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Genome wide analysis and identification of NRAMP gene family in wheat (*Triticum aestivum* L.)

Anuj Kumar, G Singh, BK Prasad, PK Sharma and Rajendera Kumar

Abstract
Bread wheat is one of the most important world food crops. Globally, wheat production was 772.64 million metric tons in 2022-21. The availability of the complete genome sequence is allowed the identification and analysis of gene family at the genome level in wheat. The natural resistance-associated macrophage protein (NRAMP) complement is a highly conserved essential membrane protein family involved in iron transport. Iron is an essential nutrient for virtually all organisms. The NRAMP proteins are widely involved in the wheat metabolic network and that they help in divalent metal ion transporter pathways in roots and shoots. This study systematically identified and characterized the wheat NRAMP gene family using three approaches, i.e. HMM (Hidden-Markov Model), Pfam (http://pfam.sanger.ac.uk) and BLASTP were used at the whole genome level. A total of 29 wheat *pTaNRAMP* genes, were identified, and they were unevenly distributed on wheat chromosomes. The conserved motif, subcellular localization and phylogenetic relationship analysis further supported the classification of *pTaNRAMP* gene in wheat. A total of 29 putative NRAMP encoding genes were divided into four clades based on phylogenetic tree analysis with *Arabidopsis* and rice genomes. The study of conserved motif analysis represents that the same clade genes have conserved motifs. Additionally, the chromosomal distribution pattern revealed that most of the NRAMP genes were distributed across cell membrane and other in chloroplast, mitochondria and nucleus. Further, detailed analysis was conducted to examine the functional role of these genes in iron transport this will enable us to exploit these genes as molecular tools for biofortification in wheat.

Keywords: NRAMP, wheat, gene family zinc, iron

1. Introduction
Common wheat (*Triticum aestivum* L. AABBDD genome, 2n = 6x = 42) is one of the most important cereal crops. It is grown in diversified environments and area. It is a staple food of millions of people. Approximately one-sixth of the total arable land in the world is cultivated with wheat. Whereas paddy is mainly cultivated in Asia, wheat is grown in all the continents of the world. It supplies about 20 per cent of the food calories for the world's growing population. Global wheat production touched 772.64 million metric tons in 2022-21(https://en.wikipedia.org/wiki/Wheat). India is the second largest producer of wheat after China. Wheat has a distinct place among the food grain crops. Carbohydrate and protein are two main constituents of wheat. On an average wheat contains 11-12% protein. Plants prerequisite metal transporters to fulfill many important functions such as metal absorption to metal sequestration. The plant roots signify the main site for the primary uptake of metals from the soils to the food chain. Therefore, the uptake and trafficking of beneficial as well as toxic heavy metals by plants determines to a large extent the quality of food. In addition to metal absorption, plants also need to be able to transport transition metals to the growing organs and to the cell compartments where they are necessary. A fine control of metal concentrations is required in chloroplasts in photosynthetic tissues, where metals play essential roles in photosynthesis but can cause serious oxidative damage. In some cases, plants also have to deal with toxic heavy metals such as cadmium, lead and mercury or toxic excess of essential metals. In this case, transporters can function either in excluding metals at the root or sequestering metals in some cell compartments such as the vacuole. The natural resistance-associated macrophage protein (NRAMP) family is a novel gene family of functional related proteins defined by a conserved hydrophobic core of ten transmembrane domains. This family of membrane proteins are has divalent cation. These NRAMPs are the integral membrane trans-porters in plants responsible for allocation of essential metals such as manganese (Mn) or iron (Fe) and non-essential metals like cadmium (Cd) or lead (Pb) (Thomine *et al*.2000) [12].

