.02ns	0.14ns	4.97**	0.04ns	0.04
	P	0.16**	0.07**	0.05**	3.43**	0.01ns	0
	K	0.2ns	0.17ns	0.08ns	3.82**	0.12ns	0.13
	Fe	258,176**	3,653,155**	45692.5**	3,025,225**	996.39ns	948.54
	Zn	527.18 **	1179.14**	393.09**	3763.72**	3.22ns	1.89
	Cu	4.05*	3.79ns	3.34*	24.81**	0.7ns	0.57
	Mn	302,100**	734,752**	238,002**	1,728,293**	893.93ns	802.78
	Ca	4E + 07**	4.7E + 07**	3.2E + 07**	2.5E + 08**	96166.9ns	73496.4
	Mg	1.1E + 07**	1.8E + 07**	7,377,555**	1.2E + 08**	2318.07ns	10845.5
	B	44.56**	96.19**	25.75**	538.36**	0.59ns	0.12
	S	482,375**	413,004**	193,041**	8,942,431**	1604.24ns	316.91
	As	0.13**	1.05**	0.08**	0.56**	0ns	0
	Cd	0.05ns	0.79*	0.02ns	0.28ns	0.08ns	0.09
	Co	132.47ns	2023.32ns	9.22ns	1815.68ns	1009ns	1014.34
	Cr	0.1**	0.4**	0.03ns	1.65**	0.02ns	0.01
	Ni	17.45**	118.45**	2.94**	337.18**	0.23*	0.02
	Pb	1.21**	0ns	1.14**	4.48**	0ns	0

ns $P > 0.05$, * $P <= 0.05$, ** $P <= 0.01$

In contrast, none of the traits showed significant variation across the blocks.

Nutritional profiling of amaranth germplasm

The nutritional profiling of grain amaranth leaves along with vegetable type checks revealed significant variation for all the studied traits (Table 3; Fig. 1a, b and c). Wide range of variation was recorded for various macro and micro nutrients. Likewise, the grain amaranth collections under study along with check cultivars also exhibited marked variation for heavy metals content. The results pertaining to primary nutrients revealed that, the nitrogen (N) content ranged from 2.88% (IC 95249) to 4.83% (EC 519523), while phosphorus (P) content varied between 0.49% (IC 17926) and 1.62% (EC 519523, IC 42358). The Potassium (K) content was highest in IC 42,356 (3.17%) and lowest in IC 17,926 (1.78%). For secondary nutrients, calcium (Ca) was particularly high in IC 95,334 (29,821.78 ppm), and lowest in IC 17,926 (12139.46 ppm), while magnesium (Mg) peaked in IC 95,334 (16,117.41 ppm) and lowest in Arka Neelachal Ruchitha (5429.59 ppm). Boron (B) was lowest in IC 17,926 (17.92 ppm) and highest in IC 42,358 (42.67 ppm), whereas, the sulphur (S) content was lowest in IC 17,926 (2266.75 ppm) and highest in IC 95,250 (4359.94 ppm). Among the micronutrients, iron (Fe) exhibited wide variation, ranging from 562.53 ppm (IC 95249) to 2151.54 ppm (Arka Neelachal Bainishi). Zinc (Zn) content varied from 131.69 ppm (IC 95250) to 216.48 ppm (IC 42358), and copper (Cu) content varied between 10.68 ppm (IC 17926) and 18.29 ppm (EC 524457). The manganese (Mn) content ranged from 515.62 ppm (IC 95250) to 2111.21 ppm (Arka Neelachal Ruchitha).

The heavy metal analysis revealed the presence of arsenic (As) only in EC 519523 (1.54 ppm) and Arka Neelachal Ruchitha (0.65 ppm), while cadmium (Cd) was detected highest in Arka Neelachal Bainishi (1.24 ppm) and lowest in IC 42,353 (0.58 ppm). Cobalt (Co) content was lowest in IC 95,250 (0.56 ppm) and highest in IC 17,926 (15.07ppm), whereas the chromium (Cr) concentration was highest in Arka Neelachal Bainishi (0.76 ppm). Nickel (Ni) content varied from 1.83 ppm (IC 95334) to 15.02 ppm (Arka Neelachal Bainishi), while the lead (Pb) accumulation was relatively higher in EC 519,512 (3.29 ppm), but was absent in majority of the amaranth collections under study. The exotic collection, EC 519,523 exhibited highest nitrogen (N) content (4.83%) followed by indigenous collections, IC 95,334 (4.42%) and IC 7836 (4.31%). The highest P content was found in EC 519,523 (1.62%) followed by IC 42,358 (1.62%) and IC 42,356 (1.59%). Potassium is one of the major nutrients which plays a key role in plant quality and defense mechanism. In the current study, IC 42,356 (3.17%) depicted highest K content followed by IC 47,434 (3.06%) and IC 47,436 (3.03%). Among the micronutrients, the vegetable type check cultivar, Arka Neelachal Bainishi exhibited the highest Fe content (2151.54 ppm) followed by grain type collections, EC 146,543 (1409.88 ppm) and IC 42,402 (1359.16 ppm). While, the highest Zn content was found in IC 42,358 (216.48 ppm) followed by Arka Neelachal Ruchitha (213.36 ppm) and EC 519,512 (207.21ppm). The current study also revealed the highest content of Cu in grain type collections under

Table 3 Nutritional composition and heavy metals content in grain amaranth leaves

Genotype	*N* %	*P* %	K %	Fe ppm	Zn ppm	Cu ppm	Mn ppm	Ca ppm	Mg ppm	B ppm	S ppm	As ppm	Cd ppm	Co ppm	Cr ppm	Ni ppm	Pb ppm
EC 519,523	4.83	1.62	2.87	645.75	167.55	18.27	967.54	18982.28	8155.35	25.54	4257.34	1.54	0.59	1.55	0.39	6.50	0.00
IC 95,247	3.68	0.92	2.24	795.7	164.28	15.6	977.73	18807.51	8280.71	30.43	3479.34	0.00	0.62	1.42	0.41	5.98	0.00
IC 47,434	4.07	1.23	3.06	959.19	200.95	12	1390.94	19977.23	8201.62	30.33	3461.10	0.00	0.68	2.86	0.45	5.75	0.13
IC 42,402	3.88	1.36	2.90	1359.16	194.78	14.37	1575.34	22850.42	9487.52	36.65	3319.51	0.00	0.78	2.92	0.59	6.42	0.00
IC 42,358	3.81	1.62	2.85	811.25	216.48	13.13	1586.47	25116.63	10730.34	42.67	3551.91	0.00	0.69	3.36	0.53	5.42	0.00
IC 42,353	4.20	1.43	2.81	1170.93	190.56	15.45	1991.03	19325.62	8962.68	35.33	3899.38	0.00	0.58	6.36	0.00	5.67	2.68
IC 95,299	3.86	1.38	2.90	1104.64	196.55	13.1	1988.05	20463.80	9005.36	40.12	4052.56	0.00	0.59	6.40	0.31	6.00	3.04
IC 95,293	4.03	1.42	2.69	1122.08	170.45	14.28	1898.40	16784.76	8499.03	37.28	3631.28	0.00	0.63	5.85	0.00	5.83	1.97
IC 17,926	2.93	0.49	1.78	1324.12	149.16	10.68	1864.32	12139.46	7746.46	17.92	2266.75	0.00	0.64	15.07	0.00	10.36	3.20
IC 95,301	4.01	1.25	2.65	970.3	175.25	11.53	1845.98	15493.06	7543.78	32.43	3254.78	0.00	0.76	6.89	0.00	5.21	1.02
IC 95,302	3.99	1.33	2.71	968.48	180.09	11.84	1870.75	15595.85	7753.89	33.34	3345.98	0.00	0.72	6.46	0.00	5.10	1.03
EC 519,512	4.09	1.45	2.93	936.89	207.21	15.61	1980.90	19400.71	9805.34	35.54	3895.25	0.00	0.81	6.83	0.00	6.73	3.29
IC 95,284	3.93	1.19	2.67	1076.89	173.11	13.36	1885.88	16681.38	9210.59	31.60	3812.23	0.00	0.80	6.63	0.00	5.60	1.22
IC 42,356	4.18	1.59	3.17	869.66	181.62	15.31	1862.39	18922.31	10102.07	34.89	4248.24	0.00	0.69	5.82	0.00	5.42	0.86
EC 524,457	4.01	1.12	2.41	764.2	142.92	18.29	1412.45	13720.86	8991.82	31.12	4104.23	0.00	0.59	4.72	0.00	6.01	0.95
IC 107,144	3.66	1.39	2.87	872.03	195.5	13.03	1637.97	19097.36	9911.49	36.33	4101.38	0.00	0.81	3.38	0.00	3.84	0.00
IC 47,436	3.70	1.25	3.03	732.2	204.84	12.01	1850.67	19322.94	9638.98	33.34	4114.87	0.00	0.84	4.74	0.00	4.90	1.13
EC 146,546	4.18	1.36	2.95	815.79	174.35	11.87	1587.15	18642.28	9737.10	31.92	3888.03	0.00	0.82	3.70	0.00	5.47	0.32
IC 42,421	4.13	1.34	3.00	737.19	179.32	11.95	1189.31	21481.73	11134.13	39.80	4162.59	0.00	0.90	1.96	0.00	3.78	0.00
IC 42,352	4.15	1.24	2.98	820.15	186.45	12.05	1204.54	20743.07	10132.12	30.32	4132.98	0.00	0.65	2.34	0.00	4.21	0.00
IC 42346-6	4.18	1.19	2.94	588.25	179.41	12.04	1269.76	18881.55	10145.75	32.79	4088.60	0.00	0.78	2.52	0.00	4.42	0.00
EC 146,543	4.24	1.21	2.83	1409.88	180.43	14.2	1043.28	21768.01	11266.92	39.24	4210.06	0.00	0.89	2.01	0.00	3.97	0.00
IC 94,661	4.23	1.29	2.91	994.5	202.29	14.21	899.85	24642.60	12982.58	38.79	3889.69	0.00	1.05	1.22	0.00	3.26	1.49
IC 93,946	4.02	1.45	2.89	935.64	192.33	14.56	874.63	28543.73	14534.19	42.49	3877.93	0.00	0.82	1.26	0.00	3.18	0.00
IC 95,315	4.23	1.27	2.54	920.56	185.34	14.82	850.06	24376.90	15432.32	35.43	4087.65	0.00	0.88	0.67	0.00	1.87	0.00
IC 95,334	4.42	1.23	2.99	850.09	171.74	13.26	699.31	29821.78	16117.41	36.74	4162.32	0.00	0.84	0.94	0.00	1.83	0.00
IC 82,625	4.02	1.15	2.53	789.8	193.56	13.89	750.53	2531.65	14324.03	35.08	3532.43	0.00	0.81	2.03	0.00	2.03	0.00
IC 95,249	2.88	1.13	2.83	562.53	148.02	13.23	544.07	26909.47	14523.63	38.85	3905.32	0.00	0.89	0.67	0.00	3.61	0.00
IC 95,250	4.00	1.14	2.91	722.61	131.69	15.08	515.62	28858.76	15411.50	35.59	4359.94	0.00	0.90	0.56	0.00	3.87	0.84
IC 7836	4.31	1.17	2.79	730.08	166.73	14.27	788.39	29396.56	15628.04	40.01	4416.45	0.00	0.97	1.41	0.00	3.97	0.00
Arka Neelachal Bainishi	3.23	0.51	1.94	2151.54	191.64	12.57	1569.09	16708.83	8088.90	29.36	2961.63	0.00	1.24	5.66	0.76	15.02	0.00
Arka Neelachal Ruchitha	3.13	0.68	2.21	942.71	213.36	11.34	2111.21	12352.66	5429.59	23.16	2555.18	0.65	0.68	13.91	0.36	8.14	0.00
SE (±)	0.13	0.03	0.23	19.48	0.87	0.48	17.92	171.46	65.87	0.22	11.26	0.02	0.19	20.14	0.05	0.09	0.00
CD (5%)	0.35	0.07	0.64	54.08	2.41	1.32	49.75	476.05	182.87	0.61	31.26	0.05	0.53	55.93	0.15	0.26	0.00
CV (%)	5.31	3.85	13.89	2.88	0.74	5.66	1.91	1.44	1.07	1.07	0.50	22.77	37.27	16.77	40.43	2.25	0.00

