Article

Not peer-reviewed version

Genome-Wide Identification and Characterization of NRAMP family members in *Miscanthus sinensis*

Wei Chen , Xiaojue Peng * , Weixiong Long * , Jiawei Niu , Suping Ying , Zhao Jian

Posted Date: 22 January 2025

doi: 10.20944/preprints202501.1599.v1

Keywords: *Miscanthus sinensis*; NRAMP; metal ion transporter

Article

Genome-Wide Identification and Characterization of NRAMP family members in *Miscanthus sinensis*

Wei Chen [1], Jiawei Niu [1], Suping Ying [1], Jianzhao [2], Weixiong Long [3,*] and Xiaojue Peng [1,*]

[1] School of Life Sciences, Nanchang University, 999 Xuefu Avenue, Nanchang City, Jiangxi Province, 330031, China

[2] Hospital of Nanchang University, Nanchang University, 999 Xuefu Avenue, Nanchang City, Jiangxi Province, 330031, China

[3] Jiangxi Academy of Agricultural Sciences, Super Rice Research and Development Center, 602 Nanlian Road, Qingyunpu District, Nanchang City, Jiangxi Province, 330200, China

* Correspondence: longweixiong@jxaas.cn (W.L.); xiaojuepeng@ncu.edu.cn (X.P.); Tel.: +86-13576993132

Abstract: *Miscanthus* is extensively cultivated as a pioneer plant, demonstrates excellent performance in coping with heavy metal stress. However, existing research on *Miscanthus* and its mechanisms for heavy metal transport is limited, particularly regarding the genetic mechanisms underlying of its tolerance to heavy metals. This study aimed to fill the knowledge gap concerning the role of NRAMP (natural resistance associated macrophage protein) genes in *Miscanthus*, as NRAMP has been widely reported to facilitate the transport of heavy metal ions in other plants. A total of seventeen *MsNRAMP* genes were identifed in *Miscanthus sinensis*, which can be classified into two major groups and are characterized by a high degree of conservation in their secondary structure, the presence of ion-binding sites characteristic of the Leu-T domain, and an average of 9-12 transmembrane domains. Our findings significantly enhance the understanding of metal ion balance and stress response mechanisms in *Miscanthus sinensis*. Specifically, we discovered that only *MsNRAMP3* and *MsNRAMP7* are expressed under normal conditions, while the remaining *MsNRAMP* genes exhibit low or no expression. Additionally, *MsNRAMP14* and *MsNRAMP8* were found to have numerous ABA and MeJA response elements in their promoter regions, suggesting a potential role in stress response.

Keywords: *Miscanthus sinensis*; NRAMP; metal ion transporter

1. Introduction

Miscanthus is a perennial allopolyploid C4 plant possesses for its rapid growth and high biomass, yielding up to 60 tons per hectare annually [1]. *Miscanthus* has an elegant appearance and that makes it suitable as an ornamental plant, it has primarily been cultivated as a fuel crop. From both economic and environmental perspectives *Miscanthus* serves as an effective plant for sustainable energy provision [2]. Despite its robust tolerance to saline-alkali stress, *Miscanthus* also demonstrates considerable tolerance to high concentrations of various heavy metals, thereby holding significant potential for production applications in saline-alkali soils [3].

Natural resistance-associated macrophage proteins (NRAMP) are divalent metal cation transporters. The NRAMP family of proteins typically features a conserved hydrophobic domain and several transmembrane regions, usually numbering between 10 and 12 [4]. These proteins possess a characteristic LeuT-like domain and exhibit a symport transport mechanism, whereby protons and metal cations are co-transported in the same direction after following their binding to specific residues [5]. For instance, the divalent metal transporter 1 (DMT1), which is essential for iron transport in the human body, is a member of the NRAMP family [6]. Furthermore, NRAMPs have been extensively studied in Arabidopsis and rice, where they have been widely reported to enhance

 doi:10.20944/preprints202501.1599.v1

plant tolerance to heavy metal stress by reducing the uptake and accumulation of lead and cadmium ions [7–9]. With the exception of OsNRAMP3, which is localized in the vacuole, the remaining of the rice NRAMP family proteins are reported to facilitate cadmium ion transport functions, and they can also transport trivalent aluminum ions [10].

As a transporter protein, NRAMP is a crucial component of the signaling pathway. Studies on Arabidopsis seeds have found that at the transcriptional level, Arabidopsis *AtNRAMP1* is regulated by the transcription factor INO, which lays a protective role for seed cells aganist excessive iron toxicity [11]. Additionally, NRAMP undergoes post-transcriptional modifications, such as the phosphorylation of AtNRAMP1 by AtCPK21 and AtCPK23, which regulates the entry of manganese ions into the vacuole, thereby protecting the plant from manganese toxicity [12].

Given the significance of *NRAMPs* in metal ion homeostasis and the lack of comprehensive analysis in *Miscanthus*, this study aims to address the existing knowledge gap regarding the role of *NRAMPs* in *Miscanthus sinensis*. Our analysis encompasses genome-wide identification, phylogenetic classification, and an investigation into their potential roles in metal ion balance and stress response.

2. Results

2.1. Identifcation of NRAMP Family Genes in Miscanthus

The genomic data obtained from JGI was Msinensis_497_v7.1. With the method of BlastP and HMMER 2.0. A total of 26 *MsNRAMP* genes were identifed in *Miscanthus*, based on 7 *OsNRAMPs* and 6 *AtNRAMPs* extracted from the National Center for Biotechnology Information (NCBI). After screening, 17 genes were found to possess the NRAMP conserved domain PF01566, and they were named *MsNRAMP1* to *MsNRAMP17*.

The physicochemical properties of proteins were summarized in Table 1. These MsNramp proteins range from 237 mino acids to 1301 amino acids in length. The subcellular localization prediction results for all these genes indicate that they are located on the plasma membrane. However, *MsNRAMP8* and *MsNRAMP9* proteins have only 4 and 6 transmembrane domains, respectively, indicating that *MsNRAMP8* and *MsNRAMP9* may not possess the full range of functions characteristic of other *NRAMP* family proteins (Figure supplementary 1).

