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Comparative genome-wide analysis of the *NRAMP* gene family in chickpea (*Cicer arietinum* L.) associated with heavy metal stress tolerance

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Abstract
Chickpea (*Cicer arietinum* L.), a widely cultivated legume, is valued for its high protein and mineral content. However, its production has been inconsistent in recent years, largely due to biotic and abiotic stresses and limited genetic diversity. Micronutrient deficiencies, particularly of iron and zinc, remain a global challenge and are often referred to as "hidden hunger". The NRAMP (natural resistance- associated macrophage protein) gene family plays a crucial role in the uptake and transport of heavy metals such as cadmium (Cd), zinc (Zn), copper (Cu), lead (Pb), iron (Fe), and manganese (Mn), and contributes significantly to plant responses under heavy metal stress conditions. In this study, a genome-wide analysis was conducted to identify and characterize *NRAMP* genes in the chickpea genome using bioinformatics approaches. Multiple sequence alignment was performed using the ClustalW method in MEGA 7 to examine conserved residues across NRAMP proteins from *Cicer arietinum*, *Nicotiana attenuata*, *Arabidopsis thaliana*, *Oryza sativa*, *Solanum lycopersicum*, and *Solanum tuberosum*. A total of nine *NRAMP* genes were identified in chickpea. The phylogenetic analysis grouped these genes into five distinct clades. Additionally, physicochemical profiling revealed that Ca-NRAMP6 has the longest protein sequence, while *Ca-NRAMP4* has the highest number of exons and introns. Protein interaction analysis indicated that only CaNRAMP 6 has a strong interaction with other proteins.

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Introduction

Iron (Fe) is an essential micronutrient involved in key cellular functions such as photosynthesis, respiration, and chlorophyll production, making it critical for plant development and growth (Ning et al., 2023; Bhat et al., 2024). It is also a core component of Fe-sulfur (S) clusters, heme groups, and various Fe-binding proteins (Mori and Nishizawa, 1987; Vallières et al., 2024). Due to the frequent unavailability of Fe in many soil types, plants have evolved sophisticated mechanisms to acquire, utilize, and store this micronutrient efficiently (Kim and Guerinot, 2007; Bhat et al., 2024). These processes are tightly regulated at both transcriptional and post-translational levels to ensure homeostasis (Mori and Nishizawa, 1987; Spielmann et al., 2023). Two main strategies have been identified for Fe uptake in plants. Strategy I, common in non-Graminaceous species, involves an iron transporter, which mediates Fe transport from the soil to the roots (Soviguidi et al., 2025). In contrast, Strategy II, employed by Graminaceous species, relies on transporters like YSL that facilitate Fe uptake through siderophore-Fe complexes (Conte and Walker, 2011; Hell and Stephan, 2003;

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Zhang et al., 2017; Martín-Barranco et al., 2021). Alongside these systems, the *NRAMP* gene family also plays a significant role in the absorption and translocation of Fe within the plant system (Ma et al., 2023).

The *NRAMP* gene family has been identified in a wide range of organisms, including bacteria, yeast, plants, mice, and humans (Qin et al., 2017; Ma et al., 2023). This gene family is responsible for transporting divalent metal ions, such as iron (Fe) and manganese (Mn), across cellular membranes, facilitated by a highly conserved transmembrane domain. The first *NRAMP* gene (*NRAMP1*) was discovered in mice, where it functions in immune responses against intracellular pathogens (Gruenheid et al., 1997). *NRAMP2,* also known as *DMT1*, is involved in the transport of various metals, including Fe, Zn, Cu, Mn, Cd, and Pb, and its mutation has been linked to microcytic anemia in both mice and Belgrade rats (Garrick et al., 2003). In plants, *NRAMP* homologs have been functionally characterized in several species. For instance, *A. thaliana* possesses six NRAMP proteins (Tripathi et al., 2018). AtNRAMP1 mediates Fe, Mn, Cd transport, while AtNRAMP6 contributes to intracellular Cd transport and localizes to vesicular endomembrane structures (Cailliatte et al., 2009; Nakanishi-Masuno et al., 2018; Li et al., 2019). *AtNRAMP3* and *AtNRAMP4* are expressed on the vacuolar membranes during seed germination, adding to vacuolar Fe mobilization (Maser et al., 2001; Yamaji et al., 2013; Li et al., 2021). Similarly, in rice, *OsNRAMP3* facilitates Mn translocation to developing leaves and panicles, even under low Mn levels (Hu et al., 2024), and is predominantly expressed in young leaves compared to root tissues.

Further evidence of *NRAMP* function under heavy metal stress has been observed in *Sedum alfredii*, where *SaNRAMP6* expression is induced in response to Cd exposure (Beaumont et al., 2006; Chen et al., 2017). In legumes, *NRAMP* gene activity is increasingly being studied. For example, Fe deficiency significantly upregulates *AhNRAMP1* in peanut, and heterologous expression of this gene in tobacco enhances Fe accumulation and confers tolerance to Fe scarcity (Li et al., 2014). The widespread conservation and metal transport functionality of the *NRAMP* genes highlight their significance in both plant nutrition and tolerance to heavy metal stress, underscoring the need for further characterization in economically important legumes such as chickpea. Due to this reason, the current study was undertaken with the principal objective to do the genome-wide analysis of the *NRAMP* family in chickpea plants stressed with different types of heavy metals.

Materials and Methods

Recognition of the *NRAMP* gene family in chickpea

To identify *NRAMP* gene family members in *C. arietinum* reference protein sequences, *AtNRAMP1-8* from *A. thaliana* was retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/nucleotide/). The conserved *NRAMP* domains were identified using the Pfam database (https://pfam.xfam.org/). These conserved domain sequences were then subjected to BLAST against the Pulse Crop Database (https://www.pulsedb.org/) to identify putative *NRAMP* genes in the chickpea genome.