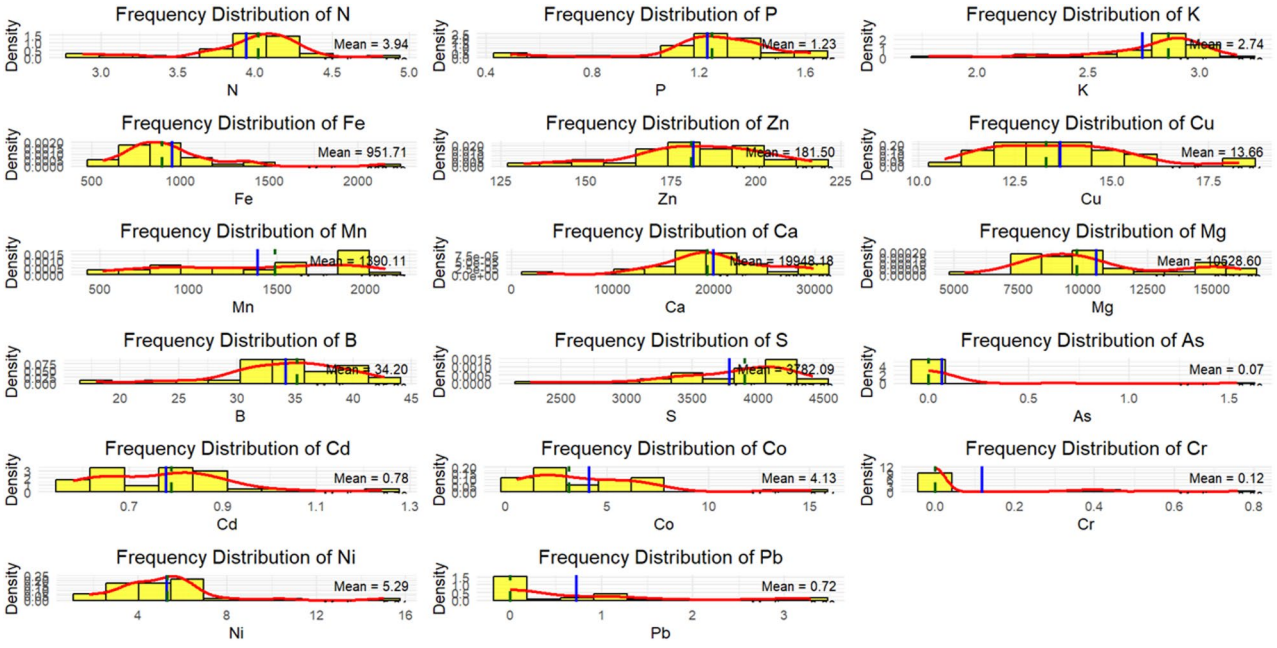

Fig. 1 Frequency distribution and density plots of mineral nutrients and heavy metals in studied germplasm. Histograms depict the variability in concentrations of macroelements (N, P, K, Ca, Mg, and S), microelements (Fe, Zn, Cu, Mn, B, and Co), and heavy metals (As, Cd, Cr, Ni, and Pb). The red curves represent kernel density estimates, while vertical lines indicate mean values, illustrating the extent of variation and skewness among the evaluated genotypes

study as compared to vegetable type check cultivars. In this context, the highest Cu content was found in EC 524,457 (18.29 ppm) followed by EC 519,523 (18.27 ppm) and EC 519,512 (15.61 ppm).

Further, the vegetable type check cultivar Arka Neelachal Ruchitha (2111.21 ppm) exhibited highest content of Mn followed by IC 42,353 (1991.03 ppm) and IC 95,299 (1988.05 ppm). The indigenous collections, IC 95,334 and IC 7836 exhibited highest content of Ca and Mg in the current study. The data analysis revealed the highest content of boron (B) in IC 42,358.

The treatment Test vs. Check contrast signifies comparing the mean of all grain types vs. the mean of vegetable types, where significant results indicated the difference between performance of test and check genotypes (42.67 ppm) followed by IC 93,946 (42.49 ppm) and IC 95,299 (40.12 ppm). Sulphur is another major macro nutrient essential for the various plant developmental processes. In the current investigation, the highest sulphur (S) content was found in IC 7836 (4416.45 ppm), followed by IC 95,250 (4359.94 ppm) and EC 519,523 (4257.34 ppm). The evaluation of vegetables for heavy metals accumulation is imperative, as heavy metals impose several health risks to human health. In the current study, the arsenic content was found only in EC 519,523 (1.54 ppm) and Arka Neelachal Ruchitha (0.65 ppm), while in the remaining collections under study this heavy metal was not detected.

For the assessment of heavy metals with corresponding FAO/WHO safe limits in the studied germplasm,

Table 4 Mean values of heavy metals accumulated across the germplasm under study along with corresponding FAO/WHO permissible limits

Heavy Metal	Mean (mg kg^{-1})	FAO_limit (mg kg^{-1})
As	0.07	0.1
Cd	0.78	0.2
Co	4.13	50
Cr	0.12	2.3
Ni	5.29	67.9
Pb	0.72	0.3

the heavy metals values were converted from ppm to mg kg^{-1} (Supplementary Table S1). The mean value of arsenic (As) content was slightly lower than the FAO/WHO safe limit of 0.1 mg kg^{-1} (Table 4; Fig. 2). Cadmium (Cd) is another non-essential heavy metal, and the present study revealed that the check cultivar Arka Neelachal Bainishi (1.24 ppm), and collections IC 94,661 (1.05 ppm) followed by IC 7836 (0.97 ppm) exhibited highest content of Cd in the leaves. The comparative analysis indicated that Cd concentration in all the studied germplasm including their mean value (0.78 mg kg^{-1}) surpassed the FAO/WHO permissible limit of 0.2 mg kg^{-1} (Table S1, Table 4; Fig. 2). Cobalt (Co) is a heavy metal present in nature, but toxic to human health and environment when it is present in higher concentration. The current investigation revealed highest content of this heavy metal (Co) in IC 17,926 (15.07 ppm) followed by Arka Neelachal Ruchitha (13.91 ppm) and IC 95,301 (6.89 ppm). Additionally, the data analysis revealed the highest content of chromium