MsNRAMPs are distributed relatively evenly across the genome. Chromosome 2 contains four MsNRAMPs, while Chromosomes 1 and 3 each house three *MsNRAMPs*. Additionally, Chromosomes 5, 6, 7, 9, 14, 15, and 18 each possess a single *MsNRAMP* (Figure 1).

Table 1. Characteristics of the identifed *MsNramp* genes.

Name	ID	Protein length(aa)	PI	MW	Transmembrane domains
MsNRAMP1	Misin01G114500	1301	6.97	1.42E+05	11
MsNRAMP2	Misin01G114600	539	6.08	5.86E+04	10
MsNRAMP3	Misin01G442700	517	6.89	5.63E+04	10
MsNRAMP4	Misin02G103100	470	7.13	5.16E+04	9
MsNRAMP5	Misin02G103200	410	7.02	4.48E+04	9
MsNRAMP6	Misin02G147400	547	6.74	6.00E+04	10
MsNRAMP7	Misin02G425400	517	6.16	5.64E+04	9
MsNRAMP8	Misin04G097000	237	6.12	2.59E+04	4
MsNRAMP9	Misin04G098700	316	5.61	3.40E+04	6
MsNRAMP10	Misin04G099000	508	8.28	5.50E+04	9
MsNRAMP11	Misin05G017900	1273	6.34	1.38E+05	11
MsNRAMP12	Misin06G008000	1278	6.26	1.39E+05	11
MsNRAMP13	Misin07G028400	527	8.30	5.73E+04	10
MsNRAMP14	Misin09G139400	573	7.33	6.20E+04	9
MsNRAMP15	Misin14G135600	547	4.80	5.90E+04	11
MsNRAMP16	Misin15G155500	601	5.55	6.52E+04	12
MsNRAMP17	Misin18G228300	548	8.31	5.93E+04	12

 doi:10.20944/preprints202501.1599.v1

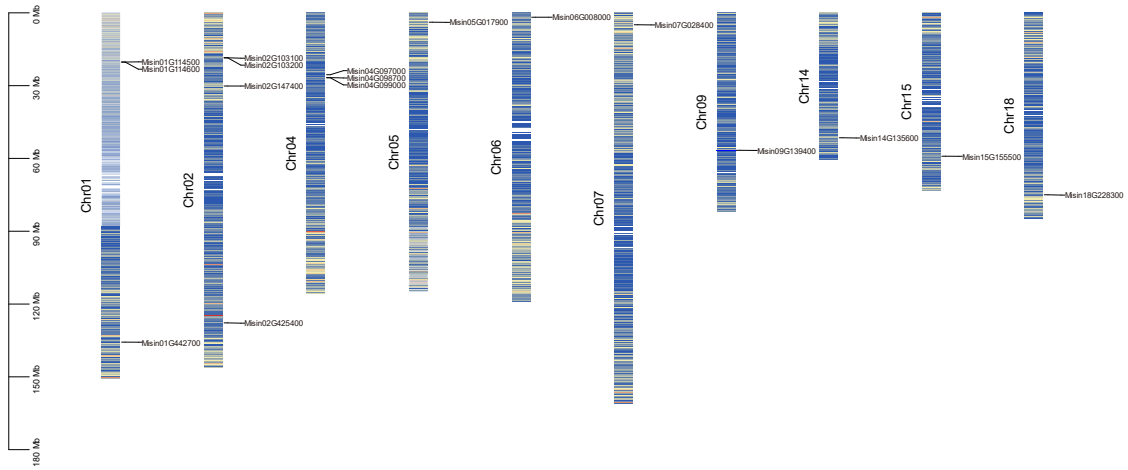


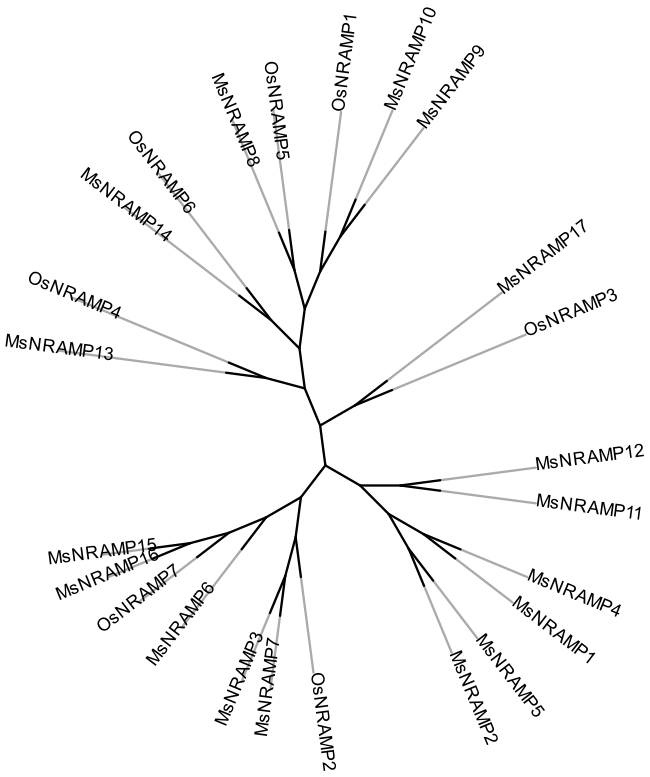
Figure 1. Genome location of the identifed *MsNramp* genes. Each box symbolizes an individual chromosome, with the lines within denoting the positions of genes that are capable of expression. The *MsNRAMPs* genes are distinctively highlighted by black lines.