Sequence alignment and phylogenetic analysis of *NRAMP*

Multiple sequence alignments of peptide sequences were conducted using the ClustalW algorithm within MEGA-7 software (https://megasoftware.net/). Moreover, the aligned sequences were visualized using GeneDoc. Protein sequences of *NRAMP* genes from *Nicotiana. attentuata*, *Oryza sativa*, *Solanum lycopersicum*, *Solanum tuberosum,* and *Cicer arietinum* were downloaded from the Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html), while *A thaliana NRAMPs* were retrieved from the TAIR database (http://www.arabidopsis.org/). A phylogenetic tree was constructed using the Neighbor-joining method in MEGA-7.

Motif analysis of CaNRAMP proteins

Motif analysis of CaNRAMP protein sequences was performed using the MEME suite (https://meme-suite.org/meme/). The resulting MEME XML output was used in the TBtools software for graphical motif visualization.

Conserved domain analysis of CaNRAMP protein

To analyze conserved domains within the CaNRAMP protein, the amino acid sequences were subjected to the NCBI Conserved Domain Database (CDD) (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The resulting domain files were then visualized using TBtools.

Gene structure analysis

Genomic and complementary DNA sequences (CDS) of *CaNRAMP* genes were obtained from the Pulse Crop Database (https://www.pulsedb.org/). These sequences were analyzed using the Gene Structure Display Server 2.0 (http://gsds.cbi.pku.edu.cn/) to determine intron-exon organization.

Subcellular localization analysis

Sub-cellular localization of CaNRAMP protein was predicted using two web-based tools:CELLO (http://cello.life.nctu.edu.tw/) and WoLF PSORT (https://wolfpsort.hgc.jp/).

Analysis of physicochemical properties

Physicochemical properties of the CaNRAMP proteins, including amino acid length, molecular weight, isoelectric point (pI), and Grand Average of Hydropathicity (GRAVY) values, were calculated using the ExPASy ProtParam tool (http://web.expasy.org/protparam/).

Synteny Analysis of *CaNRAMP*

To access synteny relationships, the genome, protein, and GFF files of *C. arietinum* and *A. thaliana* were used. Collinearity files (GFF and CTL) were prepared and visualized using synteny visualization tools integrated within TBtool.

Protein structure prediction and protein-protein interaction analysis

Predicted 3D structures of the CaNRAMP protein were generated using the trRosetta platform (https://yanglab.nankai.edu.cn/trRosetta/). Protein-protein interaction networks were explored using the STRING database (https://string-db.org/cgi/inputsessionId=bmUAl0MkLcdG and input_page_show_search=on), which provides both known and predicted interactions.

Gene expression analysis

The transcriptomic expression data of *CaNRAMP* genes across various developmental stages and conditions were retrieved from the NCBI GEOdatabase (https://www.ncbi.nlm.nih.gov/DataSets/). Heatmaps representing transcript levels were generated through TBtools.

Results

Characterization of the *NRAMP* gene family in chickpea

To identify the *NRAMP* gene family members in the *C. arietinum* genome, a BLAST search was performed using the Pfam domain NRAMPx glutathione peroxidase (CL0172) as a query. This analysis revealed a total of nine *NRAMP* genes in the chickpea genome (**Table 1**). These genes were designated as *CaNRAMP1* to *CaNRAMP9*.

Table 1: *CaNRAMP* genes identified from the genome of *Cicer arietinum* L.

Gene name	Gene ID	Gene name	Gene ID
CaNRAMP1	Ca_01573	*CaNRAMP6*	Ca_12043
CaNRAMP2	Ca_02191	*CaNRAMP7*	Ca_12639
CaNRAMP3	Ca_02923	*CaNRAMP8*	Ca_12662
CaNRAMP4	Ca_03869	*CaNRAMP9*	Ca_17802
CaNRAMP5	Ca_09322		

Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignment of NRAMP protein sequences from *C. arietinum*, *N. attentuata*, *A. thaliana*, *O. sativa*, *S. lycopersicum*, and *S. tuberosum* was carried out using Clustal W in MEGA 7. Several conserved residues were observed across all aligned sequences. To examine the evolutionary relationship among these NRAMP1 proteins, a phylogenetic tree was constructed using the maximum likelihood method in MEGA 7 software (**Figure 1**). The amino acid sequences used to build the phylogenetic tree are listed in **Table 2**. The analysis clustered the NRAMP protein into five major clades (A-E). Clade A consisted of nine proteins, including one from *C. arietinum*. Clade B was the largest, containing 17 proteins, with four from *C. arietinum*. Clade C included five proteins, all from *O. sativa,* with no chickpea members. Clade D consisted of seven members, two of which were from *C. arietinum*. Clade E contained 10 proteins, including two from *C. arietinum*. This distribution indicates that *CaNRAMP* genes are present in four of the five clades (A, B, D, and E), suggesting

potential functional conservation or divergence. For example, *OsNRAMP1* and *OsNRAMP10*, *AtNRAMP6* and *CaNRAMP6*, *S. lycoNRAMP5* and *S. tubNRAMP2* showed strong evolutionary relationships. A separate phylogenetic tree constructed using only the *C. arietinum NRAMPs* (with 1000 bootstrap replicates) confirmed internal diversification among the nine chickpea genes (**Figure 2**).