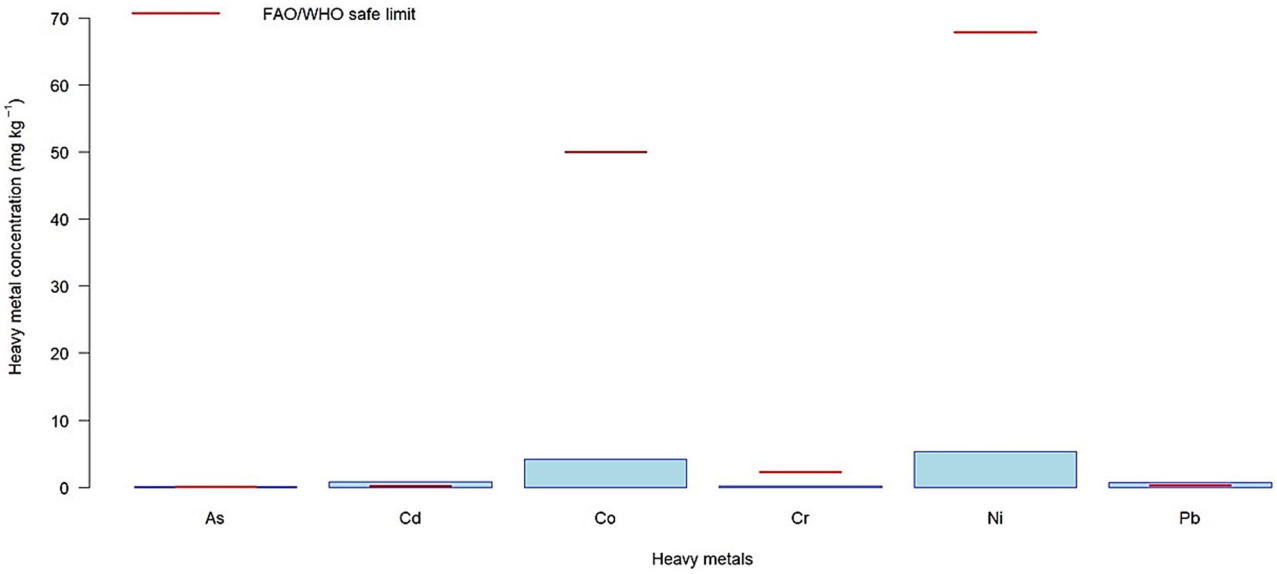

Fig. 2 Comparative analysis of mean values of toxic heavy metals observed in the studied amaranth germplasm with corresponding FAO/WHO safe limits. Blue bars represent the mean concentration (mg kg^{-1}) of arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), nickel (Ni), and lead (Pb) across evaluated germplasm collections. Red horizontal lines indicate the corresponding FAO/WHO permissible safe limits for each heavy metal

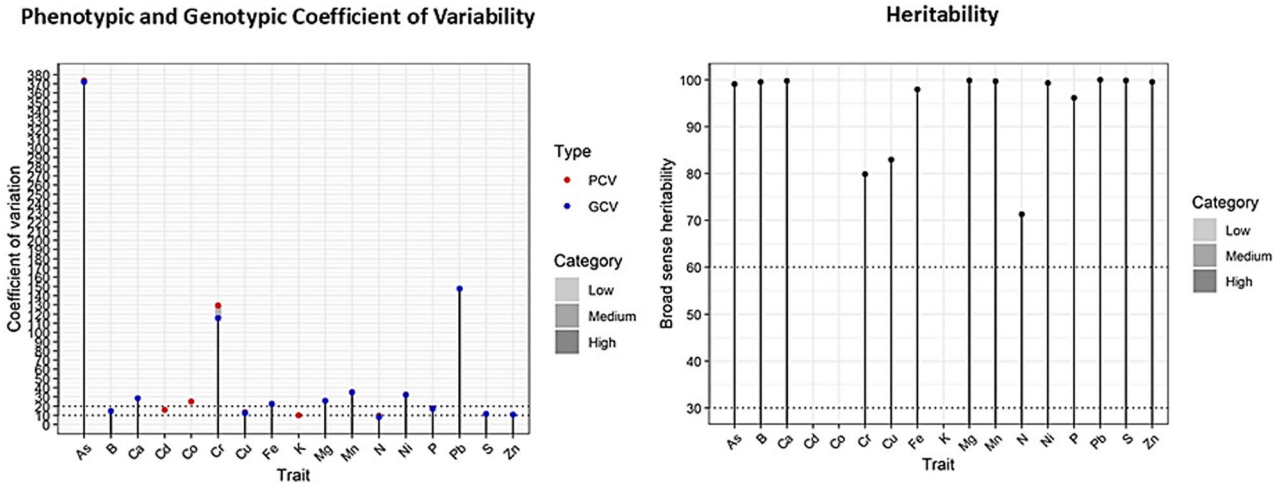

Fig. 3 Graphical representation of PCV and GCV estimates, broad sense heritability for nutritional traits under study advance was not computed for these traits, as their estimation would not be biologically meaningful under a single-environment augmented design

(Cr) and nickel (Ni) in vegetable type check cultivars Arka Neelachal Bainishi and Arka Neelachal Ruchitha. The comparative analysis further indicated that the mean concentration of Co (4.13 mg kg^{-1}), Cr (0.12 mg kg^{-1}), and Ni (5.29 mg kg^{-1}) in the studied germplasm was substantially lower than the FAO/WHO maximum permissible limit (Table S1, Table 4; Fig. 2). Lead (Pb) is another toxic heavy metal, and the data analysis revealed that among the amaranth collections under study, the highest amount of Pb was found in EC 519,512 (3.29 ppm) followed by IC 17,926 (3.20 ppm) and IC 95,299 (3.04 ppm). The comparative analysis indicated that mean Pb concentration (0.72 mg kg^{-1}) surpassed the FAO/WHO permissible limit of 0.3 mg kg^{-1} (Table S1, Table 4; Fig. 2). However, Pb content was not detected in 50% of the indigenous and exotic collections of grain amaranth under study including check cultivars (Table S1, Table 3).

Assessment of genetic components of variance

Genetic variability is a prerequisite for initiating a crop breeding programme, and analysis of genetic variability parameters gives an idea of trait architecture. In the current study, the appraisal of genetic variability parameters based on mineral nutrients among the diverse collections of grain amaranth indicated the presence of wide range of variation (Fig. 3, Table S2). The estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) revealed that, the magnitude of PCV

was slightly higher than the GCV or comparable to GCV, for all the nutritional traits under study. Further, the variable GCV and PCV estimates were recorded for the traits under study, and were categorized as low, medium and high (Table 5). The majority of the traits under study exhibited high estimates of GCV and PCV, except for the nitrogen (N) content (low GCV and PCV), and for P, Zn, Cu, B, S, and Cd content (medium PCV and GCV). Additionally, the heritability (h^2b) estimates were categorized as low (< 30%), medium (30–60%) and high (> 60%). The results revealed that for all the nutritional traits under study, the high estimates of heritability were recorded. The heritability estimates varied from 71.3% (N content) to 99.85% (Mg content). The highest value of h^2b was recorded for Mg (99.85%) content followed by S (99.84%) and Ca (99.77%). Similarly, the data analysis revealed high estimates of genetic advance as percent of mean (GAM) for majority of the traits except for the N content for which moderate (14.02%) GAM was recorded. The GAM estimates varied from moderate (14.02) to high (763.71). Overall, the appraisal of genetic parameters of variability revealed high estimates of h^2b (> 60%) coupled with GAM (> 20%) for all the nutritional traits (P, Fe, Zn, Cu, Mn, Ca, Mg, B, S) and heavy metals (As, Cr, Ni, Pb), except for the N content, where moderate GAM was recorded. Notably, for certain elements such as Cd, Co, and K, genotypic variance could not be reliably detected because the mean square due to genotypes was equal to or lower than the error mean square, resulting in negative estimates of variance. Hence, in this scenario the genetic parameters including GCV, heritability, and genetic.

Table 5 Eigen values of principal component

Prin_comp	eigenvalue	percentage of variance	cumulative percentage of variance
PC1	6.534	38.433	38.433
PC2	2.526	14.858	53.291
PC3	1.978	11.637	64.928
PC4	1.779	10.465	75.393
PC5	1.192	7.013	82.405
PC6	0.686	4.033	86.439
PC7	0.608	3.577	90.016
PC8	0.493	2.9	92.916
PC9	0.336	1.976	94.891
PC10	0.221	1.299	96.191
PC11	0.203	1.196	97.386
PC12	0.133	0.781	98.167
PC13	0.109	0.643	98.81
PC14	0.096	0.566	99.377
PC15	0.058	0.342	99.719
PC16	0.041	0.238	99.957
PC17	0.007	0.043	100

Multivariate analysis

The techniques of multivariate analysis like principal component analysis (PCA) and cluster analysis, are most common methodologies used for appraisal of genetic divergence among the germplasm collections. In the current study, the PCA and cluster analysis depicted substantial amount of variability for various mineral nutrients among the diverse collections of grain amaranth germplasms along with vegetable type checks.

Principal component analysis

In the current study only first five principal components (PC1 to PC5) were used to interpret the results, as these five PCs explained over 80% of total variation among the diverse collections of amaranth germplasms based on macro nutrients, micro nutrients and heavy metals (Table S3 and Table 5). The first two PCs (PC1 and PC2) explained more than 50% of total variation among the amaranth germplasm collections under study (Table 5; Figs. 4 and 5).

The PCA analysis indicated first five PCs (PC1 to PC5) as most informative with eigenvalues of more than 1, i.e. 6.534, 2.526, 1.978, 1.779, and 1.192, respectively (Table 5). The influence of different variables in respective principal components is presented in Table S3 and Fig. 6. The positive or negative sign indicates the positive or negative interaction between the variables. PCA analysis revealed that majority of the essential nutritional traits under study such as N, P, K, Cu, Ca, Mg, B, and S, positively influenced the PC1 (Table S3, Fig. 6). While, most of the heavy metals, As, Co, Cr, Ni, and Pb, negatively influenced the PC1 (Table S3, Fig. 6). These results indicated that PC1 mainly reflects overall mineral nutrient acquisition and storage capacity of grain amaranth leaves. The genotypes depicting high score in PC, represents robust nutrient uptake, translocation and metabolic demands. Thus, genotypes on positive side of PC1 (IC 93946, IC 95334, IC 95315, IC 94661, IC 7836, IC 95250, IC 95249) reflected scope for their utilization as donor in biofortification breeding programmes. The PC2 was positively affected by N, P, K, Zn, Cu, Mn, S, As, Co and Pb (Table S3, Fig. 6). On the other hand, PC2 was driven by contrasting loadings of Mn, Fe, Ni, Co, Pb, and As, while minimum contribution of major macronutrients was observed. Thus, PC2 genotypes can be subjected to further advance generation screening for lowering the heavy metals accumulation and breeding for micronutrient enrichment.