2.2. Phylogenetic Analysis and Motif Analysis of MsNRAMP

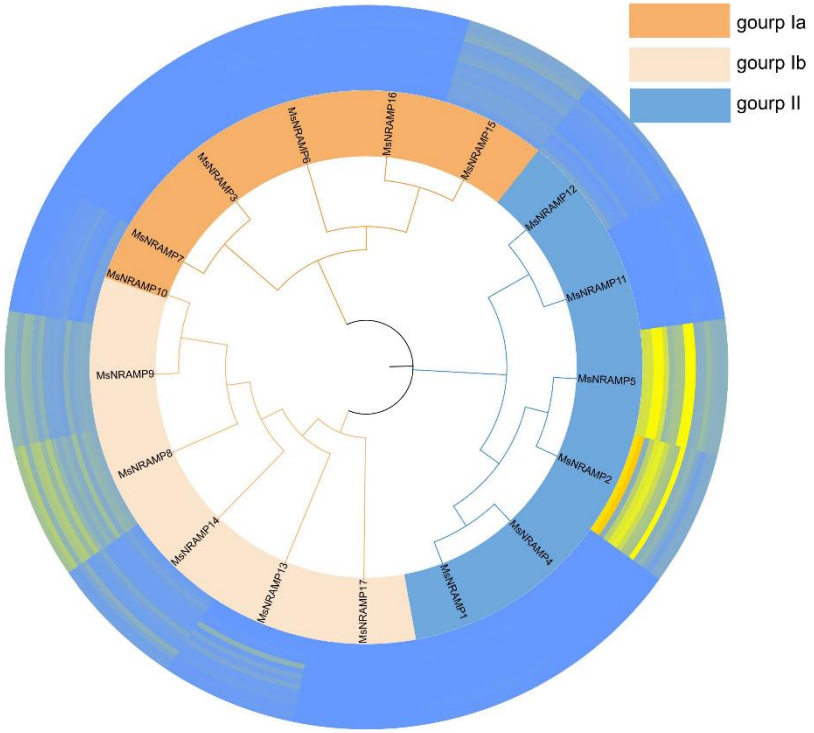
Miscanthus is closely related to sorghum and sugarcane [14]. However, given the extensive research on the functions of NRAMP in rice species, we have chosen to construct an evolutionary tree that includes the model organism Oryza sativa and *Miscanthus*, as is shown in Figure 2a, to better understand their evolution together with their function. The full length of protein sequences of seven OsNRAMPs and seventeen MsNRAMPs were used to construct the phylogenetic tree (Figure 2b). OsNRAMPs are served as a reference for classification. 12 conserved motifs were identified by MEME across the different groups of MsNRAMPs is shown in Figure 3.

Based on the results of the phylogenetic tree, the MsNRAMP family is divided into two major groups, with Group I further subdivided into two subgroups, Ia and Ib. The motifs analysis indicates that, with the exception of the presumably functionally incomplete *MsNRAMP8* and *MsNRAMP9*: all *MsNRAMPs* share motifs 1 and 14. Excluding *MsNRAMP1*, all *MsNRAMPs* share motif 4, Group I share motif 15, and Group II shares motif 11. Proteins in Group Ia have identical motifs with a tandem structure of motifs 2, 1, 6, 13, 4, 14, 7, 3, 10, 5, and 15; Group Ib proteins typically have a tandem structure of motifs 1, 6, 4. Group II displays a sequentially tandem structure of motifs 9, 4, 8, 14, 10, and 5. Genes within the same group also have similar structural characteristics: Group Ia generally consists of four exons, while Group Ib typically contains a greater number of shorter, dispersed exons. Motif 3 includes the binding site for ions on NRAMP [5].

 doi:10.20944/preprints202501.1599.v1



(**a**)



(**b**)

Figure 2. Phylogenetic tree of NRAMP members in *Miscanthus sinensis* and *Oryza sativa*. Figure a depicts the amino acid sequence homology between the *OsNRAMPs* and the *MsNRAMPs* through an unrooted phylogenetic tree. Figure b depicts the phylogenetic tree of *MsNTAMPs*, along with their expression levels in different tissues and at various time points.

 doi:10.20944/preprints202501.1599.v1

Figure 3. Figure displays the phylogenetic tree, corresponding motifs for each *MsNRAMP*, and the CDS (coding sequence) structures progressing from left to right.

2.3. Collinearity Analysis of NRAMP in Miscanthus and Closely Related Poaceae Species

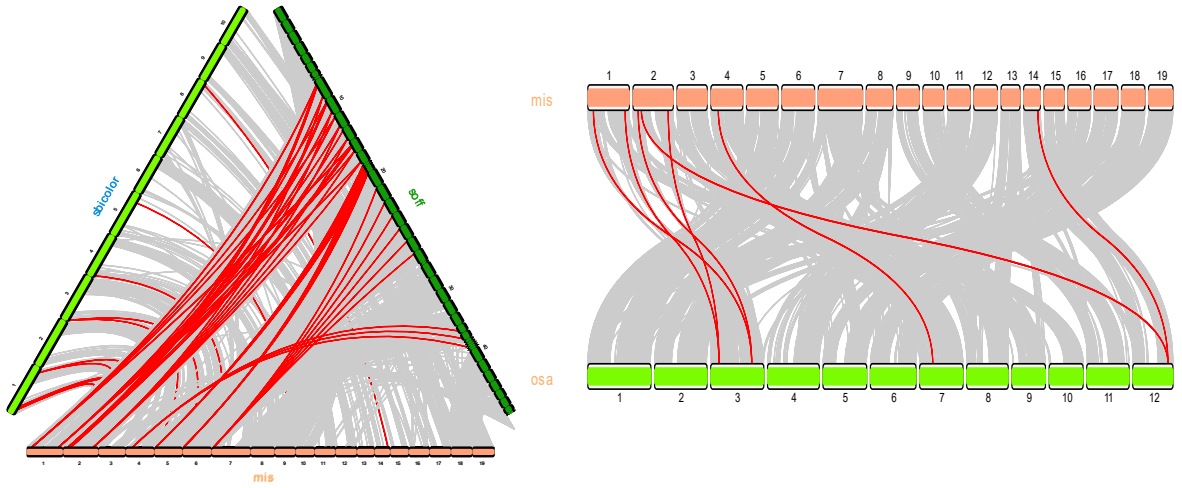
The process of divergence between *Miscanthus sinensis*, *Saccharum officinarum* and *Sorghum bicolor* is a compelling topic in the study of plant evolution. According to existed research, the ancestral species of *Miscanthus sinensis* and *Saccharinae* diverged from the ancestral species of Sorghum bicolor between 6.1 and 5.5 million years ago [13]. This process involved genome duplication and chromosomal rearrangements. To explore the evolutionary process of the NRAMP family genes within this context, collinearity analysis was performed.

