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The Structural, Functional Identification of Natural Resistance-Associated Macrophage Protein as Transporter Elements in Oryza Sativa

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Abstract: The NRAMP (natural resistance-associated macrophage protein) family of transporter proteins has four members in *Oryza sativa*. OsNRAMP1, OsNRAMP2, OsNRAMP3, and OsNRAMP4 have been reported and considered for this study. These NRAMP transporter proteins have been studied in rice plants to transport a variety of metal ions such as Mn_2+, Cd_2+, Zn_2+, Fe_2+, and others. As a result, it's critical to predicting and properties of the OsNRAMP family of transporters computationally to study and understand them. In future research, it will be important to understand their biological insights. In this research, different *in-silico* methodologies, and strategies were used for the investigation of NRAMP-transporter proteins. The protein sequences' physiochemical parameters were evaluated, putative transmembrane domains and helices, localization and hydrophobicity, phylogenetic analysis, correlated motif patterns of the transporter proteins were identified, and proteins-interaction associates were predicted. The online structure prediction method was used to obtain 3D models of all OsNRAMP transporter participants, which were then analysed. Since there is currently limited information about the functional and structural dimensions of these transporters, this research will anticipate the theoretical details regarding us.

Key words: *Oryza sativa,* interaction analysis, structure prediction, OsNRAMP transporter proteins, physiochemical properties.

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1.Introduction

Transporters play a key role in metal absorption and sequestration in plants. Different transporters adjacent to cell membrane serving as barriers and channels in plants metal-ions take up. After a considerable examination of plant transporter elements with the help of heterologous mode of expression in protein, we realize NRAMP is one of the families of transporters functioning as divalent metal transporter from bacteria to humans. NRAMP share the noticeable plant's transport range of divalent cations in metals (iron, cadmium, magnesium, and zinc), and also the sequence identity between yeast, plant, fly, and mammals (Cellier et al., 1995; Migeon et al., 2010; Nelson, 1999).

Natural Resistance-Associated Macrophage Proteins (NRAMPs) symbolize evolutionarily screened basic metal-transporter membrane proteins family engaged in iron-passage crosswise multiple living things comprising bacteria, fungi, plants, and animals. NRAMP genes are extensively dispensed in wholly plant families, chiefly encounter as a Bivalent cation conveyer. Initially, NRAMP gene recognized in vertebrates was NRAMP1 gene that inscribes a macrophage membrane protein amenable for cation compression in the phagosome in succession governing the phagocyted bacterial regulation. Family of distinct great worth is NRAMP genes of the plant as they are liable for utilization of the alimentary integral bivalent cations Ferrous ion_Fe2+, Manganese_Mn2+, Zinc_Zn2+, moreover deadly metal Cadmium^{2+}_Cd2+ enduring unfamiliar purpose in plant outgrowth and progression interposing agricultural surroundings concluding distinctive origins for-instance atomic waste, arising out of sediments or from the utilization of rock-phosphate manures possessing remarkably elevated measures of Cd2+. Human's swallow-up plant- acquired Cd^{2+} from the dietary regime, or from smudging, which at one stage of life amassed in the body. Over the last few years there lapsed much holistic directorial compulsion to lessen human susceptibility that's why there is intense consciousness on such food constituent which are the principal dawn of cadmium in the edible food comprising porridge, breakfast-cereal, vegetables, nuts and pulses, root vegetable or Irish potato, and other products. Mineral alimentation influence crop productiveness consequently, it is a core component, cognate to plant growth and progression. Certain metal cations which are crucial for cellular approaches comprising copper, iron, zinc, cobalt, nickel, and manganese they are also constituents of transcription integrant and other proteins, operating as chief cofactors for various enzymes, in addition to they are vital for both mitochondrial and chloroplast activities (Pottier et al., 2015).