Cluster analysis

The average performance data of diverse collections of grain amaranth based on nutritional traits was subjected to Mahalanobis distance (D^2) analysis to determine the genetic distance between them and grouping of diverse

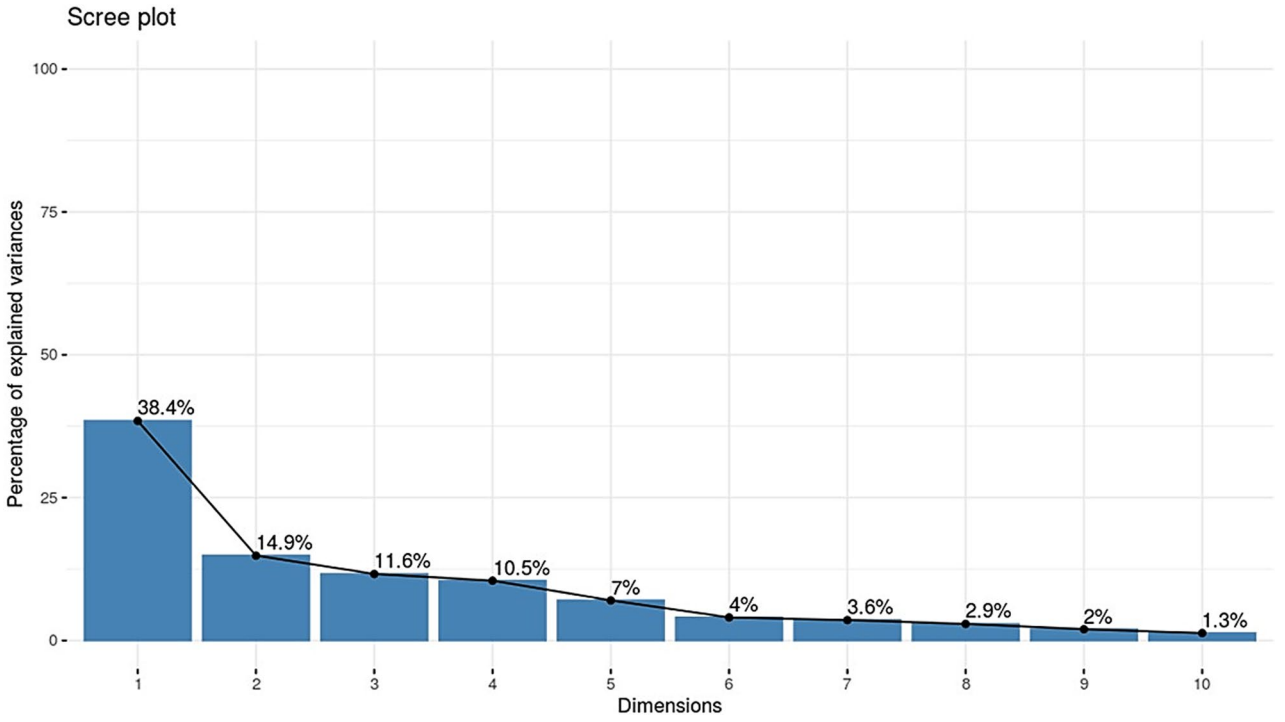

Fig. 4 Scree plot depicting the percent variation explained by first ten principal components among the diverse collections of amaranths under study based on nutritional traits

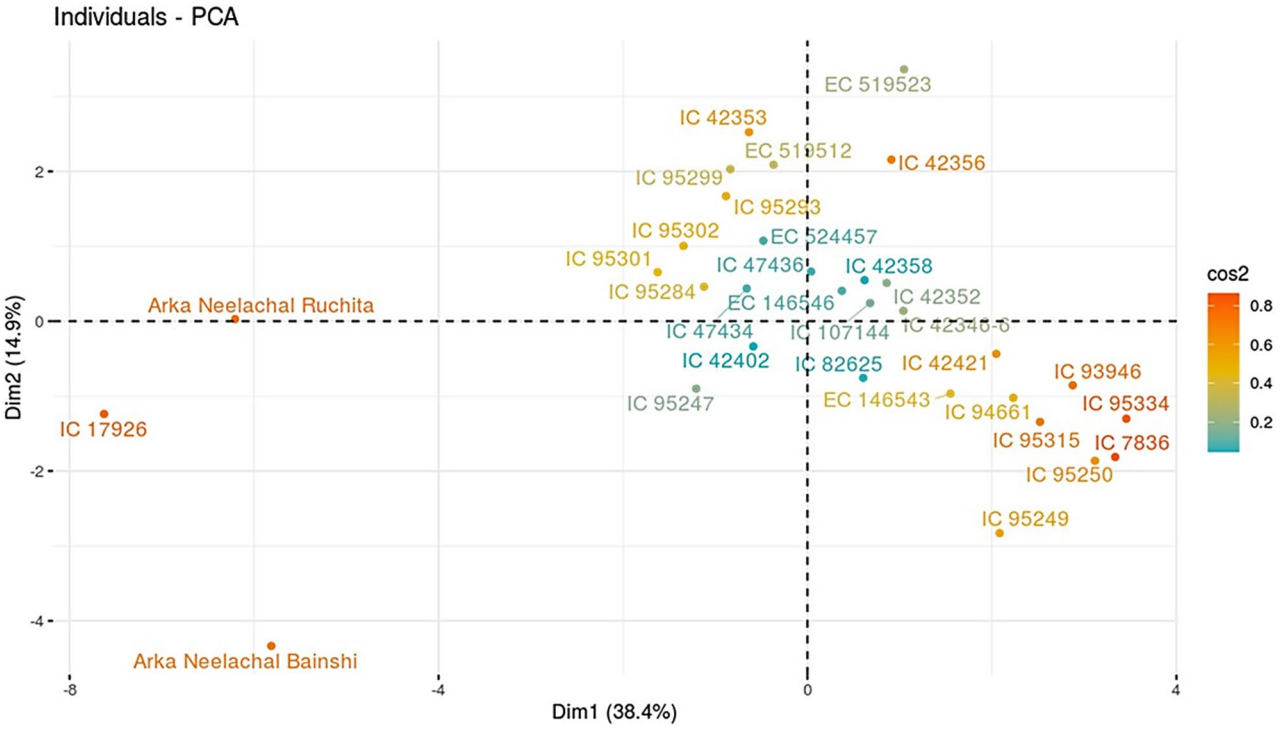

Fig. 5 PCA plot depicting different germplasm collections of grain amaranth across two dimensions (dimension 1 and dimension 2). The vegetable type check cultivars remained separate from grain type collections under study

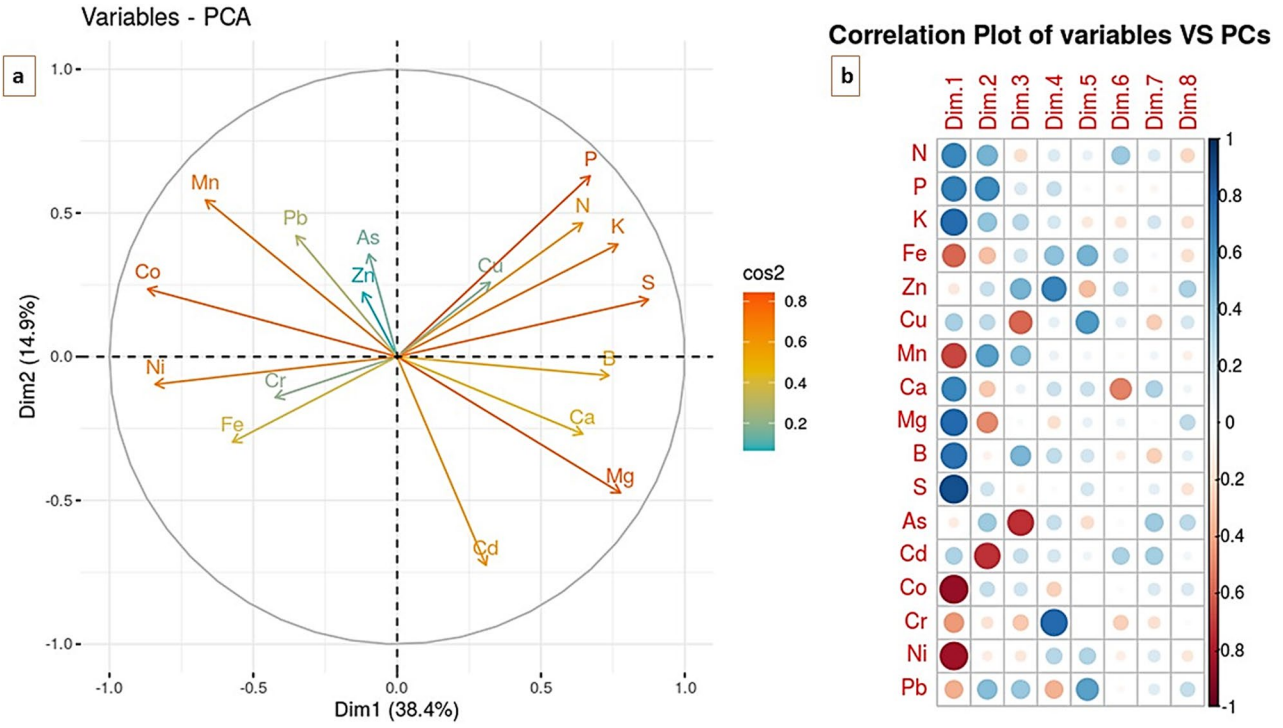

Fig. 6 **a** PCA biplot depicting the contribution of different variable in total variation across the two dimensions. The intense orange colour indicates higher contribution of variables in total variation. **b** Association of variables with five principal components. In each dimension, the intense blue colour depicted strong positive association of respective variables with their principal component and intensity towards red colour indicated negative influence of variables in respective principal components

collections under study into different clusters. The cluster analysis grouped the diverse collections under study into five basic clusters (CI to CV) (Fig. 7; Table 6). The ward D^2 dendrogram was prepared using Euclidean distance (Fig. 7). The Cluster-I, Cluster-IV and Cluster-V comprised of only single genotype viz. EC 519,523, IC 82,625 and Arka Neelachal Bainishi, respectively (Table 6; Fig. 7). While maximum number of collections (27) were grouped into Cluster-II (Table 6; Fig. 7).