As shown in Figure 4a, the identification of 12 collinear gene pairs between *Miscanthus sinensis* and *Sorghum bicolor*. A limited number of gene duplications have been observed transitioning from *Sorghum bicolor* to *Miscanthus sinensis*, suggests that *MsNRAMP1* and *MsNRAMP2*, as well as *MsNRAMP4* and *MsNRAMP5*, are the result of tandem duplication events. In contrast, 62 gene pairs exhibit collinearity between *Miscanthus sinensis* and *Saccharum officinarum*, with a substantial number of gene duplications occurring from *Miscanthus sinensis* to *Saccharum officinarum*. *MsNRAMP3* and *MsNRAMP7* are part of chromosomal segmental duplications, as indicated by their collinearity with a single homologous gene in *Saccharum officinarum*.

As shown in Figure 4c. Six pairs of *Oryza sativa* and *Miscanthus sinensis NRAMP* genes showed homology was summarized in Table 2. The Ka/Ks values of *MsNRAMP* are all less than 1, indicating that they are under purify selection during the evolutionary process, which implies that the *MsNRAMP* protein family plays a beneficial role in the adaptation of plants to their environment.

MsNRAMP3, *MsNRAMP6*, *MsNRAMP7*, and *OsNRAMP3* exhibit high homology. As *OsNRAMP3* has been reported as a regulatory gene in rice in response to manganese ions in the environment [14]. Induction of *OsNRAMP3* gene expression following chromium treatment in rice has been shown to alleviate cadmium toxicity [15].

 doi:10.20944/preprints202501.1599.v1



(**a**) (**b**)

Figure 4. Figure a shows the collinearity between the *NRAMP* family genes of *Miscanthus sinensis* and *Sorghum bicolor*, with 12 pairs of collinear segments and the collinearity between the NRAMP family genes of *Miscanthus sinensis* and *Saccharum officinarum*, with 62 pairs of collinear segments. Figure b depicts the collinearity between the *MsNRAMPs* and *OsNRAMPs*,with 7 pairs of collinear genes.

Table 2. Genomic collinear pairs of *NRAMPs* between *Miscanthus sinensis* and *Oryza sativa.*

Oryza sativa	Locus ID	Miscanthus sinensis	Locus ID	Ka/Ks
OsNRAMP7	Os12G0581600	MsNRAMP15	Misin14G135600	0.1124
-	Os03G0700800	MsNRAMP2	Misin01G114600	0.3037
OsNRAMP3	Os03G0208500	MsNRAMP3	Misin01G442700	0.1044
OsNRAMP3	Os03G0208500	MsNRAMP6	Misin02G147400	0.1625
OsNRAMP3	Os03G0208500	MsNRAMP7	Misin02G425400	0.1092
OsNRAMP7	Os12G0581600	MsNRAMP6	Misin02G147400	0.1329
OsNRAMP1	Os07G0258400	MsNRAMP10	Misin04G099000	0.1341
OsNRAMP7	Os12G0581600	MsNRAMP15	Misin14G135600	0.1124

2.3. Collinearity Analysis of NRAMP in Miscanthus and Closely Related Poaceae Species

MsNRAMPs proteins were compared with other species (*Arabidopsis thaliana*, *Escherichia coli*, *Deinococcus radiodurans*, *Eremococcus coleocola*), and it was found that their secondary structures are quite conserved. Divalent cation and proton binding sites are located at the junction of the first and sixth transmembrane domains (Figure Supplementary 1 and 2), contributing to the formation of a complete hydrophobic structure that effectively binds ligands. This indicates that the first and sixth transmembrane domains are located at the central to the transporter protein's architecture. It appears improbable that only four or six transmembrane domains can form a complete structure capable of executing the full biological process. Therefore, we assert that MsNRAMP8 and MsNRAMP9 cannot, or rather, cannot function independently as transporters.

Through sequence alignment, we identified that *Misin07G412700* and *Misin08G205700*, which are homologs of the core regulator of ethylene signaling in rice, *OsEIN2*, also contain ion-binding sites (Figure Supplemenary 3). However, it is unfortunate that their secondary structures are interrupted by a redundant sequence in the middle, and they lack distinct transmembrane domains. This observation suggests that NRAMP, as a membrane transporter protein, exhibits significant homology with transcription factors, indicating that *MsNRAMPs* may actively participate in regulatory pathways at the transcriptional level.

Divalent metal cations require six coordination bonds, protons can provide two coordination bonds, and the remaining four coordination bonds are provided by residues of NRAMP. In Figure 5a, we can see that the sites marked with purple asterisks are the metal cation binding sites reported

 doi:10.20944/preprints202501.1599.v1

in rice [16]. This figure illustrates the transmembrane domain 1 and 6 of NRAMP in rice species, which together forming an iron-binding site that symports metal ions with protons. Figure 5b, using MsNRAMP3 as an example, illustrates the binding situation of the ion site in *Miscanthus* species. Figures 5c and 5d illustrated the tertiary structure of MsNRAMP3. Demonstrating that it possesses a complete Leu-T domain, through three-dimensional structure simulation.