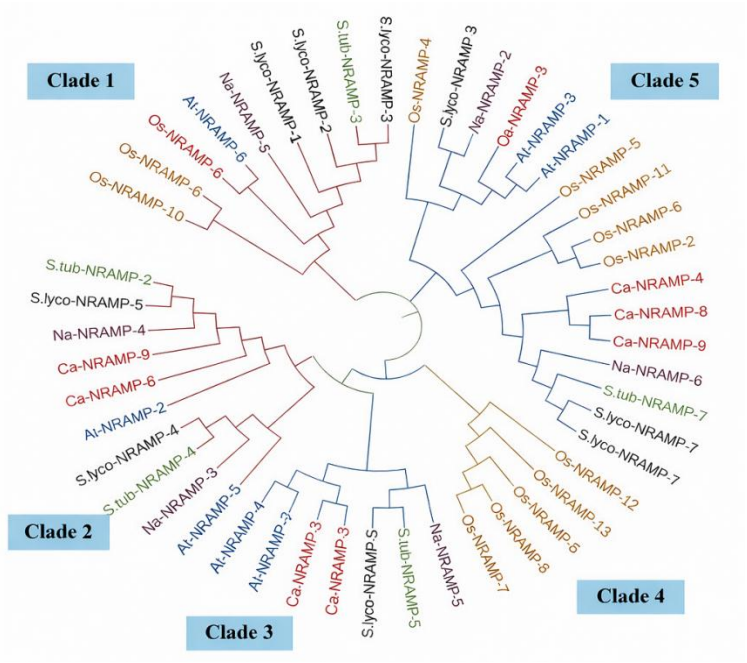

Figure 1: A phylogenetic tree of the NRAMP proteins from *Cicer arietinum, Nicotiana attenuata, Arabidopsis thaliana, Oryza sativa, Solanum lycopersicum,* and *Solanum tuberosum* was generated using MEGA7. Five colors (red, pink, green, blue, and dark blue) represent the five clades.

Table 2: Gene ID of all species (*N. attentuata*, *A. thaliana*, *O. sativa*, *S. lycopersicum,* and *S. tuberosum*) used in the phylogenetic analysis

Gene name	Gene ID	Gene name	Gene ID
CaNRAMP1	Ca_01573	*NaNRAMP3*	OIT37722
CaNRAMP2	Ca_02191	*NaNRAMP4*	OIT37723
CaNRAMP3	Ca_02923	*NaNRAMP5*	OIT30818
CaNRAMP4	Ca_03869	*NaNRAMP6*	OIT03259
CaNRAMP5	Ca_09322	*OsNRAMP1*	Os07t0155600-01
CaNRAMP6	Ca_12043	*OsNRAMP2*	Os07t0258400-01
CaNRAMP7	Ca_12639	*OsNRAMP3*	Os03t0208500-02
CaNRAMP8	Ca_12662	*OsNRAMP4*	Os06t0676000-01
CaNRAMP9	Ca_17802	*OsNRAMP5*	Os02t0131800-01
AtNRAMP1	AT1G15960.1	*OsNRAMP6*	Os07t0257200-01
AtNRAMP2	AT1G47240.1	*OsNRAMP7*	Os01t0733001-00
AtNRAMP3	AT1G80830.1	*OsNRAMP8*	Os03t0606600-00
AtNRAMP4	AT2G23150.1	*OsNRAMP9*	Os03t0607400-01
AtNRAMP5	AT4G18790.1	*OsNRAMP10*	Os03t0700800-01
AtNRAMP6	AT5G03280.1	*OsNRAMP11*	Os01t0503400-03
AtNRAMP7	AT5G67330.1	*OsNRAMP12*	Os12t0581600-01
NaNRAMP1	OIS96181	*S.lycoNRAMP1*	Solyc09g005523.1
NaNRAMP2	OIT05083	*S.lycoNRAMP2*	Solyc09g007870.3
NaNRAMP3	OIT37722	*S.lycoNRAMP3*	Solyc09g005490.1
S.tubNRAMP1	PGSC0003DMG400009247	*S.lycoNRAMP4*	Solyc04g078230.1
S.tubNRAMP2	PGSC0003DMG400021539	*S.lycoNRAMP5*	Solyc04g078250.3
S.tubNRAMP3	PGSC0003DMG400021547	*S.lycoNRAMP6*	Solyc02g092800.3
S.tubNRAMP4	PGSC0003DMG400023310	*S.lycoNRAMP7*	Solyc11g018530.2
S.tubNRAMP5	PGSC0003DMG400024976	*S.lycoNRAMP8*	Solyc11g018535.1
S.lycoNRAM9	Solyc03g116900.3		

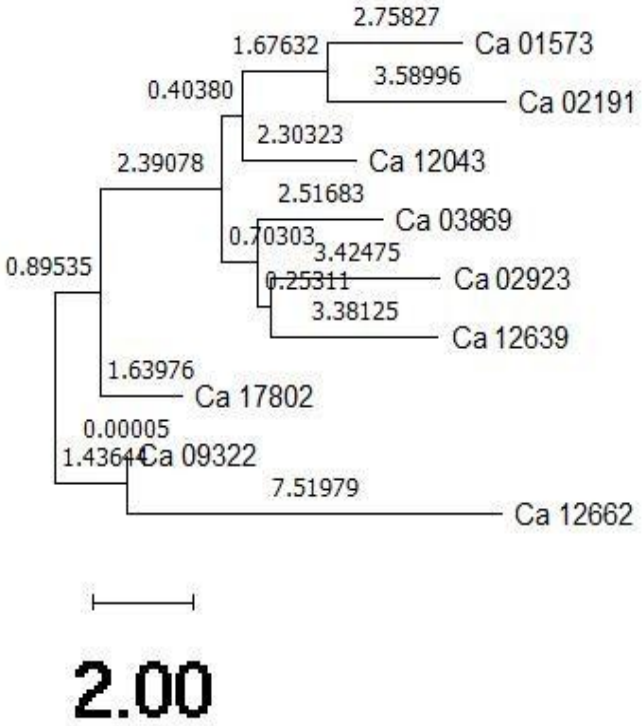

Figure 2: The phylogenetic tree from the protein sequences of CaNRAMPs. The tree was constructed using the Maximum-Likelihood method with 1000 replicates, and the evolutionary distance was computed using the Poisson correction method.