The function of metal transporters of plant NRAMP homologs has been demonstrated by expression in *Saccharomyces cerevisiae*, however their roles in metal homeostasis *in planta* have not been fully elucidated yet. The function of *AtNRAMP*1 and 3 has been investigated by the analysis of plants in which *NRAMP* genes have been disrupted or overexpressed. Curie and collaborators have constructed *Arabidopsis* lines that overexpress. Plants do not display any obvious phenotype when they are grown with sufficient iron (50 μM). However, when they are grown on high toxic concentrations of iron (0.3-0.8 mM), they appear to be significantly more resistant to iron toxicity than control plants. The same authors have also isolated plants carrying an insertion of the T-DNA from *Agrobacterium tumefaciens* in the *AtNRAMP1* gene. In agreement with the phenotype of the CaMV35S-*AtNRAMP1Arabidopsis*, the mutant plants homozygous for the disrupted *AtNRAMP*1 allele are hypersensitive to toxic iron concentrations. Taken together, these results point to a function of *AtNRAMP1* in iron homeostasis in *Arabidopsis*. This function is likely mediated by the iron transport function of *AtNRAMP*1 demonstrated by complementation of the yeast iron uptake mutant, *fet3fet4*.

The first known NRAMP protein (NRAMP1) was identified in rat's phagosomal membranes, and was found to function in natural defense against infections by intracellular parasites (Vidal *et al*., 1995) [13]. These genes are widely distributed in all plant families, acting mainly in divalent cation transport. Iron (Fe) & zinc (Zn) are essential micronutrients required in a number of biological processes in plant species. The study was undertaken to identify wheat homologs for the genes/alleles of NRAMP, responsible for divalent cation transport. Plants include diverse groups like legumes, cereals; dicots (*A. thaliana, G.max*) and monocots (*T. aestivum, Z. mays* and *O.sativa*), species. Members of the widely distributed natural resistance-associated macrophage protein (NRAMP) family of cation transporters have also been characterized in *Arabidopsis* and rice (Belouchi *et al*. 1997; Thomine *et al*. 2000) [1]. In Arabidopsis, only six members from NRAMP1 toNRAMP6 were identified, which are classified into two subfamilies based on phylogenetic analysis. *AtNRAMP*1 and *AtNRAMP*6 form the first group,while*AtNRAMP*2 to *AtNRAMP*5 fall in the second group (Maser *et al*., 2001) [8]. *AtNRAMP*1 is located in root plasma-membraneand acts as a high affinity transporter for Mn uptake in roots (Cailliatte *et al*., 2010) [4]. As a homolog of *AtNRAMP*1, *AtNRAMP*6 shows no Mn or Fe trans-port activities, but is involved in cellular Cd distribution and contributes to Cd toxicity (Cailliatte *et al*., 2009). [3] *AtNRAMP*1 protein may be related to iron subcellular transport and its targeting to storage compartments such as vacuoles or plastids (Curie *et al*. 2000) [5]. In rice seven NRAMP members have been so far identified, which contribute to uptake and translocation of metals such as Mn, Cd and Fe (Xia *et al*., 2010; Takahashi *et al*., 2011; Yamaji *etal*., 2013; Ishimaru *et al*., 2012; Ishikawa *et al*.,2012; Sasaki *et al*.,2012) [14, 7, 6, 11, 9, 16]. For example, *OsNRAMP5* was identified as a major transporter for Cd uptake in the real soil Cd contamination (Ishikawa *et al*., 2012; Sasaki *et al*., 2012) [6, 9]. In recent years, several *NRAMP* genes have been identified in legumes. For example, a peanut *NRAMP* gene, *AhNRAMP1*, is significantly induced by Fe deficiency in roots and leaves, and heterologous expression of *AhNRAMP1* in tobacco leads to Fe accumulation in young leaves and tolerance to Fe deprivation (Xiong *et al*., 2012) [15]. *NRAMPs* involve in cellular metal ions homeostasis and metal ion transports. These are found in membrane and the function is metal ions transporter activity. *NRAMPs* genes are expressed in both in root and shoots. Among the genes known to promote Fe export from the vacuole to the cytoplasm, eight NRAMP genes were recently described in wheat (Borrill *et al*., 2014) [2]. Five of these genes showed very low levels of expression in flag leaves during the time points included in our study, suggesting that they may play more important roles during other developmental stages or in other tissues. Of the three NRAMP genes with higher expression levels during senescence, *TaNRAMP3* and *TaNRAMP7* both exhibited stable expression, but *TaNRAMP2* was significantly upregulated. In this study, the NRAMP genes throughout the wheat genome were identified, classified. Subsequently, the gene structure, protein motifs, motif logos and subcellular localization pattern of these NRAMP genes were analyzed.