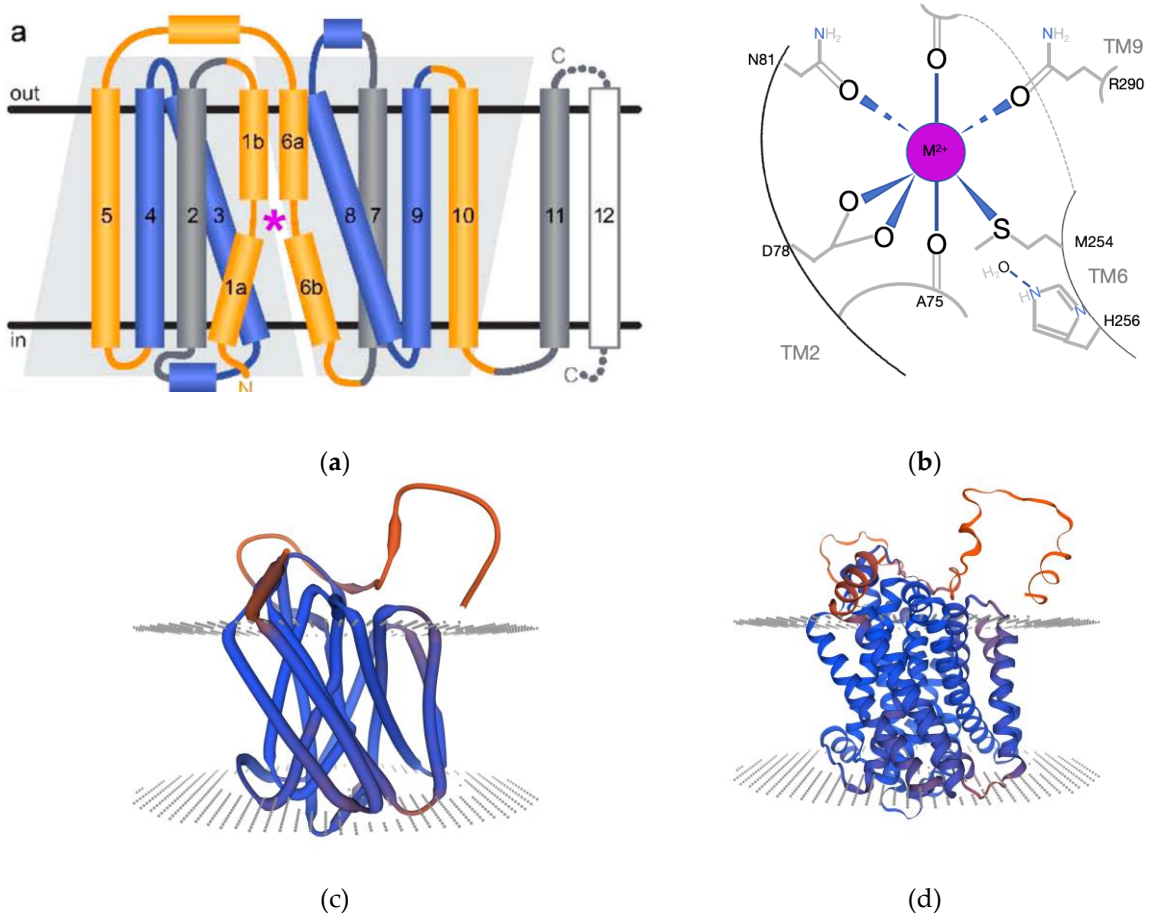


Figure 5. Structural and functional prediction of the identifed *MsNramp* genes. Figure a illustrates a schematic diagram of *NRAMPs* located on the plasma membrane. Figure b depicts the residues of *MsNRAMPs* that interact with metal ions. Figures c and d present three-dimensional structural models of MsNRAMP3. .

2.4. Cis-Acting Elements of MsNRAMPs and Transcriptomic Analysis

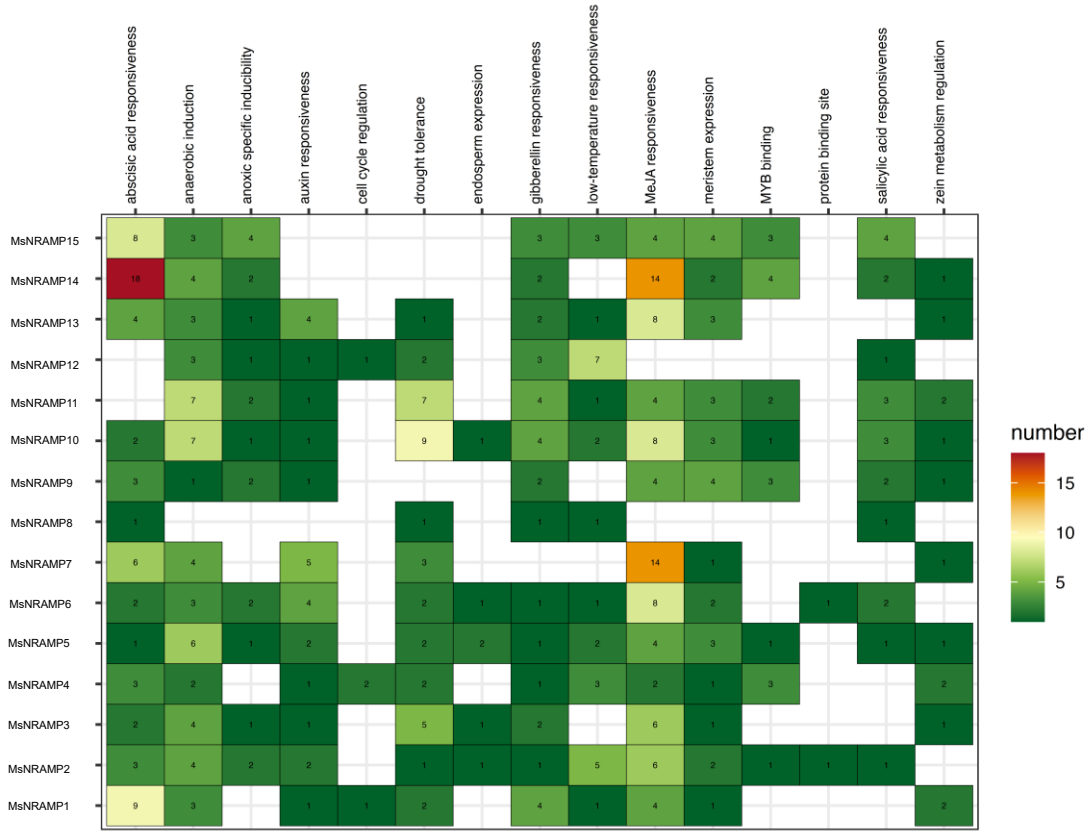
Utilizing PLANTCARE, we analyzed the promoter sequences of *MsNRAMPs*, specifically, focusing on counting the number of elements within these sequences that respond to external signals, as illustrated in Figure 6.

The raw transcriptome sequencing data was retrieved from the NCBI's Sequence Read Archive (SRA) database with the accession number PRJNA575573. The processed data is presented as a heatmap in Figure 7, which illustrates that among the many *MsNRAMPs*, only *MsNRAMP3* and *MsNRAMP7* have relatively high expression levels. These expression levels are primarily localized in leaf and internode, with comparatively low levels observed in the rhizome.