The k-means clustering also grouped the amaranth collections under study into five different clusters (Fig. 8). Further, the data analysis revealed that, like D^2 dendrogram analysis, the k-means cluster analysis also demonstrated that the vegetable type check cultivars, Arka Neelachal Bainishi and Arka Neelachal Ruchitha along with indigenous collection IC 17,926 were unique.

Additionally, the exotic collection EC 519,523 was solely allotted to a separated cluster depicted in orange colour (Fig. 8), which further supported the results of dendrogram analysis. The cluster means for each of the nutritional trait under study are presented in Table S4. The cluster mean analysis indicated that Cluster-I is represented by the genotype (EC 519523) as a good source of primary nutrients (N, P, K), Cu and S along with lower value of heavy metals Co, Cr, Ni and Pb that is below FAO/WHO safe limits (Table S4). The largest Cluster-II is represented by the genotypes with good amount of Ca, Bo and substantially lower concentration of toxic heavy metals like Co, Cr, Ni below FAO/WHO permissible limit. The cluster mean analysis further indicated that vegetable type check cultivars included in Cluster-III and Cluster-V are good source of Fe with lower concentration of heavy metals like Cd, As, Co, Ni, Pb below FAO/WHO safe limits.

The cluster distance matrix representing the inter and intra-cluster distance is presented in (Table S5). The intra-cluster distance was zero, while inter-cluster distance was variable between the clusters, presenting the scope for selection of different parents (Table S5). The maximum inter-cluster distance was recorded between Cluster I – Cluster IV followed by Cluster III – Cluster IV, implying the greatest divergence in mineral and elemental composition. The minimum inter-cluster distance was observed between Cluster II – Cluster V, suggesting high similarity in mineral composition and elemental accumulation patterns. These findings predicted preliminary breeding implications suggesting that the crosses between genotypes represented by Clusters I × IV and III × IV would be ideal combinations for enhancing genetic recombination and nutrient diversification. The cluster mean analysis also indicated that genotypes represented by Cluster-III and Cluster-V could potentially be utilized

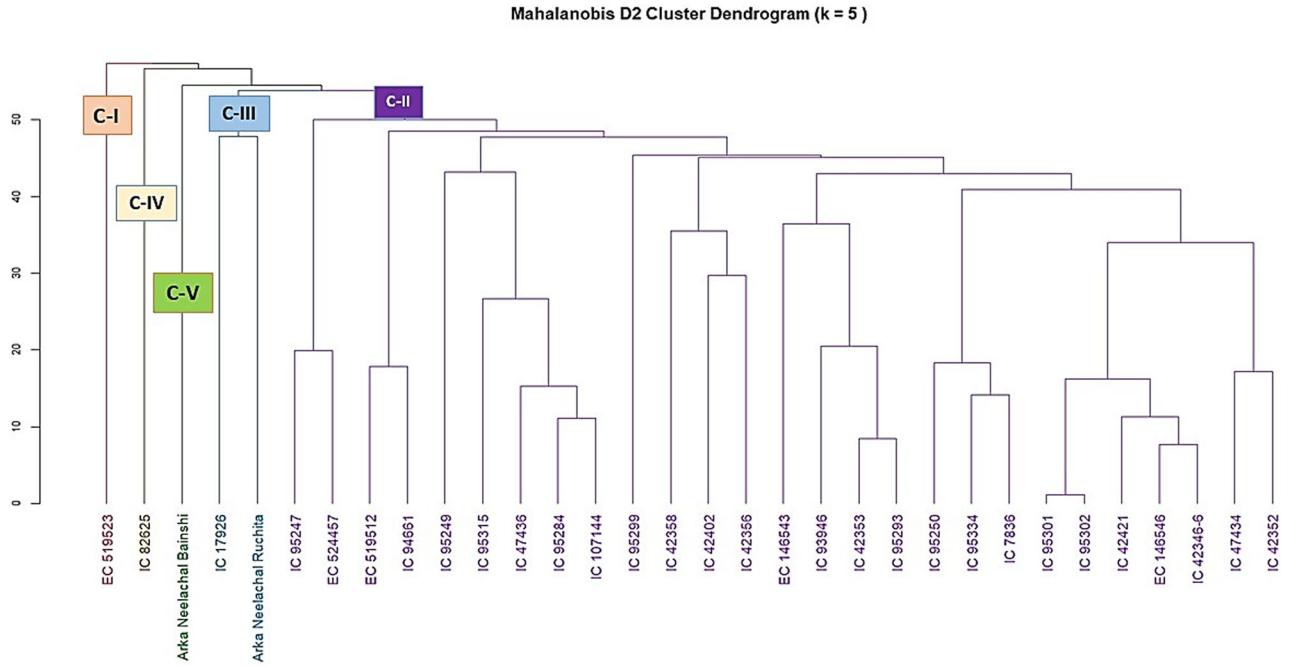

Fig. 7 Dendrogram based on the cluster analysis of different nutritional traits depicting relationship among 30 indigenous and exotic collections of grain amaranths along with 2 vegetable type check cultivars

Table 6 Clustering of exotic and indigenous grain amaranth collections along with vegetable check cultivars into five clusters by mean values of nutritional traits under study

Cluster number	Number of genotypes	Accession names
C-1	1	EC 519,523
C-2	27	IC 95,247, EC 524,457, EC 519,512, IC 94,661, IC 93,946, IC 95,249, IC 95,293, IC 42,353, IC 95,315, IC 47,436, IC 95,284, IC 107,144, IC 95,299, IC 95,334, IC 95,250, IC 7836, IC 42,358, IC 42,402, IC 42,356, EC 146,543, IC 95,301, IC 95,302, IC 42,421, EC 146,546, IC 42346-6, IC 47,434, IC 42,352
C-3	2	Arka Neelachal Ruchitha, IC17926
C-4	1	IC 82,625
C-5	1	Arka Neelachal Bainishi

as parent in Fe biofortification breeding programme of amaranth with low accumulation of heavy metals. Thus, the cross combinations Cluster V × Cluster IV and Cluster III × Cluster IV would be instrumental in Fe biofortification with low burden of heavy metals.

Further, the mahalanobis distance heatmap (Fig. 9) also confirmed the five clusters as presented by dendrogram analysis. The germplasm collections within clusters exhibited low intra-cluster distance (blue to yellow colour shades), while strong inter-cluster distance was depicted between cluster 3 and cluster 5, and cluster 1 and cluster 5 (red colour shades). The check cultivars were grouped close to each other in cluster 1.

Correlation analysis

The results pertaining to interaction analysis among different mineral nutrients under study are presented in Fig. 10. The perusal of Pearson's correlation depicted significantly strong positive association among major nutrients such as between N and P, N and K, N and S, N and Ca, P and K, P and S, K and S, and K and Ca. Among the micro-nutrients, the strong significant correlation was recorded between, Fe and Cr, Fe and Ni, Fe and Zn, Cr and Ni, Cr and Zn, and Ni and Zn (Fig. 10). Cu content also exhibited strong significant interaction with P, N and K. the heavy metal, arsenic (As) exhibited strong positive correlation with Mn content. The major.

Discussion

Amaranth, a Mesoamerican native, is gaining the attention of plant breeders and researchers, owing to its marvellous potential as a climate resilient crop and nutraceutical value [16]. Amaranth holds value both as a leafy vegetable and a pseudocereal crop, that offers high quality protein, mineral elements, essential amino acids, carotenoids, and other bioactive compounds [5]. Mostly, leafy type species like *A. tricolor*, *A. tristis*, *A. blitum*, *A. viridis*, and *A. spinosus*, are popular as leafy vegetables with a tremendous source of nutrition. While grain types such as *A. cruentus*, *A. hypochondriacus*, and *A. caudatus*, are valued as pseudocereal, that are source of gluten free protein and health promoting bioactive compounds [5, 16].