There are four NRAMP transporters in *Oryza sativa* (rice) that have been spotted. As rice is one of the enormously consumed foods crops in south Asian countries, but it is unproductive in many crucial nutrients and vitamins. Inadequacy in these micronutrients is common in flourishing countries. There are diverse NRAMP transporters comprising OsNRAMP1, OsNRAMP2, OsNRAMP3, OsNRAMP4 are functionally illustrated in rice. OsNRAMP1 engaged in cd^{2+} uptake. OsNRAMP4 does not exhibit the transport pursuit especially for divalent metal ions (Fe, Zn and, Mn) alternatively it served as trivalent Al ion transporter exhibiting inconsistency with other OsNRAMP members. OsNRAMP3 transports Mn regarding too diverse environmental swaps (Xia, Yamaji, Kasai, & Ma, 2010). It was proclaimed that NRAMPS are the extremely hydrophobic region of a membrane protein with 10 to 12 supposed TMDs with cytosolic-N_C terminals. Transport residues agreement lies on intracellular loop allying TMD-8 and 9. As transporters have an eminent role in rice plants but there is no efficient information and crystal structure accessible about transporters (Narayanan,

Vasconcelos, & Grusak, 2007; Thomine, Wang, Ward, Crawford, & Schroeder, 2000; Xia, Yamaji, & Ma, 2011).

By manipulating *in-silico* methods, we analysed thoroughly about NRAMP family of transporters within the rice. Subsequently, results are unveiled which reveals these transporters are proton synergetic metal ion transporter however, they are copiously adjacent in both prokaryotes and eukaryotes on the contrary still no accurate procedure of these proton synergetic transporters is well known and protein network analysis (Von Mering et al., 2005). We can point out the specific residues which contribute to metal tying and fetching by understanding their structure. Moreover, we can recognize the mechanism of transporter working analogous with the metal ions transfer in addition to can prognosticate transported metal ions along with transporters to whom substrate specificness still not been analyzed inside cultivated rice in virtue of additional determinations, involving stimulation, evolutionary process, certain biological assessments for mutants and gene knockout plants. Thus, our study is refocussing on the attributes of OsNRAMP family transporters their phylogenetic, physiochemical-expression pattern employing by the bioinformatics tools.

2.Material and Methods

2.1 Finding the sequences of NRAMP Transporter Protein

The NRAMP transporter protein sequences were retrieved from Rice Annotation Project Database through protein_IDs are OSNRAMP1 (Os07g0258400), OSNRAMP2 (Os03g0208500), OSNRAMP3 (Os06g0676000), OSNRAMP4 (Os02g0131800) (Sakai et al., 2013).

2.2 Physiochemical profiling of NRAMP Family Proteins

The OsNRAMP transporter family-protein physiochemical properties were investigated by Expasy-protparam tool (Gasteiger et al., 2005), protein_domains through PfamDatabase (Bateman et al., 2002), motif of proteins were analyzed through TOOL-MEME (Bailey et al., 2009), sub-cellular localization of protein through Server-CELLO (Yu et al., 2014), and the hydrophobicity or hydrophilicity measure through ExPASY-PROTSCALE (Gasteiger et al., 2005).

2.3 Prediction of transmembrane helices and phylogenetic analysis in NRAMP transporter proteins

The OsNRAMP transporter family protein transmembrane-helix of transporter protein were predicted from TMHMM-Server v. 2.0 (Atashgahi, 2019), and the evolutionary relationship of resultant sequences of OsNRAMP_transporters in rice a phylogenetic tree was constructed through MEGA-X tool by using Neighbour Joining, Maximum Likelihood methods, and the Multiple sequence alignment of OsNRAMP given Sequences in Rice was aligned by using Clustal-Omega (Sievers & Higgins, 2014).

2.4 Prediction of Protein association network analysis

The OsNRMP transporter protein associative network and functional analysis was generated through STRING Database (Szklarczyk et al., 2016). And the computationally theoretical-pl and molecular weight-Mw of OsNRAMP Transporter proteins was predicted through ExPASY-Compute pl/Mw (Ouvry-Patat et al., 2008).