Gene structure, conserved domain, and motif analysis of *CaNRAMPs*

Structural analysis of *CaNRAMP* genes revealed variation in gene length ranging from 1920 (*CaNRAMP1*) bp to 6505 bp (*CaNRAMP9*). The number of exons varied from 4 to 13. For instance, *CaNRAMP1, CaNRAMP2,* and *CaNRAMP5* had 4 exons. *CaNRAMP3* and *CaNRAMP7* had 12 and 13 exons, respectively. *CaNRAMP9* contains 5 exons. Motif analysis using MEME identified 10 conserved motifs across the *CaNRAMP* family (Figure 3). Most genes contained between 4 and 10 motifs: *CaNRAMP1, CaNRAMP2,* and *CaNRAMP5* had 10 motifs. *CaNRAMP6* and *CaNRAMP8* had 5 and 4 motifs, respectively. The domain analysis indicated the presence of the Redoxin domain in several members of *the CaNRAMP2 and CaNRAMP5 families, but not in CaNRAMP1,* suggesting possible functional divergence within the family (**Figure 4**).

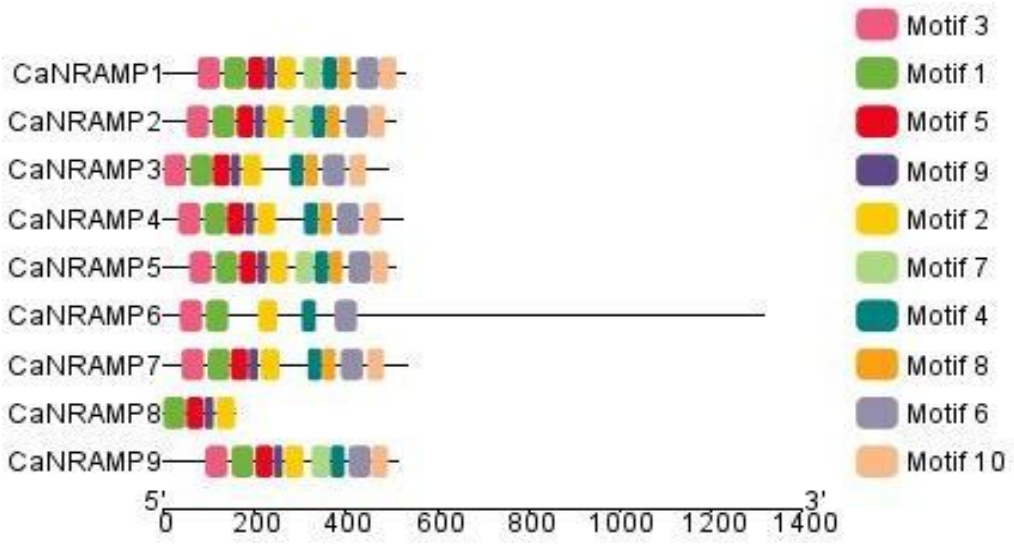

Figure 3: Genomic organization of the *CaNRAMP* gene family and motif analysis of all *CaNRAMPs* identified from the chickpea genome. The conserved motif configurations are named in the *CaNRAMPs*. Different colored boxes reveal distinct motifs.

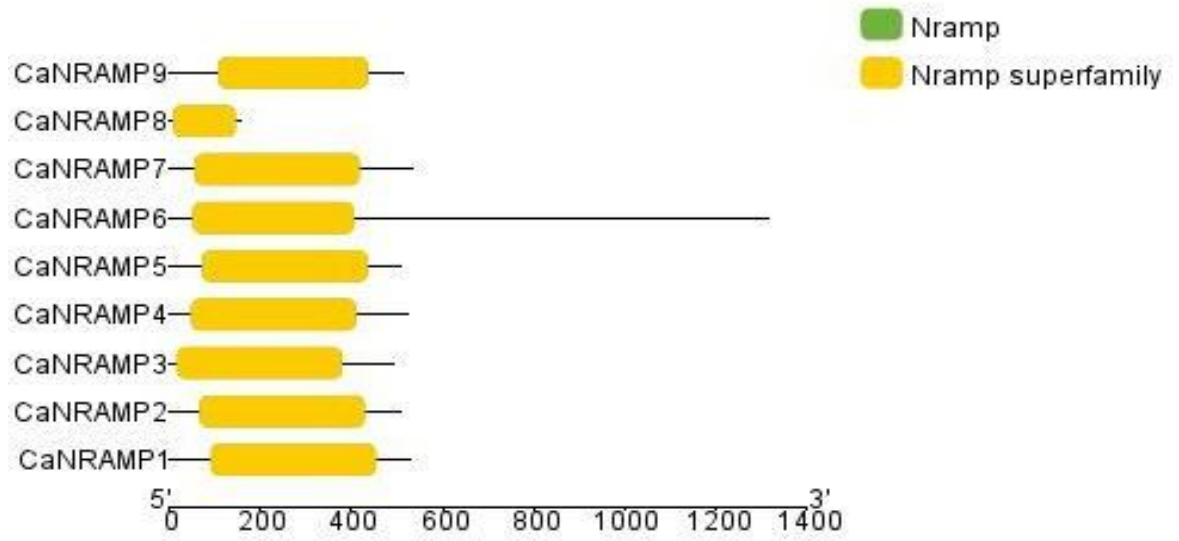

Figure 4: Conserved domain analyses of all *CaNRAMP*s.