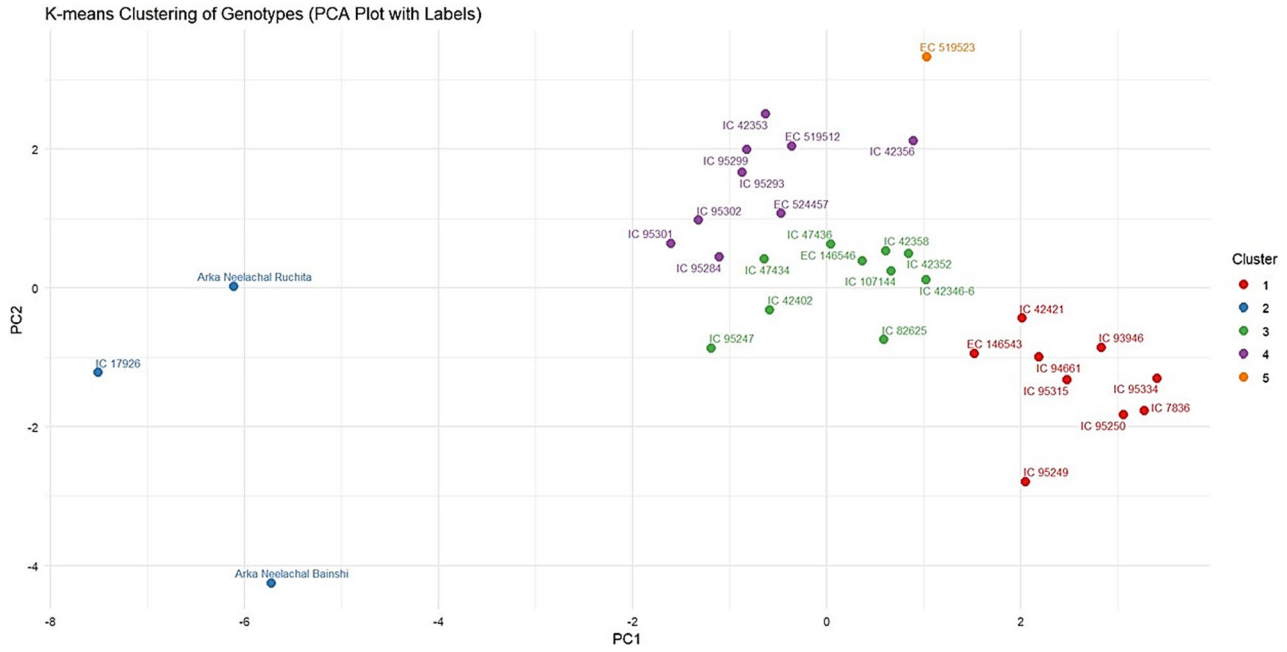

Fig. 8 K-means clustering of amaranth genotypes based on principal component analysis (PCA). Genotypes were grouped into five clusters (Cluster I–V) using K-means clustering and visualized on the first two principal components (PC1 and PC2). Cluster I, Cluster II, Cluster III, Cluster IV, and Cluster V are differentiated using unique color–symbol combinations, allowing unambiguous interpretation in both color and black-and-white formats

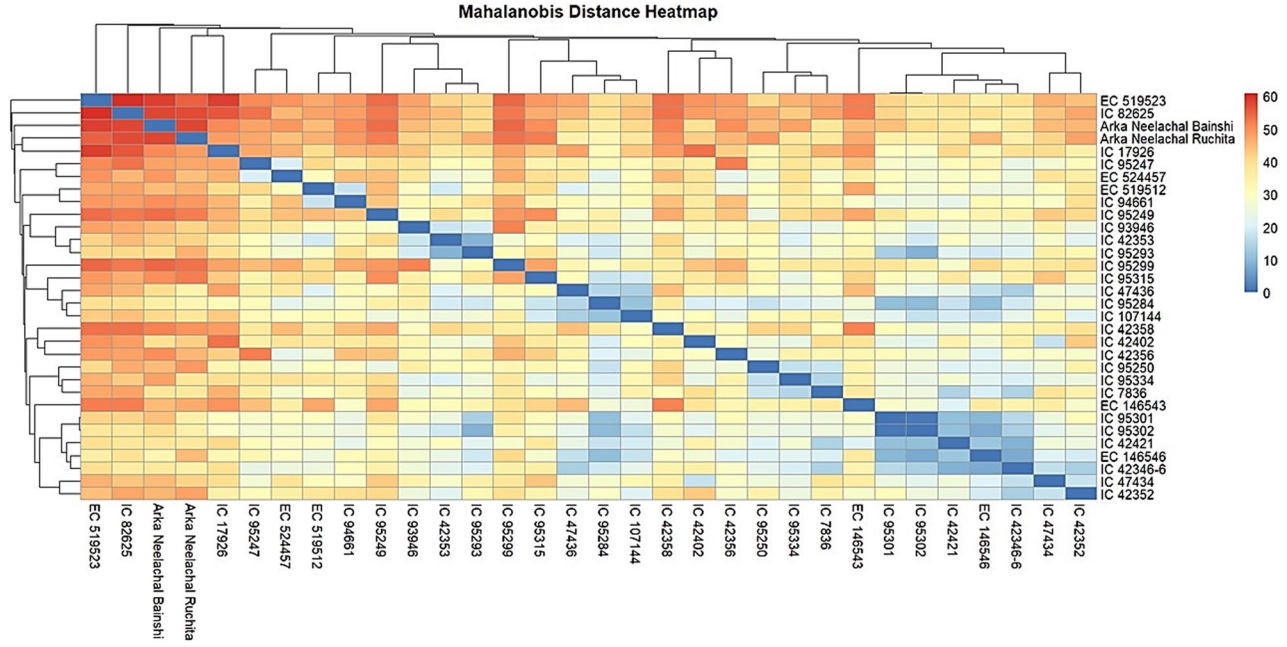

Fig. 9 Mahalanobis distance heatmap determining the genetic relationship between germplasm collections based on nutritional traits. The blue colour code represents the close association or similarity between genotypes. The yellow to orange color represents the moderate divergence between germplasm collections under study. The intense red colour represents the highly diverse collections

However, the leaves of grain types are also edible at young stage, and are potential source of nutrition [24]. The burden of hunger and malnutrition calls for in depth exploration of underexploited potential of underutilized crops like amaranth. Being a climate resilient crop and powerhouse of nutrition, amaranth is a valuable player in tackling the global hunger and nutritional security [23]. In this context, the current investigation was executed to unravel the underexploited potential of grain amaranth (*A. hypochondriacus*) as a nutritious leafy green.

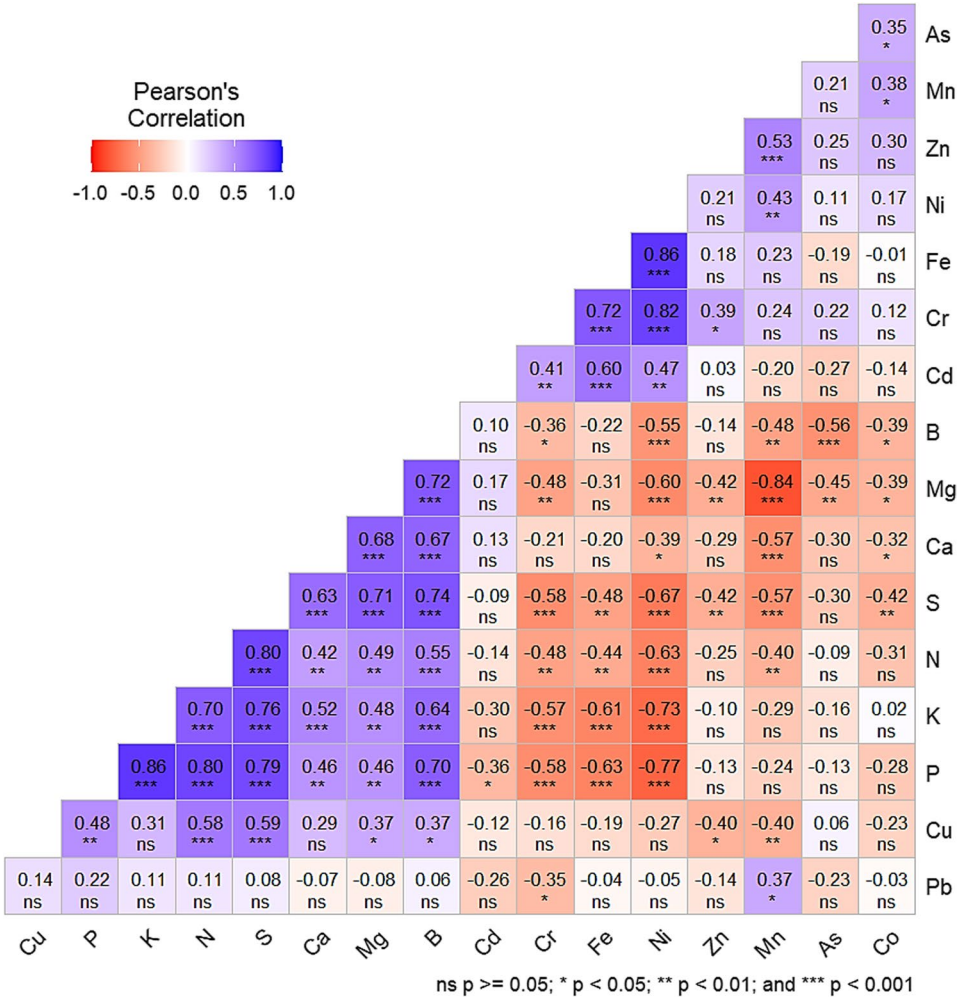

Fig. 10 Correlation analysis among macro and micro nutrients and heavy metals nutrients like N, P, K, Ca and S exhibited strong significant negative correlation with heavy metal Cr. Similarly, these major nutrients such as N, P, K and Ca also depicted significantly strong negative association with Ni content. It means, there is antagonistic interaction between macro nutrients and heavy metals (e.g. Ni and Cr). Among the micronutrients, boron (B) also exhibited significantly negative interaction with Ni and Cr

The ANOVA results demonstrated that the treatment effect was significant for majority of the traits, indicating the presence of considerable genetic variation among the diverse collections of grain amaranth under study. Among the primary nutrients, N and P exhibited significantly strong differences between test treatments and checks, while the treatment effects were non-significant for K content. The Ca displayed a significantly strong effect of treatments among the macronutrients. For the micronutrients, Fe, Zn, Cu, Mn, Mg, B, and S, the treatment effects were pronounced, suggesting the strong genetic variation for these nutrients among the test treatments. The relative magnitude of treatment effects for micronutrients as compared to macronutrients particularly K, indicated that micronutrients uptake is more responsive to agronomic and genotypic amendments that might be buffered by soil type and physiological homeostasis [44]. Among the heavy metals, As, Cr, Ni and Pb, exhibited significant treatment effects. The significant alteration in As and Pb, are particularly of concern due to their toxicity. The results are in conformity with the precious reports that leafy vegetables accumulate more heavy metals depending upon the environmental factors, soil factors, uptake mechanisms, and genotype [45, 46]. The non-significant block effects indicated the uniformity in experimental conditions, and the observed variation was due to genetic factors. Hence, huge genetic variation is present among the studied germplasm, that is crucial for breeding programs, as it provides the opportunity for selection and improvement of desirable traits [47]. In the check cultivars (Arka Neelachal Bainishi and Arka Neelachal Ruchitha), N, K, and Cu, did not show significant variation, which may be due to the narrow genetic base. Previously, studies on grain types including *A. cruentus* and *A, caudatus*, has demonstrated genotypic variation for mineral nutrients like Fe, Cu,

Zn, and antioxidant compounds [24, 29]. The observed variation in the nutrient composition of grain amaranth leaves suggests wide scope for selection of nutrient dense accessions. Further, the strong treatment effects for macronutrients and micronutrients are consistent with the multiple reports suggesting amaranth species accumulate more nutrients than other leafy vegetables [48, 49]. The biofortification of staple cereals for Fe and Zn, has been a major breeding objective in the past decades, in line with this, the current investigation indicates amaranth meaningfully as a nutri-dense leafy vegetable. The variability with respect to Ca content indicates the utility of amaranth for the populations with low dairy intake.