2.5 3D structure prediction and structural visualization

The OsNRAMP Family of transporter proteins models by using given sequences was predicted through Swiss-Model (Schwede, Kopp, Guex, & Peitsch, 2003), the obtained targeted model-quality assessment by ProQ-Protein Quality prediction tool (https://proq.bioinfo.se/cgi-bin/ProQ/ProQ.cgi), the structural refinement and structural analyzed through UCSF-Chimera Tool (Pettersen et al., 2004), Discovery Studio Visualizer (Rizvi, Shakil, & Haneef, 2013), and also structural validation through Rampage Tool (Lovell et al., 2003), SAVES v6.0 Tool (https://saves.mbi.ucla.edu/).

3.Results and Discussion

The physicochemical properties of molecular insight of the OsNRAMP transporter family-protein with domain family of PF01566 with encode amino acid 518 to 550 (residues) their molecular weightage from 55.814-59.708kDA and pI value is 5.19 to 8.48. The NRAMP-Family proteins localisation through CELLO-Server with contains 10 to 12 TMDs via cytoplasmic_N-C regions and localized in plasma-membrane are shown in Table no 01.

Table 01: The characteristics of NRAMP Transporter proteins

Proteins IDs	Length (amino acid)	Molecular weight Kda	pI	TMD	GRAVY	Domains	CELLO-Localization
OsNRAMP 1	518	55.814	7.58	11	0.684	PF01566	Plasma membrane
OsNRAMP 2	524	56.928	6.17	10	0.564	PF01566	Plasma membrane
OsNRAMP 3	550	59.708	8.33	12	0.514	PF01566	Plasma membrane
OsNRAMP 4	545	59.244	7.00	12	0.558	PF01566	Plasma membrane

The hydropathy of obtained sequences of NRAMP-proteins through ExPASY-Protscale tool with the plotting of every amino-acid using graphical ways size in 19-amino acid by using principals of Algorithm of kyte-DOOLITTLE. With the solubility factors of NRAMP proteins membrane consensus motif of transport localization along via this query; GQSSTITGTYAGQY-V-MQGFLD-E/N present in OsNRAMP1,2, and 3. The top consensus sequences of NRAMP protein motifs were obtained by MEME Tool, with results are that's the motifs: 1-2-3, 4-29, 5-41 with 50 amino acids long in length of the objectives sequences of NRAMP other than 03 motifs are shown in Table no 02.

Table 02: The top conserved motifs of NRAMP transporter family through MEME Tool

Motif/Ranges	Site of identified	E-Values	Sequences
1/50	07	6.8-135	QSLSANLGVVTGRBLAELCKTEYPVWVKTCLWLLAE LAVIASDIPEVIGT
2/50	07	8.5-119	PAWKRFLSHIGPGFMVCLAYLDPGNMETDLQAGANB KYELLWVILIGLIF
3/50	06	2.6-110	SGQSSTITGTYAGQYVMQGFLDIKMKQWLRNLMTRS IAIVPSLIVSIIGG

4/29	07	4.3-71	GALVMPBNLFLHSALVLSRNTPASAKGMK
5/41	07	1.7-71	PVWTGVLIAGSSTLLLLGLQRYGVRKLEVVVALLVFV MAGC

These present most consensus sequences of typically ranging-residues in structural transporter protein of NRAMP Family with indicates: smf_1,2 and 3 in *C. elegans* and, with motif-4 with the functions of metal-divalent cations mechanism of *pseudomonas aeruginosa* and *C. actobutylicium* in apart of transporter protein NRAMP from rice. The family of NRAMP transporter proteins inside transmembrane helices regions of obtained targeted sequences; OSNRAMP1-Os07g0258400, OSNRAMP2-Os03g0208500, OSNRAMP3-Os06g0676000, and OSNRAMP4-Os02g0131800 using TMHMM-server to predicted the inner protein part of helices with OsNramp-1,2,3,4-Integral membrane protein to metal ionic transporter on irgsp1locus, and chromosomal positions no. 02, 07, 03, 06 on -, -, -, + strands of DNA. Also, the targeted transporter proteins water solubility through innovagien-peptide calculator and the obtained results are OSNRMAP1,2,3,4 with (1.3, -2, 3.3 and 0.1) (Lear & Cobb, 2016) are shown in figure no 01.