2.Material and Method

2.1 Identification of *NRAMP* gene in wheat genome

In order to identify the *NRAMP* genes in wheat genome(http://plants.ensembl.org/Triticum_aestivum/)using three approaches, i.e. HMM (Hidden-Markov Model), Pfam (http://pfam.sanger.ac.uk)and BLASTP. The Hidden-Markov Model (HMM) profile for NRAMP was created using HMMER 3.1 (http://hmmer.janelia.org/) software packages and search against the *T. aestivum* genome with parameter e-value<=1e-5 was done. These identified sequences were verified through the Pfam (http://pfam.sanger.ac.uk) database and the sequences having NRAMP domain were selected. The sequence retrieved using BLAST program in BioEdit7.0.2.with e-value ≥1e-20 and Identity ≥95% against *T. aestivum, Z. mays, B. distachyon, O. sativa, T. urartu* and *H. vulgare* to get the putative NRAMP protein sequences in wheat genome. As some of the NRAMPs genes were previously identified and named (Borrill P *et al*., 2014) [2], and we here used *pTaNRAMP* (Putative *T. aestivum* NRAMP) refer to the previous study in *Arabidopsis* and rice.

2.2 Chromosomal distribution and subcellular localization of NRAMP gene in wheat genome

To explore the subcellular localization of the *pTaNRAMP* genes, putative subcellular localizations were identified by ExPASy(https://www.expasy.org/) analysis.The protein sequences of wheat were downloaded from Ensembl Plants (ftp://ftp.ensemblgenomes.org/pub/plants/release31/fasta/triticum_aestivum/). All the identified *NRAMP* genes were BLAST (E-value _ 1 e-20, percent identity _ 95%). Twenty-nine *pTaNRAMP* genes were identified as members of the wheat NRAMP gene family. Multiple sequence alignment was conducted for the predicted proteins. Based on these findings, the chromosomal location, chromosomal distribution, molecular weights, isoelectric points and locations of *pTaNRAMP* genes was determined.

2.3 Prediction of orthologs and paralogs of wheat *NRAMPs* gene

In this study, orthologs are genes that are related by vertical descent from a common ancestor and encode proteins with the same function in different species, while the paralogs were defined as two or more different genes in the same species which are so similar in their sequences that they are assumed to have originated following the duplication of a single ancestral gene having significant hits of bit scores of >100, E-

values of >e−20, percent identity>95% and genome coverage > 80%. All Twenty-nine *pTaNRAMP* genes of *T. aestivum* separately BLAST searched against five crop plants using default parameters. All the hits meeting this criteria for each of the29*T.aestivum* chromosomes was counted and tabulated using Microsoft Excel. Genes from different species that grouped together within each tree were designated as orthologs. Based on these orthologs of NRAMP a collinear map of the *T. urartu O. sativa, H. vulgare, B. distachyon* and *Z. mays*was created using genome visualization tool CIRCOS (http://mkweb.bcgsc.ca/tableviewer/). Syntenic relationship was inferred NRAMP genes of five genomes by finding orthologs among them.

For determining orthologs, we performed all against-all BLAST search of the genes on one genome against the other. Only significant hits meeting the criteria of BLAST bit score>100, E-value1e-20 and 95% identity between gene sequences over at least 50% of the gene length were choosen for the analysis. If two significant BLAST hits match the above mentioned parameter and have bidirectional hits with each other, then they were considered as orthologs to each other and were counted as single orthologous pair. The BLAST search output was processed using BLAST Parser software (http://geneproject.altervista.org/) The number of orthologous pairs were detected, tabulated in excel worksheets and then represented in the form of figure circos (Figure 1)