The mineral composition analysis revealed that, the N content, which is an indicator of protein concentration, was significantly high in exotic collection 'EC519523'. Besides, 'EC519523' was also high in P content along with appreciable level of S and Cu content. P is important component of cellular energy metabolism through ATP (adenosine triphosphate) and plays a critical role in cell division, plant physiological processes under abiotic stresses, and synthesis of nucleic acids [50]. Hence, EC519523, can be used in phosphorus and nitrogen efficient breeding programme in amaranth. High content of K is linked with disease resistance, high quality, stomatal regulation imparting stress resilience [51]. In this context, the current study revealed that the indigenous collections such as IC 42,356, IC 47,434, and IC47436 could contribute in breeding nutrient rich and stress resilient amaranth cultivars. Moreover, the high variability for Ca and Mg content, that are crucial for bone health and multiple enzymatic activities [52], revealed the nutritional promise of locally adapted cultivars e.g. IC 95,334 and IC 7836. It is noteworthy that the vegetable type check cultivar, Arka Neelachal Bainishi, exhibited highest Fe accumulation alongside grain type collections like, EC146543 and IC 42,402. Similarly, Mn content was high in vegetable type check cultivar, Arka Neelachal Ruchitha followed by grain types. The availability of high Fe and Mn in vegetable type and grain type collections, revealed the scope for dual-purpose role of amaranth in nutritional security and diet diversification. These findings also highlight conspicuous nutritional and agronomic trade-offs between leafy and grain type amaranths while leaves are used as dietary component. The evaluation of grain types for leaf nutrient composition at tender leaf stage is imperative to identify dual-purpose genotypes that can enhance cropping system flexibility and farmer income. Besides, vegetable type check cultivars excelled in Fe and Mn content only, grain types exhibited broader variability and more balanced profile for primary and secondary nutrients such as N, P, K, Ca, Mg, and S, that is further influenced by G × E interaction. Moreover, the micronutrients concentration is also in acceptable range as depicted in grain type accessions. Hence, nutritional potential should be examined holistically rather than based on a single element. It is apparent that complex mixture of nutrients in food is important for a good health than a single isolated element as nutritional value of food is complex interplay of nutrients, phytochemicals and other bioactive compounds [53]. Additionally, some of the grain amaranth accessions may harbour novel allele combinations for accumulation of essential mineral nutrients that can be utilized in biofortification of leafy amaranths. Additionally, grain types might be slightly lower in certain elements but offers distinct advantage as dual-purpose and agronomically resilient source of nutrition. The inclusion of grain types in food systems broadens the breeding and nutritional potential of amaranth germplasm rather than competing with specific vegetable type amaranths [24, 54]. Such trade-offs are relevant for low input farming systems, where grain amaranth serve as essential source of leafy nutrition besides seed production [24, 54]. This is in consistent with previous claims of Hoidal et al. [54] and Habtemariam and Dana [24], who demonstrated the dual-purpose of amaranth leaves and seeds for healthy life. Simultaneously, for the Zn biofortification, both vegetable type and grain type cultivars, Arka Neelachal Ruchitha and IC 42,358, respectively, exhibited their candidature in breeding programmes. Marked variation was recorded for the Cu content among the amaranth germplasm collections under study. Cu is essential for plant growth and development through lignin synthesis and act as a cofactor for photosynthesis [55]. The current study revealed that the leaves of grain type have more Cu content than vegetable type check cultivars. Cu is crucial for human health and plays role in energy production, Fe metabolism and proper functioning of cardiovascular system [56]. High content of boron (B) in the accessions like IC 42,358 and IC 93,946, also revealed substantial variation among the diverse collections, as B plays a significant role in reproductive development, membrane integrity and cell wall structure [57]. Besides, boron also promotes the human health by supporting nervous system functioning and hormones balance. Breeding of dual-purpose cultivars of grain types require a comprehensive understanding of the trade-offs between leaf and grain yields along with mineral nutrition and other antioxidant quality traits [58]. Further, the monitoring the accumulation of heavy metals in leafy vegetables is imperative, as amaranth is utilized both as vegetable type and grain type. The heavy metals composition analysis indicated that in large numbers of amaranth collections under study, such as IC 95,247, IC 47,434, and IC 42,402 heavy metals like As, Pb and Cr were not detected. While, some accessions depicted accumulation of heavy metals, although mostly within permissible limits reported in leafy vegetables [59]. The comparative

analysis indicated that the mean concentration of heavy metal arsenic (As), Co, Cr, and Ni, was within FAO/WHO threshold limits [60, 61]. In the present investigation, the mean values of Pb and Cd surpassed the FAO/WHO maximum permissible limits. Simultaneously, in 50% of the germplasm under study the Pb content was not detected. Thus, the results of present study depicted significant genotypic variations where several accessions remained within limits, while the mean Cd and Pb values exceeded the referenced permissible thresholds. These findings suggested that suitability for consumption should therefore be considered genotype and environment dependent rather than universally safe. The variation in accumulation of heavy metals might be attributed to genetic factors, climatic and soil conditions. Sarker and Oba [32] also studied the macro and micro elements composition in the leaves of *A. hypochondriacus*, and reported wide variation for mineral nutrients composition. They reported that K content ranged from 5.19 mg g^{-1} to 8.56 mg g^{-1} FW, Ca content from 1.89 mg g^{-1} to 3.01 mg g^{-1}, Mg content from 3.20 mg g^{-1} to 3.88 mg g^{-1}. The past studies have also demonstrated wide variation for macro and micro nutrients, protein content, and antioxidant compounds in amaranth, which strengthens the functional food value of amaranth [24, 32, 54, 62, 63].

The existence of substantial genetic variation in the target germplasm is a prerequisite for exploiting the breeder's skills to breed promising cultivars with high nutrition and quality attributes. In the current investigation, the moderate to high estimates of GCV and PCV for majority of the mineral nutrients except for N content, indicated the scope for improvement of nutritional value through selection procedures [64]. The understanding of heritability provides information about possibility of trait improvement through selection [11, 65]. The estimates of heritability (h^2b) accompanied with genetic advance (GA) enables scope of trait improvement through selective breeding [66]. The present study revealed inflated estimates of heritability and GA for macro nutrients, micro nutrients, and heavy metals composition indicating scope of simple selection procedures for the improvement. The results are in line with the previous estimates of heritability and genetic advance for K, Ca, Mg, and protein content in vegetable type amaranth genotypes of Bangladesh [67]. However, in the present investigation, the inflated estimates of heritability were observed for majority of the traits, which could be attributed to single season evaluation, where G × E interaction variance cannot be distinguished from genotypic variance, hence is confounded within the variance components. The absence of genotype × environment interactions, and negative genotypic variances for some traits (K, Cd, Co) indicated design limitations. Heritability estimates are environment specific; the results should be validated under multilocation trials. Additionally, the heavy metal accumulation is also soil dependent. These results must be interpreted with caution. The field experiment was conducted in augmented block design with replicated checks but test entries remain unreplicated [68]. Thus, the structure of augmented design often underestimates the environmental variance for test entries, further contributing to inflated estimates of heritability. Therefore, the scope of selection must be further validated with multi-location experiments. In the present study, the results also indicated the scope for selection of ideal genotypes with zero accumulation of heavy metals like As, Pb, Cr, and Ni. Additionally, the estimates of genetic components of variance were not computed for K, Cd and Co, which is attributed to negative estimates or non-estimation of genotypic variance (GV) for these traits. these findings indicated the strong influence of environment in the expression of these traits, suggesting the need of further multi-location or multi-environment experiments. Further, the results of PCA and cluster analysis also supported the presence of substantial variation among the diverse genotypes of grain amaranth under study. The PCA is useful to determine the major principal components linked with desirable traits in diverse germplasm and to estimate the correlation level [31]. In addition, PCA is utilized to reduce the dimensions and provides the information about contributing variables in total variation among the studied germplasm [31, 69]. The PCA analysis in the current study, depicted five major principal components (PCs) explaining over 80% of total variation, where macro and micro nutrients like N, P, K, Cu, Ca, Mg, B and S contributed more in total variation. Similar trends were recorded by Abrar et al. [31], who also suggested first five major principal components (PCs) based on physiological traits and macro and micronutrients like N, P, K, Ca, Fe, and Zn in amaranth germplasm. Likewise, in grain amaranth (*Amaranthus hypochondriacus)* four major PCs were identified while studying nutritional and agro-morphological traits in Nepal [70]. The significant correlation between PC1 and mineral nutrients can be utilized for identification of ideal indigenous or exotic collections of grain amaranth [31, 69].