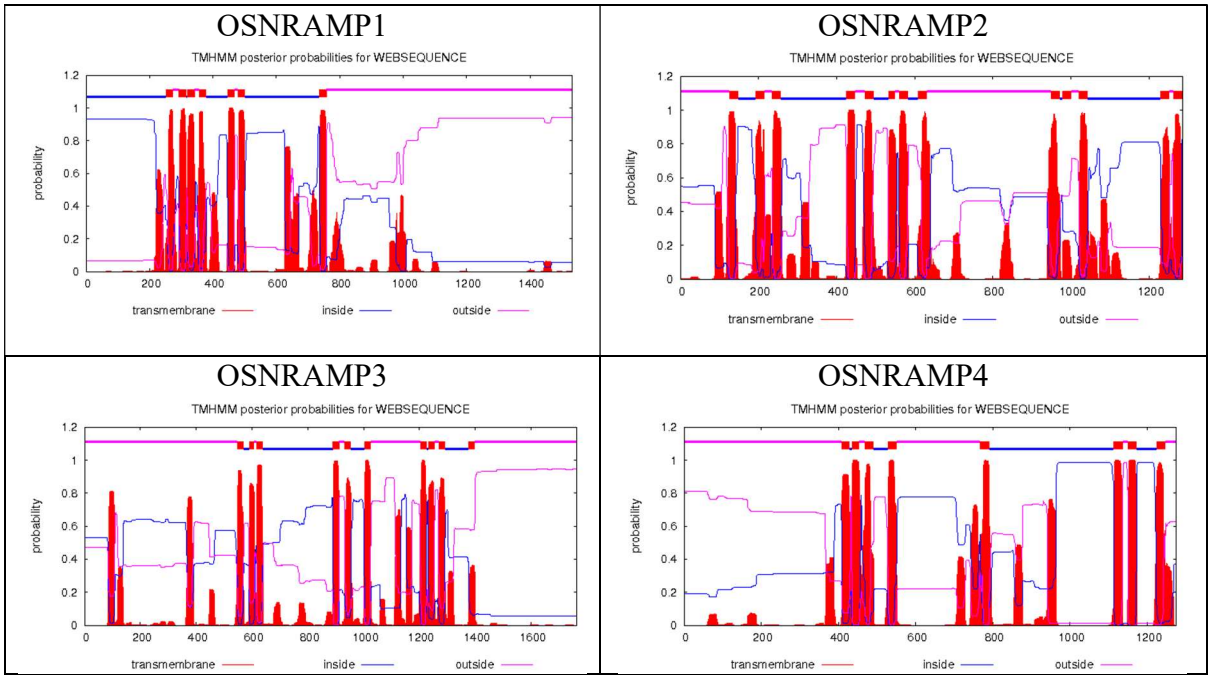


Figure no 01: The 04-OsNRAMP transporter protein families of transmembrane-helices with color shows: transmembrane-helices: **RED**, inside: **BLUE**, outside: **PINK**

The MS (Multiple Sequence) sequences alignment by using Clustal-Omega against the four OsNRAMP proteins to generate alignment sequences as shown in figure no 02.

Figure 02: The Multiple sequences alignment of NRAMP-transporter proteins with highlighted conserved portion through **Clustal Omega**

And the variability of sequences and sequences-conservation identity percentage of transporter proteins are shown in Table no 03.