2.4 Conserved motifs and Phylogenetic analysis of the *pTaNRAMP* genes in wheat genome

Identified wheat *pTaNRAMP* protein sequences were used to predict the motif pattern using the online software MEME 4.10.02(http://meme-suite.org/tools/meme). Motifs were predicted with the parameters such as optimum motif widths sets of 6–200 residues, number of repetitions and a maximum of 20 motifs. The *Arabidopsis* genomes contain a rather ancient diversity of NRAMP genes family. A total of already identified 6 and 7 *NRAMP* genes of *A. thaliana* and *O. sativa* respectively were selected for the purpose of classifying the types of *NRAMP* genes in wheat. The Phylogenetic tree of NRAMP gene pairs from *A. thaliana, O. sativa* and *T. aestivum* of *NRAMP* genes was constructed based on coding sequences in Clustal X (http://www.clustal.org/) using the neighbor-joining method with 1, 000 bootstrap replicates. The alignment result was used to find the best model test by MEGA and confirm by JMODEL test. The phylogenetic tree was visualized with the online software tool iTol (https://itol.embl.de/). Phylogenetic trees are used to infer the relationship among the identified NRAMP genes was also constructed by coding sequences using the neighbor-joining method. The genes that grouped together within the gene tree but were derived from different crop plants regarded as homeologous sequences of same *NRAMPs.*

3. Results and Discussion

3.1 Identification and classification of *pTaNRAMP* genes in Wheat genome

The availability of the complete genome sequence allowed the identification and analysis of gene family at the genome level in wheat. A database search for *NRAMP* homologous genes in plant species identifies a large number of genes. Overall the plant *NRAMP* proteins show high amino acid sequence conservation with NRAMP from other kingdoms. *NRAMP* gene family, as one of the plant-specific metal ion transporter gene families, for divalent metal ion transporter in root and shoots. We identified and characterized the wheat *pTaNRAMP* gene family based on a genome-wide search approach. In total, twenty-nine *pTaNRAMP* unique genes were identified by HMM analysis, Pfam search and BlastP search. We named them*pTaNRAMP*1 to*pTaNRAMP*29 according to the already identified Arabidopsis *NRAMP* proteins with highest sequence similarity and following the nomenclature system applied to *Arabidopsis*. Based on the classification scheme of *NRAMP* genes in *A. thaliana* and *O.Sativa* total 6 and 7 genes were identified respectively (Maser *et al*., 2001) [8]. (Table1). Furthermore, an un-rooted tree of the twenty-nine *pTaNRAMP* genes was constructed to determine their phylogenetic relationships.

Table 1: NRAMP gene family in wheat genome representing following characteristics: *pTaNRAMP* Name, sequence I.D, Isoelectric Point, Molecular Weight, Length and chromosomal location. Protein molecular weights and theoretical isoelectric point *pI* values were computed using ExPASy.

Name	Seq-ID	Length	PI	MW	Localization	Chromosome location
TaNRAMP1	TRIAE_CS42_5AS_TGACv1_394226_AA1279000.1	547	5.2	59126	Cell membrane	5AS
TaNRAMP2	TRIAE_CS42_5BS_TGACv1_423603_AA1380480.1	547	5.2	59078	Cell membrane	5BS
TaNRAMP3	TRIAE_CS42_4AL_TGACv1_290083_AA0981450.1	531	4.99	58077	Cell membrane	4AL
TaNRAMP4	TRIAE_CS42_4DS_TGACv1_361580_AA1170390.1	530	4.99	58022	Cell membrane	4DS
TaNRAMP5	TRIAE_CS42_4BS_TGACv1_328307_AA1085990.1	598	5.53	65191	Cell membrane	4BS
TaNRAMP6	TRIAE_CS42_4DL_TGACv1_344138_AA1144210.1	519	6.17	56699	Cell membrane	4DL
TaNRAMP7	TRIAE_CS42_4BL_TGACv1_320879_AA1050740.1	521	6.17	56916	Cell membrane	4BL
TaNRAMP8	TRIAE_CS42_4AS_TGACv1_307585_AA1021690.1	521	6.48	56824	Cell membrane	4AS
TaNRAMP9	TRIAE_CS42_5DS_TGACv1_456926_AA1479950.1	445	8.89	48738	Cell membrane	5DS
TaNRAMP10	TRIAE_CS42_7BL_TGACv1_576780_AA1854780.1	548	7.98	59600	Cell membrane	7BL
TaNRAMP11	TRIAE_CS42_7AL_TGACv1_556173_AA1757430.1	525	7.57	57026	Cell membrane	7AL
TaNRAMP12	TRIAE_CS42_7BL_TGACv1_577015_AA1862810.1	525	8.08	56742	Cell membrane	7BL
TaNRAMP13	TRIAE_CS42_4AS_TGACv1_306761_AA1013050.1	540	6.41	58514	Cell membrane	4AS
TaNRAMP14	TRIAE_CS42_4BL_TGACv1_322206_AA1069640.1	540	6.67	58428	Cell membrane	4BL
TaNRAMP15	TRIAE_CS42_3B_TGACv1_222100_AA0757350.1	546	7.02	48113	Cell membrane	3B
TaNRAMP16	TRIAE_CS42_5BL_TGACv1_404828_AA1312170.1	270	6.13	29316	Cell membrane	5BL
TaNRAMP17	TRIAE_CS42_7DL_TGACv1_605996_AA2008810.1	405	6.09	35150	Cell membrane	7DL
TaNRAMP18	TRIAE_CS42_6AS_TGACv1_487188_AA1568800.1	684	6.45	75348	Mitochondrian	6AS
TaNRAMP19	TRIAE_CS42_6BS_TGACv1_514283_AA1657800.1	404	8.9	43888	Cell membrane	6BS
TaNRAMP20	TRIAE_CS42_7AL_TGACv1_557364_AA1780410.1	300	9.99	33088	Cell membrane	7AL