14 out of the 15 *MsNRAMPs* have gibberellin response elements in their upstream promoters, while thirteen possess MeJA response elements. This finding suggests that *MsNRAMPs* may be regulated by stress signaling pathways. A recent study indicates that gibberellins (GA) can negatively regulate the expression of *OsNRAMP5*, subsequently reducing rice's tolerance to aluminum toxicity [17].

MsNRAMP14 and *MsNRAMP8* feature numerous ABA and MeJA response elements in their promoter regions, however, their transcripts are scarcely expressed, implying the potential for their induced expression.

 doi:10.20944/preprints202501.1599.v1



(**b**)

Figure 6. Number of cis-element in the promoter of *MsNRAMPs*, with red indicating high and green low.

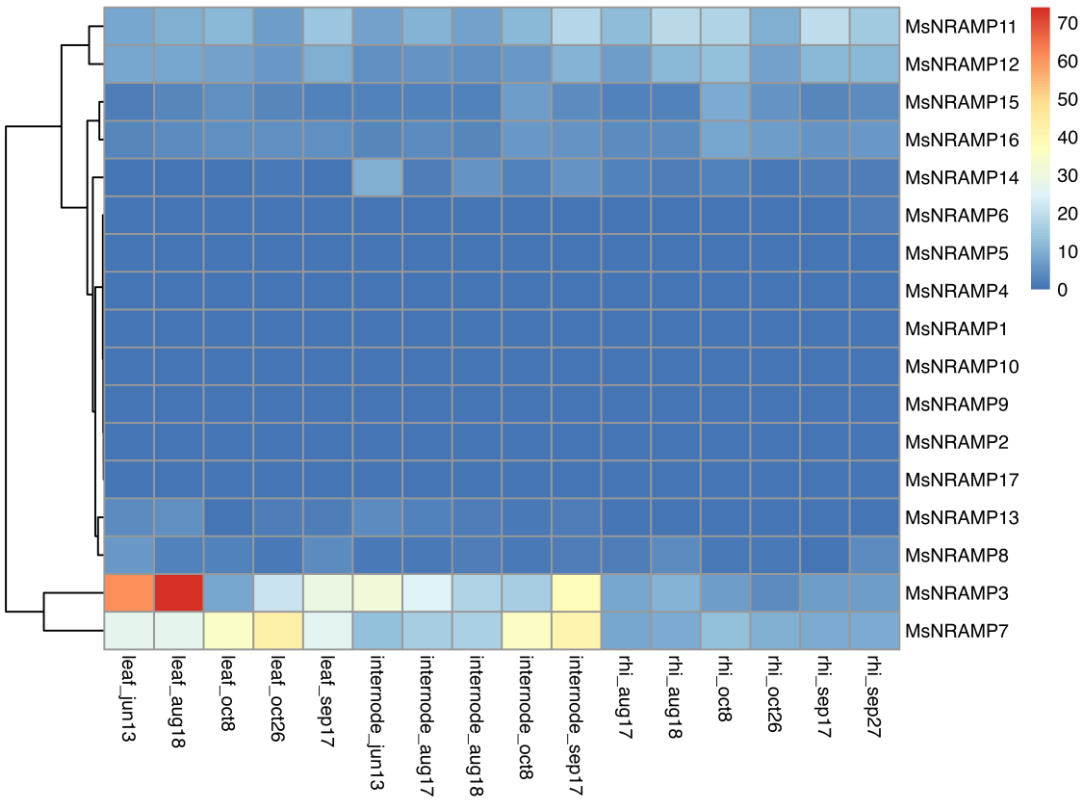


Figure 7. Expression of *MsNRAMPs* in diffenet tissues(leaf, internode and rhizome), with red indicating high and blue low.

 doi:10.20944/preprints202501.1599.v1

3. Discussion

3.1. From Transcription Factor EIN2 to NRAMP

OsEIN2 is the core regulatory factor in the ethylene (ET) defense signaling pathway of *Oryza sativa* [18]. Multiple sequence alignments reveal high homology between the NRAMP family in *Miscanthus sinensis* and the *OsEin2* homologous genes, with *OsEin2* containing an NRAMP domain. In *Arabidopsis*, salt stress downregulates the expression of *AtEin2* [19], and *Oryza sativa* overexpressing *OsEin2* exhibit increased sensitivity to salt stress [20]. Future research could explore whether *NRAMP* expression is upregulated under salt stress or heavy metal treatment and further investigate the potential negative feedback mechanism in the EIN2-NRAMP pathway. Additionally, many ABA (abscisic acid) and MeJA (methyl jasmonate) response elements have been identified upstream of the *MsNRAMP* promoter. In Arabidopsis, AtEin2 can respond to ABA [20], but there is no direct molecular evidence demonstrating the involvement of NRAMP transporters in the ABA pathway. In *Miscanthus*, NRAMP exhibits specialized expression, with only two of its many homologous genes expressed at normal levels, making it an excellent model for studying this pathway.

3.2. Transporter Function Inference Based on Expression Data

After being absorbed by plants, heavy metals such as cadmium and mercury tend to accumulate in the leaves through the process of transpiration, while lead preferentially deposits at the leaf base or in the roots. In rice, NRAMP5 is known to mediate the transport of the heavy metal cadmium. Both leaves and roots are sites of highly active metabolic processes [8]. However, it has been observed that MsNRAMP3 and MsNRAMP7 exhibit minimal expression in the roots, while their expression levels in the leaves are significantly higher than those in the rhizome. Based on these observations, it is reasonable to speculate that NRAMP transporters may be activated by signals or molecules with high mobility.

4. Materials and methods

4.1. Identification of NRAMP Family Genes and Protein Property Prediction

The genome and annotation data of *Miscanthus sinensis* were obtained from the Plant JGI database Phytozome v13. Putative *MsNRAMP* genes were identified based on NRAMP genes from *Oryza sativa* and *Arabidopsis thaliana*. The candidate *NRAMP* genes were identified using the BLASTP method, retaining those with an e-value of 10-5 and scores greater than 200. The Conserved Domain Database(CDD) was used for trimming the obtained protein sequences [21]. Meanwhile, the R packages Peptides was employed to caculate the physicochemical properties of the proteins, TMHMM v2.0 was used to predict transmembrane domains [22], CELLO v.2.5 was utilized for subcellular localization [23], and TBtools v.2110 was used for visualization [24].