Table 03: Percentage identity of OsNRAMP transporter proteins

	OsNRAMP1	OsNRAMP2	OsNRAMP3	OsNRAMP4
OsNRAMP1	100%	35.96%	58.75%	56.40%
OsNRAMP2	00.00%	100%	37.97%	36.34%
OsNRAMP3	00.00%	00.00%	100%	48.97%
OsNRAMP4	00.00%	00.00%	00.00%	100%

The phylogenetic analysis and evolutionary relationship between OsNRAMP-Transporter proteins through MEGA-X tool by using original, consensus tree bootstrap values-1000 in Neighbour Joining method, and another tree to make with the method of Maximum likelihood by no. of bootstrap: 1000. We observe that the group of OsNRAMP 1,2 and 03 are closely

related relationships between these proteins in rice and common ancestry relation with OSNRAM-2 are shown in figure no 03.

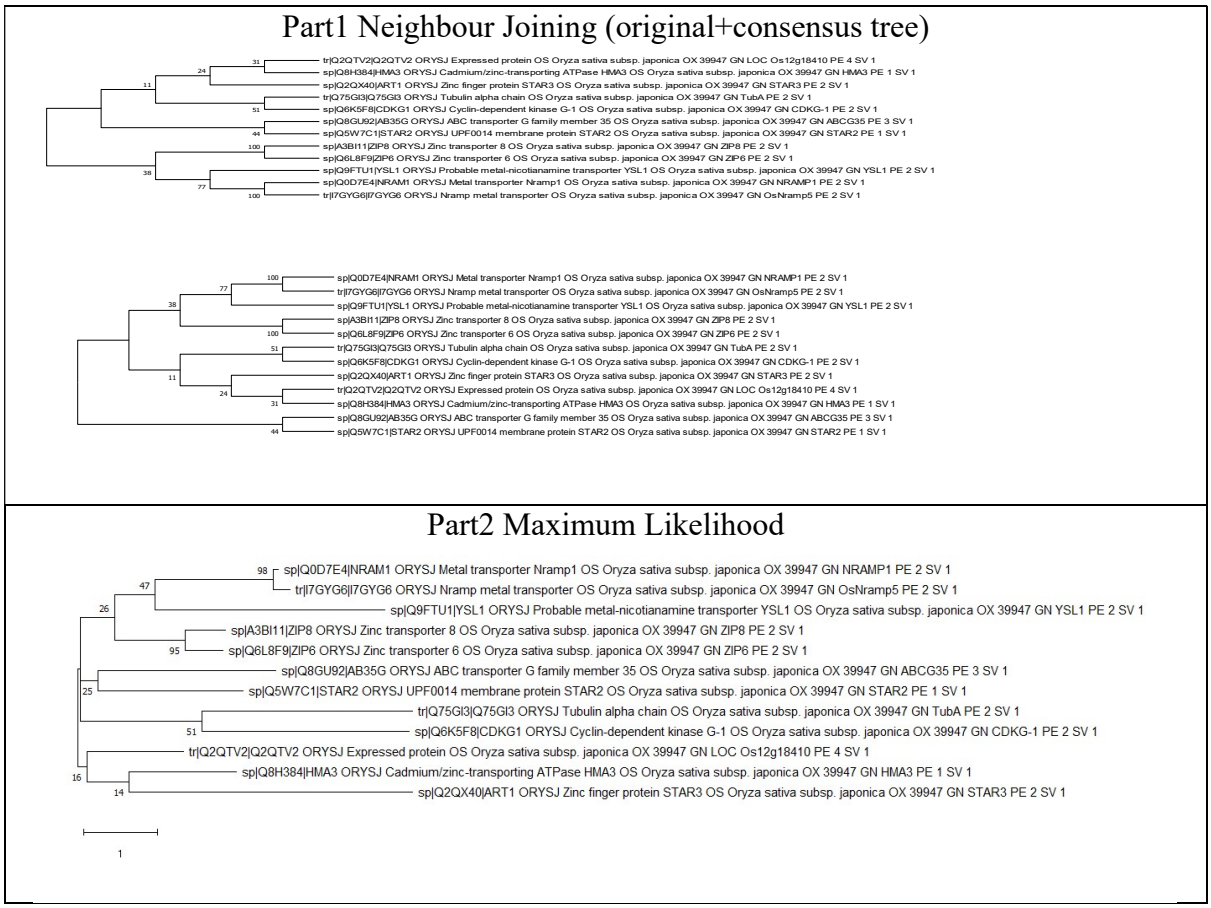
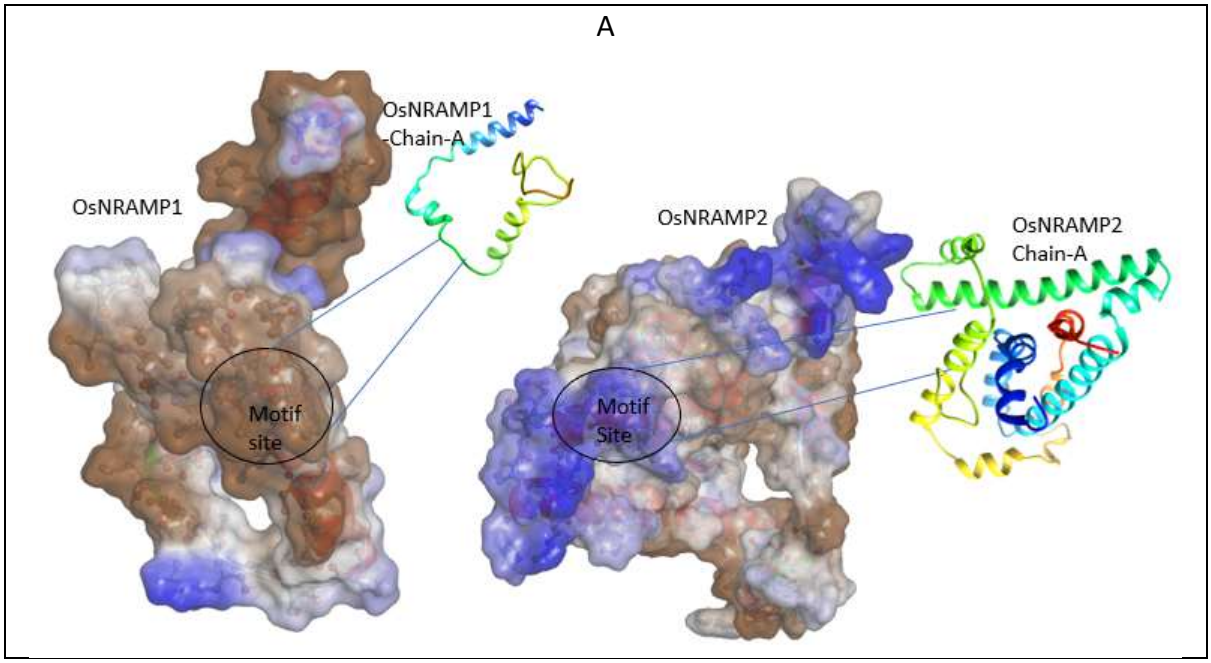


Figure no 03: Phylogenetic tree analysis of OsNRAMP transporter proteins by using MEGA-X, **Part1**: bootstrap values-1000 in Neighbour Joining method, **Part2**: bootstrap values-1000 in Maximum Likelihood method.