TaNRAMP21	TRIAE_CS42_7DL_TGACv1_603581_AA1985930.1	309	9.34	33700	Cell membrane	7DL
TaNRAMP22	TRIAE_CS42_U_TGACv1_640782_AA2074070.1	337	8.27	36719	Cell membrane	U
TaNRAMP23	TRIAE_CS42_U_TGACv1_640782_AA2074000.1	163	5.27	17471	Chloroplast, Mitochondrion, Peroxisome.	U
TaNRAMP24	TRIAE_CS42_3AS_TGACv1_211973_AA0696160.1	196	5.11	21282	Cell membrane	3AS
TaNRAMP25	TRIAE_CS42_3AS_TGACv1_211973_AA0696140.1	440	8.87	48209	Chloroplast.	3AS
TaNRAMP26	TRIAE_CS42_4AL_TGACv1_289219_AA0967510.1	1042	8.53	113012	Chloroplast.	4AL
TaNRAMP27	TRIAE_CS42_4BL_TGACv1_321202_AA1057100.1	487	5.95	53071	Cell membrane	4BL
TaNRAMP28	TRIAE_CS42_4BS_TGACv1_329963_AA1105090.1	380	7.03	41475	Cell membrane	4BS
TaNRAMP29	TRIAE_CS42_4DS_TGACv1_362329_AA1178870.1	1023	6.98	111576	Chloroplast, nucleus	4DS

TaNRAMP=Tritivum aestivum NRAMP; MW=molecular weight; PI=isoelectric point of amino acids

3.3 Chromosomal distribution and subcellular locations of *NRAMP* gene family members in wheat

In plants, several *NRAMP* genes are up-regulated under Fe starvation, indicating a function in Fe nutrition. NRAMP proteins likely localize on intracellular membranes such as the plastid envelope and the vacuolar membrane,(Sebastien Thomine and Julian I Schroeder). The *pTaNRAMP* proteins ranged in length from 163 to 1042 amino acids, with molecular weights ranging from 17471 kDa to 75347.72 kDa and the isoelectric points ranged from 5.2 to 9.99. Subcellular localization analysis indicated that 25*pTaNRAMP*are localized in the cell membrane three in the chloroplast, whereas only one were found in the peroxisome, mitochondrion, chloroplast and nucleus (Table 1).