Physicochemical properties and subcellular localization

The CaNRAMP proteins ranged in length from 159 to 1318 amino acids. Their coding sequences ranged from 477 bp to 3954 bp, and their molecular weights ranged from 17.2 kDa to 144.1 kDa. The predicted isoelectric point (pI) ranged from 4.8 to 8.88, while Grand Average of Hydropathicity (GRAVY) values varied between -0.08 and 0.885, indicating a range of hydrophobicity among the proteins (**Table 3**). The subcellular localization predictions (via CELLO) revealed that *CaNRAMP1* is localized to the plasma membrane, *CaNRAMP2* is predicted in both the extracellular space and mitochondria, *CaNRAMP3* in extracellular regions, *CaNRAMP4* in the cytoplasm, and *CaNRAMP5* is found both in the nucleus and the cytoplasm.

Table 3: Physiochemical properties of the 9 *CaNRAMP* genes present in the chickpea genome

Gene Name	***Ca-NRAMP1***	***Ca-NRAMP2***	***Ca-NRAMP3***	***Ca-NRAMP4***	***Ca-NRAMP5***	***Ca-NRAMP6***	***Ca-NRAMP7***	***Ca-NRAMP8***	***CA-NRAMP9***
Gene ID	Ca_01573	Ca_02191	Ca_02923	Ca_03869	Ca_09322	Ca_12043	Ca_12639	Ca_12662	Ca_17802
Chromosome	Ca5	Ca8	Ca1	Ca5	Ca7	Ca3	Ca5	Ca3	Ca7
Start	36458622	3829452	8853955	44243578	11739724	33049715	44165239	43927747	35992095
End	36462109	3832943	885754	44248693	11743174	33056220	44169159	43930343	35994015
Strand	Reverse	Reverse	Forward	Reverse	Reverse	Forward	Reverse	Forward	Reverse
Genomic sequence	3487	3491	3585	5115	3450	6505	3920	2596	1920
Transcript sequence	1593	1521	1485	1569	1533	3954	1605	477	1542
CDS(bp)	1593	1521	1485	1569	1533	3954	1605	477	1542
Protein Length	531	507	495	523	511	1318	535	159	514
Amino Acids	530	506	494	522	510	1317	534	158	513
Protein Mol. Wt.	58412.12	55356.07	53621.59	56867.21	55833.57	144135.4	57923.55	17216.75	56904.27
PI	4.97	5.12	8.51	8.87	5.4	5.43	7.12	8.88	4.8
GRAVY	0.45	0.67	0.653	0.62	0.606	-0.08	0.7	0.885	0.503
Introns	3	3	11	12	3	5	12	5	4
Exons	4	4	12	13	4	6	13	6	5
Subcellular Localization (Sig)	4.935	4.93	4.959	4.964	4.94	3.684	4.948	4.806	4.929

Protein-protein interaction analysis

To explore protein interaction networks, structural modeling of all CaNRAMPs was performed using trRosetta, and interaction analysis was conducted using the STRING database. Among all, only CaNRAMP6 showed significant interaction with other proteins such as LOC101508964, LOC101492486, LOC101512832, LOC101509265, and LOC101492847 (**Figure 5**). Notably, LOC101512832 contains Pyr-redox-3 and Pyr-redox-dim domains, suggesting a role in redox regulation and potential participation in oxidative stress responses.