The protein interaction association in 04 transporter proteins of OsNRAMP with highly potential interaction-confidential values approximately greater than equal to 0.7 for the OsNRAMP1,2,3,4 through STRING-Database (Von Mering et al., 2005), associate another network analysis of highly putative linkage with expresses the protein of tubulin domains, expressed protein, ATPase in OsNRAMP2 along with scores: 0.713, 0.704, and 0,705. And zinc transporter proteins that can mediate the zinc through rhizosphere in translocation of zinc elements in plant with defensive protein and express in cation transporter of metal in cyclic-kinase1 dependent with scores: 0.71, 0.73, 0.786 with one of the most recognized adverse high score in OsNRAMP-3: 0.76 with metals zinc, cadmium protein, and zinc-finger acts as transcriptional factor-activator proteins that's may help to maintain the expression level of Al (Aluminium tolerance) within the roots UDP-Glucose and detoxifying mechanism through aluminium ions in protein-mitochondrial ATP binding process. The computationally isoelectric points; theoretical-pl and molecular weight-Mw of OsNRAMP Transporter proteins of OsNRAMP_1 (9.15/175660.82), OsNRAMP_2 (4.78/340957.82), OsNRAMP_3 (4.72/477243.96), OsNRAMP_4 (4.80/341436.99) through ExPASY-Compute pl/Mw tool.

The predicted models of OsNRAMP transporter proteins through SWISS-Model; the stereochemistry characteristics of predicted 3D structure of NRAMP proteins was evaluated by RAMPAGE, its shows that the probability of amino acids (%) in selective OSNRAMP1,2,3,4: MolProbity Score: 2.73, 1.95, 2.17, 0.72, clash score: 7.89, 6.05, 11.56,

00.00 Ramachandran favoured region: 76.71,94.95, 88.30, 96.61 outliers: 6.85,1.83, 4.15, 00.00, rotamers: 4.84, 2.26, 0.81, 00.00. and the ERRAT-SAVES Tool results of these four predicted structures the overall quality factors (OQF) are OSNRAMP1_59.70%, OSNRAMP2_90.04%, OSNRAMP3_73.93%, and OSNRAMP4_77.50% (Lovell et al., 2003), the structural refinement and optimization of predicted models by using Chimera-Tool, and the protein-native models of NRAMP in rice plant was predicted with the range of LG-Score greater than 1.5, 1.4, but in predicted ProQ maximum Subscore in the range of >0.5 to >0.8 and that results; 3D-Structure of NRAMP1 [5.705, 0.128], NRAMP2 [5.2, 0.12], NRAMP3 [5.70, 0.14], NRAMP4 [5.46, 0.13]. The OsNRAMP Family of transporter proteins (OsNRAMP1,2,3,4) is predicted through SWISS-Model on the basis of evaluation, structural properties of the amino acid structure. And the structural visualization through UCSF-Chimera Tool and Discovery Studio Visualizer tool with the Chain-A and motifs of protein sequences in transporter proteins as shown in figure no: 04 (Part A-B).