3.4 Identification of *pTaNRAMP* Orthologs and Paralogs

We identify orthologs and paralogs of all twenty-nine*p* *TaNRAMP* genes of *T. aestivum* separately BLAST searched was conducted against five crop plants i.e. *O. sativa, H. vulgare, B. distachyon, T. urartu* and *Z. mays* using default parameter of >100, E-values of > e−20, percent identity > 95% and genome coverage > 80%. All the hits meeting these criteria for each of the 29*T.aestivum* chromosomes were counted and tabulated using Microsoft Excel where 297, 583, 501, 600 and 343 orthologs of *pTaNRAMP* were identified in *B. distachyon, H. vulgare, O. sativa, Z. mays* and *T. Urartu* respectively. A total of 595 paralogs of *pTaNRAMP* are identified in wheat genome. Based on these orthologs of NRAMP a collinear map of the *T. urartu O. sativa, H. vulgare, B. distachyon* and *Z. mays* are created using genome visualization tool CIRCOS (http://mkweb.bcgsc.ca/tableviewer/).

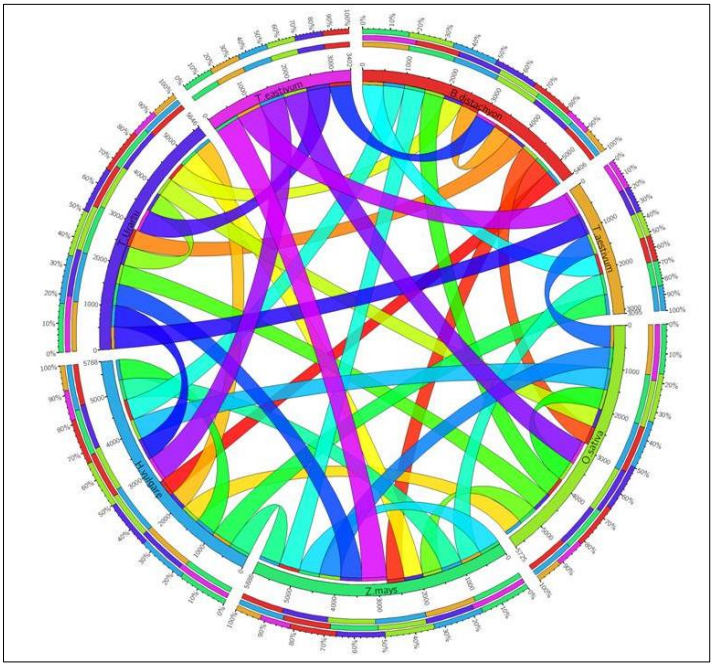

Fig 1: CIRCOS representing the orthologs and paralogs of all 29 NRAMPgenes of *T. Aestivum* separately, BLAST search was conducted against 5 crop plants i.e. *O. sativa, H. vulgare, B. distachyon, T. urartu* and *Z. mays* using default parameters of >100, E-values of > e−20, percent identity > 95% and genome coverage > 80%.

3.5 Motifs prediction and phylogenetic analysis

To understand the phylogenetic relationships of the NRAMP gene family, 29 NRAMP proteins, together with 5 and 7 publicly available Arabidopsis and rice NRAMP proteins, were selected for phylogenetic analysis with Neighbour-Joining method on the basis of multiple sequence alignment. The alignment result was used to find the best model test by MEGA and confirm by JMODEL test. The bootstrap values (0.5) for the nodes of the phylogenetic tree of NRAMP gene family were reconstructed with maximum likelihood and minimal Evolution. The trees produced by these methods mentioned above were almost identical with only minor differences at same

branches. Besides, the analysis of gene conserved motif structure were also used to confermed the validity of the phylogenetic tree.Motifs are the most distinctive features of the proteins. A total of 10 motifs containing 6–53 amino acid residues were identified. The phylogenetic tree was visualized