Cluster analysis is another important multivariate technique to assess the genetic variation and grouping the genotypes into different clusters based on their trait merits. In our study, 30 exotic and indigenous collections of grain amaranth along with vegetable type checks were grouped into 5 clusters based on Mahalanobis D^2 analysis. The results were supported by K-means cluster analysis. The vegetable types remained in a separate cluster. The cluster analysis enabled the selection of genotypes for accelerating breeding for nutritional quality in amaranth, for instance, the genotypes in cluster-1 can be

selected in biofortification breeding for Fe and Zn. The categorization of 30 grain amaranth genotypes in to separate clusters based on nutritional parameters analysis at edible leaf stage, indicated broad genetic spectrum of amaranth germplasm [31]. The distance matrix further revealed the minimum intra-cluster distance and maximum inter-cluster distance. These findings are in agreement with Abrar et al. [31] who reported similar trends in grain and leafy amaranth genotypes based on nutritional and morphological traits. The hybridization programme between the genotypes from clusters having maximum inter-cluster distance would be fruitful for getting high heterosis for macro and micro nutrients. It will facilitate the creation of high genetic diversity and genetic recombination in the advance generations [31]. The adaptive diversity may be different between different populations of different species of amaranth [71].

Further, the corrplot analysis suggested synergistic and antagonistic association among macro and micro nutrients in amaranth. The correlation studies indicated significant association among macro nutrients (N, P, K, Ca, Mg, and S). These are the essential nutrients for the plant growth and development, and are absorbed by the plants in large amounts [72]. The strong positive interaction between micro nutrients indicated shared metabolic or uptake mechanisms. Both N and P are essential for plant growth and development, where N assimilation for protein synthesis and cell division increases P demand as P is vital component of nucleotides like rRNA and phospholipid membrane [73, 74]. Thus, shared uptake and signalling pathways contribute to positive association between N and P [73, 74]. Moreover, both N and S are essential components of amino acids and proteins. The positive interaction of N–S is triggered by their obligatory co-incorporation into proteins via sulphur-containing amino acids viz. cysteine and methionine [75]. The N–P and N–K associations are attributed to integrated metabolic stoichiometry, synthesis of nucleic acids, enzymatic activation, and accumulation of biomass during rapid vegetative growth phase [76]. Additionally, the antagonistic interaction between major nutrients and heavy metals can be explained by competition for transport channels, chelation and sequestration of nutrients, soil pH alteration, and cross-talk between nutrient and metal homeostasis [77–79]. Similarly, in *A. blitum*, and *A. hypochondriacus*, positive or negative association was reported previously between macro nutrients, micro nutrients and phytochemicals [32, 63].

Our study highlighted the broader perspective of including grain type amaranths in cropping and food systems to enhance food and nutritional security. It is important to acknowledge the physiological cost of multiple leaf harvestings on source-sink balance. Region specific preferences for consuming tender leaves of grain type amaranth species have been reported [5, 16, 17, 24, 54, 58]. Albeit, fewer studies have demonstrated the evaluation of grain amaranth accessions with respect to their ability to maintain both leaf and grain yield under multiple harvests. However, multiple leaf harvests disrupt source-sink balance and photosynthetic efficiency by reducing carbohydrate production essential for foliage growth and grain filling [58]. The decrease in assimilate availability during grain filling stage owing to foliage defoliation limits the production of carbon for proper seed development [58]. The grain yield reduction of up to 50% have been reported in amaranth cultivars due to multiple leaf harvests [58]. Dramatic reduction in foliage mass during crop reproductive stage potentially contribute to increase in grain yield loss [58]. Although, it is also reported that sink is supplied by the source continuously with the development of new sources and concurrently the photosynthetic capacity of older leaves reduces [58, 80]. However, genotypic differences might be present among the different germplasm accessions with respect to their photosynthetic capacity, foliage restoration ability or recovery physiology. The reduction in number and intensity of leaf harvests could probably decrease grain yield loss [33, 58]. Thus, further studies on source-sink trade-offs in grain amaranth germplasms determining the optimum frequency and intensity of leaf harvestings in contrasting seasons, and assessment of genotype × management interactions, are needed for optimum leaf and grain yields to strengthen the dual-purpose potential.

The present study determined the response of grain amaranth germplasm for mineral nutrients and heavy metals accumulation at edible tender leaf stage. However, mineral bioavailability was not estimated that is strongly influenced by presence of anti-nutrients like oxalate and phytates. Further studies are needed to address this significant gap as absorption of mineral nutrients is inhibited due to antinutritional factors [49, 81]. Both oxalate and phytate hinder Ca, Fe, and Zn absorption, and impair with digestion of starch [49, 81]. Thus, results of our study depicting high concentration of mineral elements must be considered as compositional indicator of studied germplasm. Therefore, the studied grain amaranth germplasm represents a valuable resource for enhancing mineral density in leafy vegetables, however understanding of this bioavailability–concentration trade-off is especially relevant in amaranths for contribution in nutritional security.

The nutritional quality of amaranth leaves is often suggested to be comparable or higher in relation to common leafy vegetables like spinach, lettuce etc., along with promising ability to heat and drought tolerance. Unlike spinach, which is sensitive to elevated temperatures and more prone to nitrate accumulation, amaranth exhibits broader environmental adaptability and efficient nutrient

uptake under marginal soil conditions. Future breeding efforts should prioritize (i) the selection of genotypes with consistently high essential nutrient concentrations and low accumulation of toxic heavy metals; (ii) hybridization between nutritionally superior but genetically divergent clusters to exploit heterosis and transgressive segregation; and (iii) evaluation of advanced breeding lines under diverse agro-ecological conditions to ensure nutrient stability. Overall, this study provides robust evidence that grain amaranth (*Amaranthus hypochondriacus*) harbours significant and exploitable genetic variation for leaf nutritional traits at the edible vegetative stage.

Concluding remarks

The present pioneering study on establishing dual-purpose role of *Amaranthus hypochondriacus* in India, revealed the nutritional potential of leaves of this grain type species. The substantial variability was reported in the leaves of diverse germplasm collections of grain amaranth for macro nutrients (N, P, K, Ca, Mg, S) and micronutrients (Fe, Mn, Zn, Cu). The study highlighted the need of better understanding of nutritional and agronomic trade-offs between leafy and grain-type amaranths, emphasizing the importance of grain amaranth types to complement nutritional resources. The comparative analysis of toxic metal concentration with corresponding FAO/WHO limits indicated the high genotypic variation for the accumulation of heavy metals. The mean values of toxic metals in the studied germplasm for As, Cr, Ni, and Co, were below FAO/WHO permissible limits, while for the Cd and Pb content, mean values surpassed the maximum permissible limit. Although, the results also enabled the identification of specific genotypes such as EC519523, IC95247, IC42402, IC42358, IC42421, IC42352, and EC146543, where the individual Pb content was either not detected or within permissible limits. Our study paved the way for breeding of amaranth cultivars with dual purpose value and enabled the identification of specific accessions exhibiting comparatively lower accumulation of heavy metals within in permissible limits. High genotypic variation for accumulation of heavy metals indicated the need of further multilocation experiments targeting Cd and Pb for identification of germplasm accessions with stable performance regarding lower accumulation of heavy metals which is within FAO/WHO permissible limits. The study revealed the potential genotypes like EC519523, IC 42,356, and IC 47,434 for enhancing primary nutrients content (N, P and K) in amaranth for optimum plant growth and development, stress resilience, and quality improvement. The current investigation also suggested that both vegetable types (Arka Neelachal Ruchitha, Arka Neelachal Bainishi) and grain types (EC146543, IC 42402) can be exploited in biofortification breeding programme for Fe, Mn and Zn in amaranth. The balanced level of macro- and micro-nutrients in grain types suggested the need for holistic nutritional assessment rather than single-element enrichment. The novel allelic combinations valuable for biofortification might be present in grain amaranths and offer advantages as dual-purpose. The inflated estimates of heritability may be biased upwards and further necessitate the need of multi-location testing for reliable selection response. The multivariate analysis further supported the presence of sufficient genetic variation among the studied germplasm of grain amaranth for nutritional attributes in their leaves. The multivariate analysis enabled the selection of ideal genotypes from distinct clusters for achieving high heterosis in amaranth to breed climate resilient and nutrient rich cultivars. The correlation analysis revealed the significant association between macro and micro nutrients in *A. hypochondriacus* leaves. Overall, the identified nutrient-rich genotypes could be exploited in breeding and nutritional security programs, while careful screening is essential to minimize the heavy metal accumulation. The present study paved the way for integrating identified accessions into systematic amaranth breeding programme for biofortification. Further studies on G × E interactions would be instrumental in confirming the nutritional stability in selected genotypes across the diverse geographical locations. Integrating bioavailability, anti-nutritional factors, and genotype × management interactions will be critical to fully exploit the nutritional and breeding potential of grain amaranth for enhanced food and nutritional security. In the genomics era, molecular markers and genomics tools could further accelerate the selection process of ideal genotypes with high mineral nutrient composition and lower accumulation of heavy metals. The dual-purpose production system of amaranth must be promoted among marginal farmers globally for improving local nutrition while maintaining high economic benefits from grain production.

Supplementary Information

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Supplementary Material 1.

Authors' contributions

**Srija Priyadarsini: ** Writing original draft, Methodology, Data recording; **Gobinda Chandra Acharya: ** Supervision, Resources, Conceptualization. **Saurabh Singh: ** Writing – review & editing, Writing – original draft, Data curation, Data analysis, Software; **Pushpajeet Choudhari: ** Formal analysis, Data recording; **Chetna Nimje: ** Data recording, Formal analysis; **Alok Nandi: ** Review and editing.

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Data availability
All the data is included in the manuscript.

Declarations

Ethics approval and consent to participate
The authors declare that the research work described in this manuscript was conducted in accordance with the ethical standards of academic research and integrity. The study did not involve human participants or experimental animals.

Competing interests
The authors declare no competing interests.

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