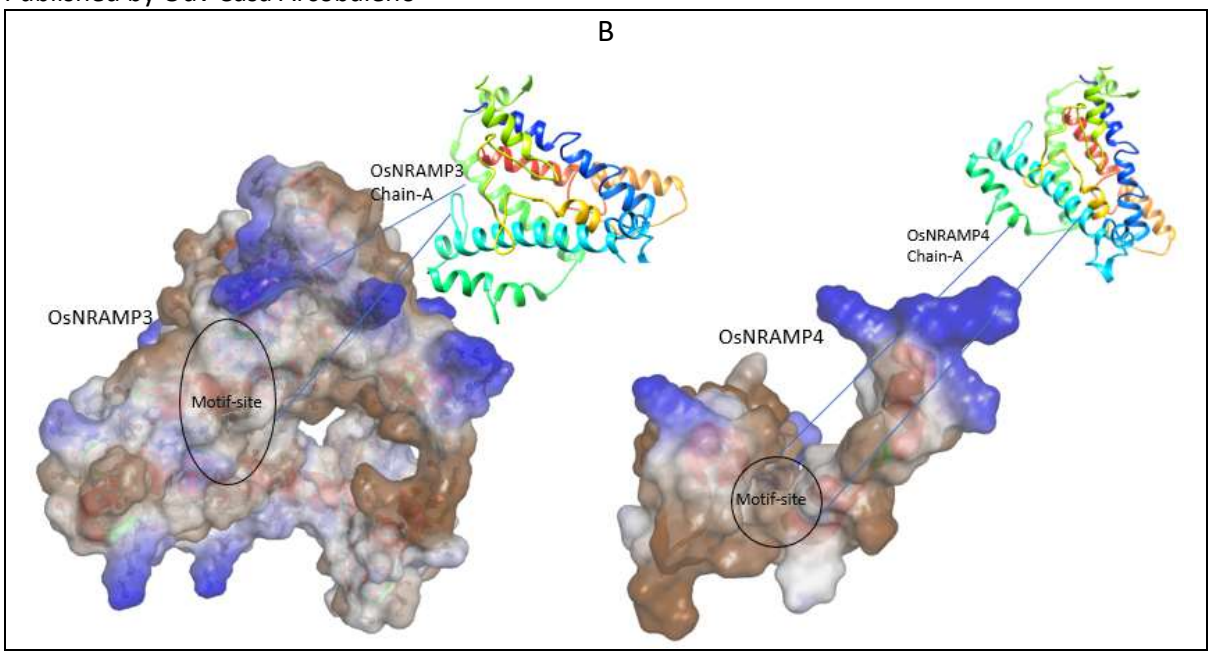


Figure no 04: The structure analysis of OsNRAMP Transporter protein of *Oryza sativa* (Rice), OsNRAMP1, OsNRAMP2, OsNRAMP3, OsNRAMP4 (A-B) with their **Chain-A**, and **Motif sites** inside the protein highlighted along **black circles** and separate colors sequences **portion: red**_motif1**, blue**_motif2**, cyan**_motif3**, light brown, red**_motif4-5 visualized through UCSF-Chimera Tool, Discovery Studio Visualizer.

We present the results of a bioinformatics study of all four OsNRAMP transporters found in rice plants. We now have a better understanding of their physiochemical, phylogenetic relationship, and molecular properties due to this research. Their presence on the plasma membrane propounds that they may be incriminated in metal ion transmembrane transport. Furthermore, their less water solubility, as expected by hydropathy plots, predicts that they are membrane proteins. OsNRAMP1, 2, and 3 are extremely conserved, although another member of the OsNRAMP lineage of transporter proteins is indeed partially conserved. Every one of the 4 leading OsNRAMP transporters current warming using both techniques have structural similarities to the recently determined crystal structure of *E. coleocola* manganese-transporter. It's a 511-amino-acid protein with 12 transmembrane helixes that helps transport Mn2+, and these transporters are identical to Cd/Zn transporters and may also be implicated in Al (Aluminium) tolerance. Since a cell is a complex structure, a single molecule may engage in a variety of ways with several molecules in response to various sensations (Ehrnstorfer, Manatschal, Arnold, Laederach, & Dutzler, 2017). The overall functions of OsNRAMP transporter genes are suitable and major role in the nutritional metal ions intake from the soil and vegetative growth of roots plant in rice.

4.Conclusion

The conclusions made here are the results of a comprehensive overview using *in-silico* methods to study the NRAMP class of rice transporters. The proton-coupled metal ion transporters belong to the NRAMP family of transporters and, despite being abundant in both prokaryotes and eukaryotes, the exact mechanism of these proton-coupled transporters is about to be clearly understood. Consequently, knowing their structure will enable us in determining the precise region or residues concerned in metal-binding and transport, along with phylogenetic relationships between protein-transporter families. It would be vital to examine the transporter working mechanism followed by metal ion transport in rice plants through further research, simulations, and further biological experiments involving gene knockout plants. This will also

assist to determine the metal ions that are transported by the transporter's substrate specificity hasn't even been observed.

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