4.2. Chromosome Location and Phylogenetic Analysis

Chromosome locations and coding sequences of candidate *MsNRAMP* genes were obtained from genomic annotation data. The visualization of *MsNRAMP* genes was accomplished using by TBtools v.2110.

Phylogenetic analysis was conducted using MEGA11 [25]. The method involved aligning the protein sequences of selected genes with Multiple Sequence Comparison(muscle), constructing a phylogenetic tree using the Neighbor-Joining model with 1000 bootstrap replicates, and visualizing the relationships by ITOL v2.

 doi:10.20944/preprints202501.1599.v1

4.3. Motif Analysis

The Multiple Em for Motif Elicitation v5.5.7 (MEME) was used to predict the motifs of the selected genes (https://memesuite.org/me-me/tools/meme; accessed on 6 November 2024) [26]. The number of motifs to be identified was set to 13. Subsequently, TBtools version 2.1.1 was employed for visualization.

4.4. Cis-acting Elements Analysis of MsNRAMP Genes

The 2000 bp upstream of the coding sequences (CDS) were extracted for promoter analysis. The sequences were submitted to the online website PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare; accessed on 6 November 2024) [27]. The results obtained were further filtered, and the R package ggplot2 was employed to plot and count the number of cis-acting elements.

4.5. Genome Collinearity Analysis

Genome and annotation data for Oryza sativa , Saccharum officinarum and Sorghum bicolor was obtained from the Plant JGI database Phytozome v13 (https://phytozome-next.jgi.doe.gov; accessed on 9 November 2024). The Software Diamond was utilized for aligning sequences between species, with an e-value threshold set at 10^{-5} [28]. Subsequently, the collinear gene pairs were calculated using the quick run MCScanX wrapper module in TBtools [29], and visualization was accomplished with the R package Rcircos.

4.6. Transcriptional Expression Analysis

The raw transcriptome sequencing files were obtained from NCBI's Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA575573; accession number: PRJNA575573). For the preprocessing of Miscanthus expression levels, we employed sequence alignment software HISAT2, sequence editing software SAMtools, and transcript quantification software Subread [30–32].

Finally, genes from different periods and tissues were visualized in a heatmap, with the data based on transcript FPKM values. (FPKM = 10^9 * C / (N * L), in which C represents the read count of the gene, N represents the total read count, and L represents the length of the gene.)

5. Conclusions

In this study, we identified 17 NRAMP family genes in *Miscanthus sinensis*, designated as *MsNRAMP1* to *MsNRAMP17*. Identification reveals that these genes are widely distributed across the *Miscanthus* genome and exhibit a high degree of conservation in their secondary structure and ion-binding sites, characteristic of the Leu-T domain.

The expression analysis indicated that only *MsNRAMP3* and *MsNRAMP7* are significantly expressed under normal conditions, suggesting potential functional specialization within the family. Nevertheless, the expression of all *OsNRAMPs* is reportedly inducible, as evidenced by both literature reviews and the analysis of standard rice transcriptome data [33–35].

Notably, *MsNRAMP8* and *MsNRAMP14*, which harbor numerous ABA and MeJA response elements in their promoters, may be involved in stress signaling pathways, highlighting the complexity of Miscanthus's response to environmental cues.

Phylogenetic analysis and motif prediction have allowed us to classify the *MsNRAMPs* into two major groups, with Group I showing closer homology to *OsNRAMPs* from *Oryza sativa*. This classification, along with the identification of conserved motifs, provides a foundation for further functional characterization of these genes. Collinearity analysis between *Miscanthus sinensis* and *Oryza sativa* revealed 7 homologous gene pairs of *NRAMPs*, reinforcing the evolutionary relationship between these species and the conservation of NRAMP function.

 doi:10.20944/preprints202501.1599.v1

The multiple sequence alignment of *MsNRAMPs* with those from other species highlights the conservation of their secondary structures, which is essential for their role in metal ion transport. The identification of key residues involved in metal cation and proton binding provides insights into the molecular basis of their transport activity. Our results on the potential roles of *MsNRAMP8* and *MsNRAMP9*, which lack the typical number of transmembrane domains, suggest that not all NRAMP family members may function independently as transporters.

In conclusion, this study provides a comprehensive characterization of the NRAMP family in *Miscanthus sinensis*, shedding light on their potential roles in metal ion homeostasis and stress response. The identified genes and their functional annotations lay the groundwork for future studies aimed at enhancing our understanding of *Miscanthus*'s adaptability to heavy metal stress and its implications for phytoremediation and bioenergy production.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Weixiong Long and Xiaojue Peng designed the study and wrote the manuscript, Wei Chen performed most of the experiments and helped write the manuscriptand wrote the manuscript, Jiawei Niu, Suping Ying and Jianzhao assisted in the data analysis.

Funding: This research was supported by grants from the National Natural Science Foundation of China (No. 32172074, 31760377), Key Projects of Jiangxi Natural Science Foundation (No. 20224ACB205005).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are available in this article and the Supplementary Materials.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ABA	Abscisic Acid
GA	Gibberellin
MeJA	Methyl Jasmonate
NRAMP	natural resistance associated macrophage protein

References

1. MiMitros T, Session A M, James B T, etal. Genome biology of the paleotetraploid perennial biomass crop Miscanthus [J]. *Nature Communication*, **2020**, 28(11):5442.
2. Lewandowski I, Scurlock J M O, Lindvall E, etal. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe [J]. *Biomass and Bioenergy*, **2003**, 25(1): 335-361.
3. Wanat N, Austruy A, Joussein E, etal. Potentials of Miscanthus giganteus grown on highly contaminated Technosols [J]. *Journal of Geochemical Exploration*, **2013**, 126:78-84.
4. Cellier M, Prive G, Belouchi A. Nramp defines a family of membrane proteins [J]. *Proceedings of the National Academy of Sciences of the United States of America*, **1995**, 92(22):10089-10093.
5. Bozzi A T, Gaudet R. Molecular mechanism of Nramp-family transition metal transport [J]. *Journal of Molecular Biology*, **2021**, 433(16):166-178.
6. Mackenzie, Bryan, Hediger M A. SLC11 family of H+-coupled metal-ion transporters NRAMP1 and DMT1 [J]. *European Journal of Physiology*, **2004**, 447(5):571-579.
7. Kanwal F, Riaz A, Ali S, etal. NRAMPs and manganese:Magic keys to reduce cadmium toxicity and accumulation in plants [J]. *Science of The Total Environment*, **2024**, 921:17105.
8. Chang J, Gao W, Wang P. OsNRAMP5 is a Major Transporter for Lead Uptake in Rice [J]. *Environmental science & Technology*, **2022**, 56(23):17481-17490.