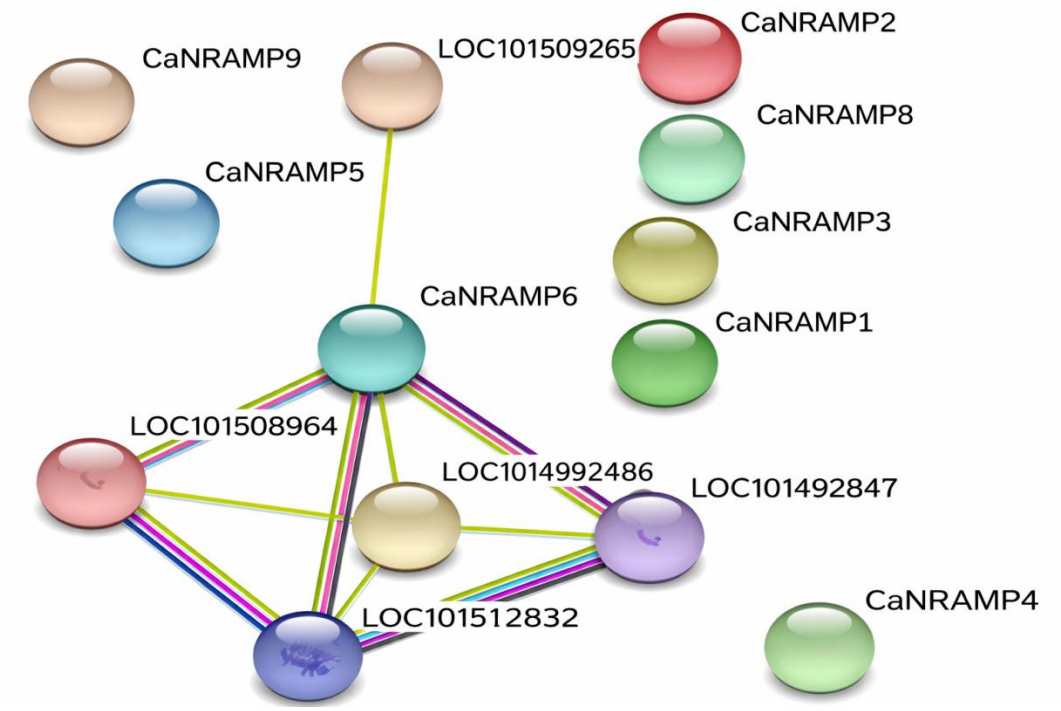

Figure 5: Protein-protein interaction analyses

Gene expression analysis

To assess the gene expression pattern, the data from the Gene Expression Omnibus (GEO) database were analyzed for root tissues at both vegetative and reproductive stages under metal stress conditions. The transcriptomic analysis revealed differential expression among the *CaNRAMP* genes (**Figure 6**). Notably, *CaNRAMP9* (Ca_17802) exhibited significantly higher expression levels, suggesting a central role in metal ion transport and nutrient regulation in the chickpea roots.

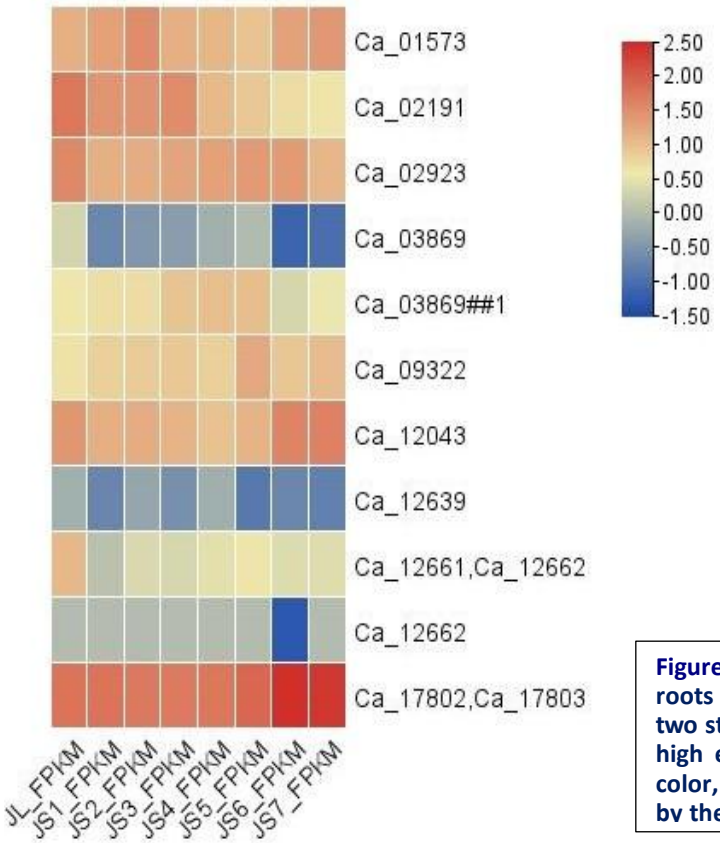

Figure 6: Expression of *CaNRAMP* genes in roots for metal ion transporter conditions at two stages (vegetative and reproductive). The high expression level is indicated by the red color, and the low expression level is indicated by the blue color.

Discussion

To investigate residue conservation patterns within the NRAMP family, the full-length protein sequences from multiple plant species, *C. arietinum, N. attentuata, A. thaliana, O. sativa, S. lycopersicum*, and *S. tuberosum,* were aligned using ClustalW in the MEGA7 software. The alignment provided insights into conserved motifs and functional domains across the species, indicating evolutionary conservation of NRAMP proteins analogous to what has been observed earlier (Kumar et al., 2016; Zhao et al., 2024). A phylogenetic analysis was then conducted using the maximum likelihood method to explore the evolutionary relationship between chickpea NRAMP proteins (CaNRAMP1-CaNRAMP9) and previously characterized NRAMPs from *A. thaliana* (*AtNRAMP1-AtNRAMP7*), *N. attentuata* (*NaNRAMP1-NaNRAMP6*), *O. sativa* (*OsNRAMP1-OsNRAMP12*), *S. lycopersicum* (*SlNRAMP1-SlNRAMP9*), and *S. tuberosum* (*S.tubNRAMP1-S.tubNRAMP5*). The resulting phylogenetic tree grouped these proteins into five distinct clades, suggesting functional conservation with the family, as similarly reported earlier (Letunic and Bork, 2021). The expression analysis indicated that the *CaNRAMP* genes exhibit specific patterns, distinguishing them from their orthologues in other plant species. Notably, these genes responded strongly to heavy metal stress, including exposure to Pb, Cu, Zn, Ni, and Cd. The expression of several *CaNRAMP* genes in the leaf tissues decreased between 6 and 24 hours post-Cd exposure, but subsequently increased, suggesting an adaptive regulation mechanism. In contrast, the *NRAMP* expression in stem tissues showed reduced sensitivity to the same stress conditions, implying differential stress responses across tissues.