with the online software tool iTol(https://itol.embl.de/). The average lengths of the *NRAMP* genes varied among the different *NRAMP* types. The phylogenetic tree show that in the first clade *pTaNRAMP16*, *pTaNRAMP18* and *pTaNRAMP19*are closely related to *OsNRAMP4* and *OsNRAMP6* rice genes. The second clade are common there is no relation of other genes. In third clade the *pTaNRAMP1*, *pTaNRAMP2*, *pTaNRAMP3*, *pTaNRAMP4*, *pTaNRAMP5*, *pTaNRAMP6*, *pTaNRAMP7*, *pTaNRAMP8* and*pTaNRAMP9* are closely related to *AtNRAMP2*, *AtNRAMP3* and *AtNRAMP4Arabidopsis* genes and other is *OsNRAMP7* rice genes. In fourth clade *pTaNRAMP10*,*pTaNRAMP20* and *pTaNRAMP21* are closely related to *OsNRAMP3* and *AtNRAMP1* and *AtNRAMP5Arabidopsis* genes. In last clade *pTaNRAMP11*, *pTaNRAMP12*, *pTaNRAMP13*, *pTaNRAMP14* and *pTaNRAMP17* are related to *OsNRAMP1* and *OsNRAMP5* rice genes.As expected, the distribution in wheat was similar to those in *Arabidopsis* and rice. Closely related genes are generally more similar and the most obvious differences only exist in lengths. Unknown motifs of set of 29*pTaNRAMP*protein sequences between 163 and 1042 in length (average length 493.9) from the NRAMP wheat were also identified by MEME motif analysis (Figure 2) where motif width is between 6 wide and 50 (inclusive).The phylogenetic are shown in Figure 3.

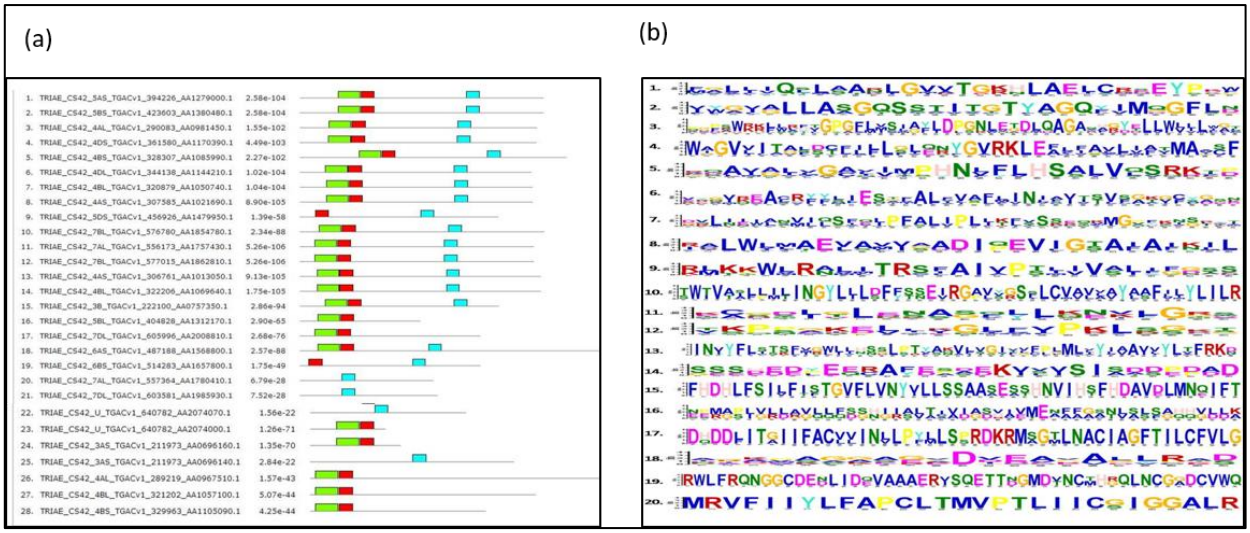

Fig 2: The (a) conserved protein motifsand (b) motif logos in the NRAMP family were identified using the online software MEME 4.10.02 tool. Motifswere predicted using the parameters such as optimum motif widths sets of 6–200 residues, number of repetitions and a maximum of 20 motifs.