9. Zhang W, Guan M, Chen M, etal. Mutation of OsNRAMP5 reduces cadmium xylem and phloem transport in rice plants and its physiological mechanism [J]. *Environmental Pollution*, **2023**, 341:122928.
10. Ramanadane K, Márton Liziczai, Markovic D, etal. Structural and functional properties of a plant NRAMP-related aluminum transporter [J]. *Proceedings of the National Academy of Sciences of the United States of America*, **2021**, 107(20), 9281-9286.
11. Sun L, Wei Y, Wu K, etal. Restriction of iron loading into developing seeds by a YABBY transcription factor safeguards successful reproduction in Arabidopsis. [J]. *Molecular Plant*, **2021**, 14(10):1624-1639.
12. Fu D, Zhang Z, Wallrad L, etal. Ca2+-dependent phosphorylation of NRAMP1 by CPK21 and CPK23 facilitates manganese uptake and homeostasis in Arabidopsis. [J]. *Proceedings of the National Academy of Sciences of the United States of America*, **2022**, 4(119):e2204574119.
13. Zhang G, Ge C, Xu P, etal. The reference genome of Miscanthus floridulus illuminates the evolution of Saccharinae [J]. *Nature Plants*, **2021**, 7(5):608-618.
14. Yamaji N, Sasaki A, Ji Xing Xia;Kengo Yokosho, et al. A node-based switch for preferential distribution of manganese in rice [J]. *Nature Communications*, **2013**, 4(1):2442-2451.
15. Sun X, Wang J, Zhang M. Combined application of biochar and sulfur alleviates cadmium toxicity in rice by affecting root gene expression and iron plaque accumulation [J]. *Ecotoxicology and Environmental Safety*, **2023**, 259(1).
16. Ehrnstorfer I, Geertsma E, Pardon E. Crystal structure of a SLC11 (NRAMP) transporter reveals the basis for transition-metal ion transport [J]. *Nature Structural & Molecular Biology*, **2014**, 20:990.
17. Lu L, Chen X, Tan Q, etal. Gibberellin-Mediated Sensitivity of Rice Roots to Aluminum Stress [J]. *Plants*, **2024**, 13:543.
18. Qiu J, Liu Z, Xie J, etal. Dual impact of ambient humidity on the virulence of Magnaporthe oryzae and basal resistance in rice[J]. *Plant, Cell & Environment*, **2022**, 45(12):3399-3411.
19. Wang Y, Liu C, Li K, etal. Arabidopsis EIN2 modulates stress response through abscisic acid response pathway[J]. *Plant Molecular Biology*, **2007**, (6):633-644.
20. Yang C, Ma B, He S, etal. MAOHUZI6/ETHYLENE INSENSITIVE3-LIKE1 and ETHYLENE INSENSITIVE3-LIKE2 Regulate Ethylene Response of Roots and Coleoptiles and Negatively Affect Salt Tolerance in Rice.[J]. *Plant Physiology*, **2015**, 169(1):148-165.
21. Yang M, Derbyshire M K, Yamashita R A, Marchler-Bauer A. NCBI's Conserved Domain Database and Tools for Protein Domain Analysis [J]. *Current Protocols in Bioinformatics*. **2020**, 69:90-98.
22. Krogh A, Larsson B, Heijne G. Predicting Transmembrane Protein Topology with a Hidden Markov Model: Application to Complete Genomes [J]. *Journal of Molecular Biology*, **2001**, 305:567-580.
23. Yu C, Lin C, Hwang J. Predicting subcellular localization of proteins for Gram-negative bacteria by support vector machines based on n-peptide compositions [J]. *Protein Science*, **2004**, 13:1402-1406.
24. Osorio D, Rondon-Villarreal P, Torres R. Peptides:A package for data mining of antimicrobial peptides [J]. *The R Journal*, **2015**, 7(1):4–14.
25. Tamura K, Stecher G, & Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, **2021**, 38(7), 3022-3027.
26. Bailey T, Boden M, Buske F, etal. MEME SUITE: tools for motif discovery and searching [J]. *Nucleic Acids Research*, **2009**, 37:202-208.
27. Lescot M, Déhais P, Thijs G, etal. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences [J]. *Nucleic Acids Research*, **2002**, 30(1):325-332.
28. Buchfink, Benjamin, Reuter K, etal. Sensitive protein alignments at tree-of-life scale using diamond [J]. *Nature Methods*, **2021**, 18(4):366-368.
29. Wang Y, Tang H, Debarry J D, etal. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity [J]. *Nucleic Acids Research*, **2012**, 40(7):147-149.
30. Buchfink B, Reuter K, Drost H. Sensitive protein alignments at tree-of-life scale using DIAMOND [J]. *NatureMethod*, **2021**, 18(4):366-368.
31. Kim D, Paggie J, Park C, etal. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. [J]. *Nature Biotechnology*, **2019**, 37(8):907-915.

 doi:10.20944/preprints202501.1599.v1

32. Liao Y, Smyth G, Shi W. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote [J]. *Nucleic Acids Research*, **2013**, 1(41):10-18.
33. Chu C, Huang R, Liu L, etal. The rice heavy-metal transporter OsNRAMP1 regulates disease resistance by modulating ROS homoeostasis [J]. *Plant, Cell & Environment*, **2022**, 45(4):1109-1126.
34. Li Y, Li J, Yu Y, etal. The tonoplast-localized transporter OsNRAMP2 is involved in iron homeostasis and affects seed germination in rice [J]. *Journal of Experimental Botany*, **2021**, 72(13): 4839-4852
35. Sasaki A, Yamaji N, Yokosho K, etal. Nramp5 is a Major Transporter Responsible for Manganese and Cadmium Uptake in Rice [J]. *The Plant Cell*, **2012**, 24(5):2155-2167.