The structural characterization of the *CaNRAMP* genes revealed details about their intron-exon organization. The presence of conserved domains, including the thioredoxin-like domain, involvement in redox regulation associated with glutathione peroxide activity, indicates potential involvement in redox regulation (Ravet et al., 2009; Souza et al., 2025). Chromosomal mapping localized *CaNRAMP1*, *CaNRAMP4* and *CaNRAMP7* on chromosome 5; *CaNRAMP5* and *CaNRAMP9* on chromosome 7; CaNRAMP6 and *CaNRAMP8* on chromosome 3; *CaNRAMP3* on chromosome 1, and *CaNRAMP2* on chromosome 8. The synteny analysis revealed colinear gene pairs between *C. arietinum* and *A. thaliana,* suggesting conserved genomic regions (Wang et al., 2012). The Ka/Ks (non-synonymous/synonymous substitution) analysis demonstrated that all *CaNRAMP* genes had Ka/Ks values below 1, indicating purifying selection pressure and evolutionary conservation of function (Yang and Nielsen, 2000). The subcellular localization prediction suggested that the CaNRAMP proteins are distributed across the plasma membrane, mitochondria, chloroplasts, extracellular space, cytoplasm, and nucleus, suggesting their functional versatility in metal transport and stress signaling. The protein structure prediction and protein-protein interaction (PP1I) analysis showed that CaNRAMP6 interacts strongly with other proteins, including LOC101508964, LOC101492486, LOC101512832, LOC101509265, and LOC101492847. These interactions may underpin coordinated responses to metal ion stress and suggest involvement in broader signaling or transport networks (Szklarczyk et al., 2023). Overall, the *CaNRAMP* gene expression was significantly modulated under heavy metal stress conditions, with coordinated regulation observed for specific gene members. Additionally, the expression profiles varied among different tissues and nutritional conditions, suggesting functional specialization and a potential role in ion homeostasis, detoxification, and stress adaptation in chickpea.

Conclusion

This study highlights the potential role of the *NRAMP* gene family in mediating cross-talk between multiple nutritional stress pathways in chickpea. Subcellular localization analysis using *Arabidopsis* protoplasts confirmed that several NRAMP proteins are localized to the tonoplast or plasma membrane, supporting their role in metal ion transport. These findings suggest that specific *CaNRAMP* members may contribute to both metal uptake and maintenance of metal homeostasis, particularly in chickpea nodules. Overall, this study provides valuable insights for future functional characterization of the *NRAMP* genes in chickpea and sets the stage for exploring their role in plant nutrient stress responses. Moreover, this research offers a genetic foundation for developing crop varieties with reduced heavy metal accumulation, enhancing food safety and crop resilience.

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Contribution of authors
Conceived the idea: ZA, MABS. Supervision of the study: MABS; Conduct of research, ZA. Data analysis: ZA. Preparation of first draft: ZA, MABS, AM, KP, ZUN. Revision of the manuscript and reading of the proof: ZA, AM, MAB, KP, ZUN.

Permissions and ethical compliance
This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Supplementary material
No supplementary material is included with this manuscript.

Conflict of interest
The authors declare no conflict of interest.

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent
All authors have critically read this manuscript and agreed to publish in IJAaEB.

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References

Bhat, M.A., Mishra, A.K., Shah, S.N., Bhat, M.A., Jan, S. et al. (2024). Soil and mineral nutrients in plant health: A prospective study of iron and phosphorus in the growth and development of plants. *Current Issues in Molecular Biology 46*(6):5194-5222. https://doi.org/10.3390/cimb46060312

Beaumont, C., Delaunay, J., Hetet, G., Grandchamp, B., De Montalembert, M. et al. (2006). Two new human DMT1 gene mutations in a patient with microcytic anemia, low ferritinemia, and liver iron overload. *Blood* 107:4168–4170.

Cailliatte, R., Lapeyre, B., Briat, J.-F., Mari, S., Curie, C. (2009). The NRAMP6 metal transporter contributes to cadmium toxicity. *Biochemical Journal* 422:217–228.

Chen, S., Han, X., Fang, J., Lu, Z., Qiu, W. et al. (2017). *Sedum alfredii* SaNRAMP6 metal transporter contributes to cadmium accumulation in transgenic *Arabidopsis thaliana*. *Scientific Reports* 7(1):13318. https://doi.org/10.1038/s41598-017-13463-4

Conte, S.S., Walker, E.L. (2011). Transporters contributing to iron trafficking in plants. *Molecular Plant* 4:464–476.

Garrick, M.D., Dolan, K.G., Horbinski, C., Ghio, A.J., Higgins, D. et al. (2003). DMT1: a mammalian transporter for multiple metals. *BioMetals* 16:41–54.

Gruenheid, S., Pinner, E., Desjardins, M., Gros, P. (1997). Natural resistance to infection with intracellular pathogens: the Nramp1 protein is recruited to the membrane of the phagosome. *The Journal of Experimental Medicine* 185(4):717–730. https://doi.org/10.1084/jem.185.4.717

Hell, R., Stephan, U.W. (2003). Iron uptake, trafficking and homeostasis in plants. *Planta* 216:541–551.

Hu, R., Limmer, M.A., Seyfferth, A.L. (2024). How manganese affects rice cadmium uptake and translocation in vegetative and mature plants. *Plant and Soil* 504:941–954. https://doi.org/10.1007/s11104-024-06669-9

Kim, S.A., Guerinot, M.L. (2007). Mining iron: iron uptake and transport in plants. *FEBS Letters* 581:2273–2280.

Kumar, S., Stecher, G., Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870–1874.

Letunic, I., Bork, P. (2021). Interactive Tree of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research* 49:W293–W296.

Li, J.-Y., Liu, J., Dong, D., Jia, X., McCouch, S.R. et al. (2014). Natural variation underlies alterations in NRAMP aluminum transporter (NRAT1) expression and function that play a key role in rice aluminum tolerance. *Proceedings of the National Academy of Sciences of the United States of America* 111:6503–6508.