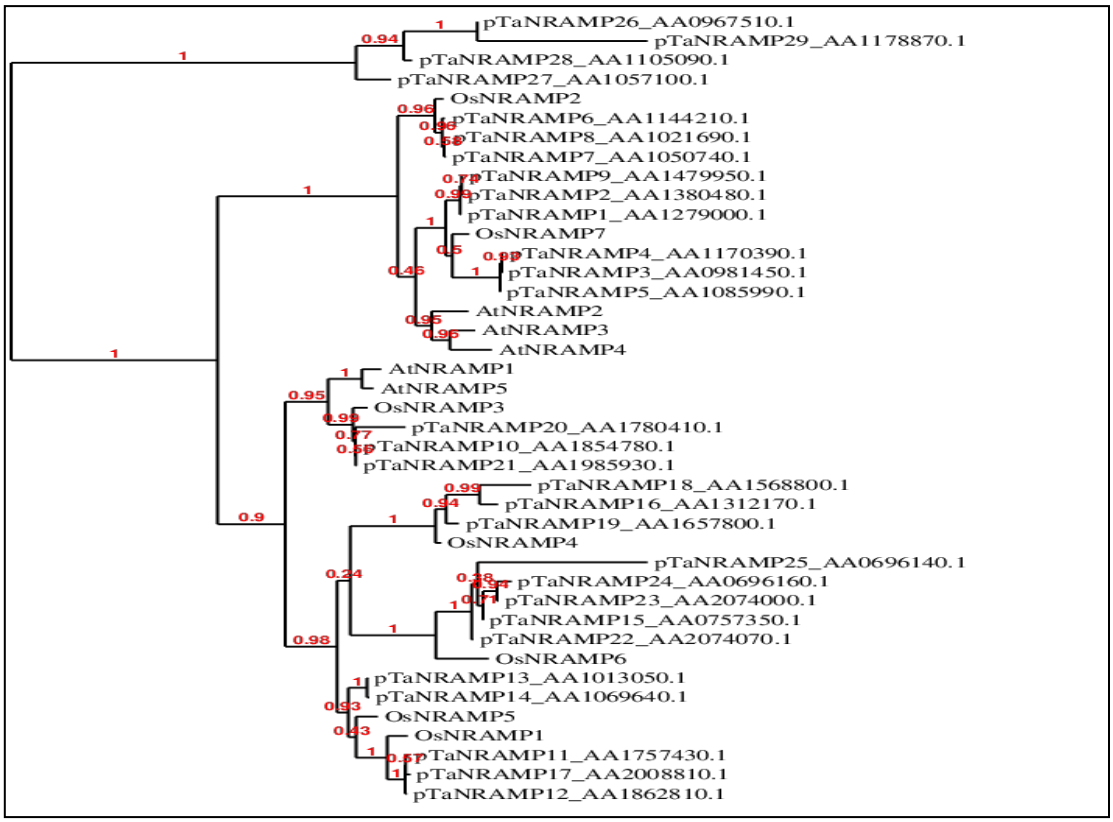

Fig 3: Phylogenetic relationships of NRAMP genes from *T. aestivum*, *A. thaliana* and *O. sativa*. The phylogenetic tree was constructed using ClustalX in MEGA5.0 by the neighbour joining method and 1000 bootstrap replicates.

4. Conclusions

This study systematically identified and characterized the wheat NRAMP gene family at the whole genome level. A total of 29 wheat *pTaNRAMP* genes, were identified, and they were unevenly distributed on wheat chromosomes. The conserved motif, subcellular localization and phylogenetic relationship analysis further supported the classification of *pTaNRAMP*gene in wheat. Our current knowledge suggests that plant *NRAMP* encode intracellular metal transporters with putative subcellular localization as diverse as the plastid envelope or the vacuolar membrane. In the future, it will be important to determine systematically the subcellular localization of all plant NRAMP proteins. With such localizations, plant NRAMP proteins are expected to play important functions in intracellular metal homeostasis. The NRAMP proteins are widely involved in the wheat metabolic network and that they help in divalent metal ion transporter pathways in roots and shoots. It is known to mediate transport of divalent metal ions, such as Fe and manganese (Mn) across cellular membranes. NRAMP proteins likely localize on intracellular membranes such as the plastid envelope and the vacuolar membrane. Over-expression or disruption of *NRAMP* genes in *Arabidopsis* leads to changes in Fe or Cd sensitivity.

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6. Author Disclosure Statement

No competing statement financial interests exits

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