Li, J., Wang, Y., Zheng, L., Li, Y., Zhou, X. et al. (2019). The intracellular transporter AtNRAMP6 is involved in Fe homeostasis in *Arabidopsis*. *Frontiers in Plant Science* 10:1124. https://doi.org/10.3389/fpls.2019.01124

Li, J., Duan, Y., Han, Z., Shang, X., Zhang, K. et al. (2021). Genome-wide identification and expression analysis of the *NRAMP* family genes in tea plant (*Camellia sinensis*). *Plants* 10(6):1055. https://doi.org/10.3390/plants10061055

Ma, X., Yang, H., Bu, Y., Zhang, Y., Sun, N. et al. (2023). Genome-wide identification of the NRAMP gene family in *Populus trichocarpa* and their function as heavy metal transporters. *Ecotoxicology and Environmental Safety* 261:115110. https://doi.org/10.1016/j.ecoenv.2023.115110.

Martín-Barranco, A., Thomine, S., Vert, G., Zelazny, E. (2021). A quick journey into the diversity of iron uptake strategies in photosynthetic organisms. *Plant Signaling & Behavior* 16(11):1975088. https://doi.org/10.1080/15592324.2021.1975088

Mäser, P., Thomine, S., Schroeder, J.I., Ward, J.M., Hirschi, K. et al. (2001). Phylogenetic relationships within cation transporter families of *Arabidopsis. Plant Physiology* 126:1646–1667.

Misson, J., Raghothama, K.G., Jain, A., Jouhet, J., Block, M.A. et al. (2005). A genome-wide transcriptional analysis using *Arabidopsis thaliana* Affymetrix gene chips determined plant responses to phosphate deprivation. *Proceedings of the National Academy of Sciences of the United States of America* 102:11934–11939.

Mori, S., Nishizawa, N. (1987). Methionine as a dominant precursor of phytosiderophores in Graminaceae plants. *Plant and Cell Physiology* 28:1081–1092.

Nakanishi-Masuno, T., Shitan, N., Sugiyama, A., Takanashi, K., Inaba, S. et al. (2018). The *Crotalaria juncea* metal transporter CjNRAMP1 has a high Fe uptake activity, even in an environment with high Cd contamination. *International Journal of Phytoremediation* 20:1427–1437.

Ning, X., Lin, M., Huang, G., Mao, J., Gao, Z. et al. (2023). Research progress on iron absorption, transport, and molecular regulation strategy in plants. *Frontiers in Plant Science* 14:1190768. https://doi.org/10.3389/fpls.2023.1190768

Qin, L., Han, P., Chen, L., Walk, T.C., Li, Y. et al. (2017). Genome-wide identification and expression analysis of *NRAMP* family genes in soybean (*Glycine max* L.). *Frontiers in Plant Science* 8:1436. https://doi.org/10.3389/fpls.2017.01436

Ravet, K., Touraine, B., Boucherez, J., Briat, J.F., Gaymard, F. et al. (2009). Ferritins control interaction between iron homeostasis and oxidative stress in *Arabidopsis. The Plant Journal* 57:400–412.

Spielmann, J., Fanara, S., Cotelle, V., Vert, G. (2023). Multilayered regulation of iron homeostasis in *Arabidopsis. Frontiers in Plant Science* 14:1250588. https://doi.org/10.3389/fpls.2023.1250588

Souza, P.V.L. (2025). The redox power of thioredoxins: evolution, mechanisms, and redox regulation of carbon–nitrogen metabolism in plants. *Discover Plants* 2:94. https://doi.org/10.1007/s44372-025-00181-9

Soviguidi, D.R.J., Duan, Z., Pan, B., Lei, R., Liang, G. (2025). Function, structure, and regulation of iron regulated transporter 1. *Plant Physiology and Biochemistry* 219:109457. https://doi.org/10.1016/j.plaphy.2024.109457.

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F. et al. (2023). The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research* 51(D1):D638–D646. https://doi.org/10.1093/nar/gkac1000

Tripathi, D.K., Singh, S., Gaur, S., Singh, S., Yadav, V. et al. (2018). Acquisition and homeostasis of iron in higher plants and their probable role in abiotic stress tolerance. *Frontiers in Environmental Science* 5:DOI=https://doi.org/10.3389/fenvs.2017.00086.

Vallières, C., Benoit, O., Guittet, O., Huang, M.-E., Lepoivre, M. et al. (2024). Iron-sulfur protein odyssey: exploring their cluster functional versatility and challenging identification, *Metallomics* 16(5): mfae025, https://doi.org/10.1093/mtomcs/mfae025

Wang, Y., Tang, H., DeBarry, J.D., Tan, X., Li, J. et al. (2012). MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Research* 40(7):e49. https://doi.org/10.1093/nar/gkr1293

Yamaji, N., Sasaki, A., Xia, J.X., Yokosho, K., Ma, J.F. (2013). A node-based switch for preferential distribution of manganese in rice. *Nature Communications* 4:2442. https://doi.org/10.1038/ncomms3442.

Yang, Z., Nielsen, R. (2000). Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models. *Molecular Biology and Evolution* 17:32–43.

Zhang, C., Cao, S., Jin, Y., Ju, L., Chen, Q. et al. (2017). Melon 13-lipoxygenase CmLOX18 may be involved in C6 volatiles biosynthesis in fruit. *Scientific Reports* 7(1):2816. https://doi.org/10.1038/s41598-017-02559-6

Zhao, Y., Xie, Q., Yang, Q., Cui, J., Tan, W. et al. (2024). Genome-wide identification and evolutionary analysis of the *NRAMP* gene family in the AC genomes of *Brassica* species. *BMC Plant Biology* 24(1):311. https://doi.org/10.1186/s12870-024-04981-1