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Research article

PHYLOGENETIC ANALYSIS (*IN-SILICO*) OF NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN (NRAMP) AND IDENTIFICATION OF ITS HOMOLOG IN BREAD WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT: The natural resistance-associated macrophage protein (NRAMP) constitutes a highly conserved integral membrane protein family involved in iron transport in several organisms, including bacteria, fungi, plants, and animals. 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 (*Arabidopsis thaliana, Glycine max, Brassica juncea and Cicer arietinum*) and monocots (*Zea mays* and *Oryza sativa*), species. In the present study four homologues for bread wheat (*Ta-OsNramp2 &7 (homologous to O. Sativa); and Ta-ZmNramp3 & 6 like (homologous to Z. mays)* were found most significant (BLAST hits were considered most significant with bit score ≥400 and E-value $\leq e^{-162/0}$) among different alleles. This study indicates that higher sequence variability for Nramp alleles exist in both *O. Sativa* and *Z. Mays* because polymorphic site, nucleotide diversity and positive Tajima's D test were found highest in O. Sativa and Z. Mays among studied species. One of the key findings of this study is presence of four wheat Nramp homologs and can help in the understanding of the variability present in Nramps gene in the different genomic background of bread wheat that can be employed in the selection of better alleles for management of divalent metal (Fe & Zn) transport in wheat improvement programmes.

Key words: Natural resistance-associated macrophage protein (NRAMP), iron, zinc, homologs, *Triticum aestivum*

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INTRODUCTION

The natural resistance-associated macrophage protein (NRAMP) constitutes a highly conserved integral membrane protein family involved in iron transport in several organisms, including bacteria, fungi, plants, and animals (Cellier et al. 1995). These genes are widely distributed in all plant families, acting mainly in divalent cation transport (Curie and Briat 2003). The first occurrence of NRAMP genes were identified in mammals (NRAMP1). This gene encodes a macrophage membrane protein responsible for cation concentration in the phagosome, thus regulating the phagocited bacterial regulation (Williams et al. 2000).

Mineral nutrition is a major factor related to plant growth and development, and consequently affects crop productivity. Copper, iron, zinc, cobalt, nickel, and manganese are metal cations essential for cellular processes, since they act as important cofactors for many enzymes, are components of transcription factors and other proteins, and are essential for both mitochondrial and chloroplast functions.

However, when present at high concentrations, along with nonessential metals such as cadmium, mercury, silver, and lead, essential metals can become extremely toxic, since they can cause oxidative damage or compete with other essential ions. Heavy metals are present in soil as natural components or as a result of human activity. Iron is a key plant micronutrient, taking part in redox centers of proteins that are essential for photosynthesis and respiration (Taiz and Zeiger 2010).

Iron deficiency causes a metabolic imbalance that is deleterious to plant development (Briat and Lebrun 1999). In iron-rich environments, such as flooded soils, the excess of this element induces production of hydroxyl radicals involved in oxidative stress and may cause damage to several cellular structures, eventually leading to cell death (Guerinot and Yi 1994; Briat et al. 1995). Thus, plants must balance iron concentration in a homeostatic way, providing the necessary amounts of iron and preventing internal cation excess (Briat and Lobréaux 1997; Grusak et al. 1999).

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). AtNRAMP1 protein may be related to iron subcellular transport and its targeting to storage compartments such as vacuoles or plastids (Curie et al. 2000). The identification of orthologues and paralogues is prerequisite for the understanding of the evolutionary relationships within this gene family.

If we are looking to unravel the history of a gene family, the accurate relation between genes in the gene family must be determined across the species of interest. These relations can be described either in terms of orthology or paralogy, which are two key concepts of evolutionary genomics (Koonin 2005). The orthologs are genes that diverged because of speciation event, whereas in paralog sequence, divergence follows gene duplication (Fitch 1970). Hence, orthologs are the genes that, at present, exist in different species but earlier have originated from a single gene in the last common ancestor of these species and have often retained identical biological functions (Remm et al. 2001). One can also conclude that the fundamental function of orthologous pairs/groups may have been conserved across evolutionary related species. The high percentage of orthology for a gene family between two species may reflect high conservation of their function in those species (O'Toole et al. 2008). However, Philippa et al. 2014 identified NRAMP homologs in bread wheat using rice (OsNramp1 to OsNramp7) Nramp alleles as query. This type of genome-wide analysis for Nramp alleles has not been performed across varied systems like (monocot, dicot and legumes) so far. The objectives of the present study were as follows: (i) to understand their phylogenetic relation, with monocot and dicot and (ii) to understand homologous relation between different bread wheat genomes.

METHODS

The multiple sequence alignment (MSA) of all identified Nramp genes was performed to construct a phylogenetic tree by MEGA version 6.0 using default parameters (Tamura et al. 2013). The neighbour-joining distance trees were constructed separately for Nramp gene families using default settings and 1000 bootstrap replications to ensure a high confidence range and accuracy. Bootstrap analysis was performed to evaluate the degree of support for each homologous group in the tree.

Multiple Expectation-Maximization for Motif Elicitation (MEME) is a suite of tools for motif discovery and searching. This suite is quite often used by previous researchers for the support of phylogenetic trees and to find the conserved motif structures. The second tree prepared was that for 27 Nramp's allele sequences by MEGA 6.0, and that was further supported by MEME 10.04.02 (Timonthy et al. 1994) results. About twenty different sub domains or motifs between 8 and 50 residues were detected and distributed by MEME software. An overlay of phylogenetic tree and motif distribution from MEME can be used to find the correlation (Martınez et al. 2005; Di et al. 2010). Expect values (E-values) were analyzed for putative Nramp's gene family. The E-values, which are calculated by the BLAST software, indicate the probability that the observed similarity between the query protein and any other protein detected by the BLAST search arose by chance (Altschul et al. 1997). The trees are thus found to be correlated and well supported, then further represented for each plant species, Nramp genes were identified by systematic BLAST (Fitch 1970) searches of each of the query gene sequence against the gene sequences of all six plant species separately. For each and every BLAST search, BLAST default settings were used, and BLAST hits were considered significant with bit score $\geq$100 and E-value $\leq e^{-20}$.

In silico homology analysis was conducted for these identified genes by BLAST search against the Nramps sequences of respective plant species downloaded in local database. For each gene, we counted the number of significant Nramp hits (those having bit score ≥100 and E-value $\leq e^{-20}$) and categorized these genes as "not homolog," "less homolog," "moderately homolog," and "highly homolog expressed" if there was no hit, 1 to 100 hits, 101 to 400 hits, and more than 400 hits, respectively.

RESULTS

The NRAMP family members function as divalent metal transporters in a wide range of organisms, from bacteria to Homo sapiens (Cellier et al. 1995). The identified Nramp genes belong to highly diverged plant species, ranging from monocots to dicots and including grains and legumes. We have analyzed Nramp genes to evaluate their evolutionary relations between monocots and dicots. For Nramp genes, MSA was obtained, and NJ distance tree was constructed and supported with bootstrap values using default settings and parameters. The neighbour-joining (NJ) method was preferred by the earlier researcher because of its reasonable accuracy and cubic running time which makes this method a widely used one for phylogenetic tree construction (Studier and Kepler 1988; Saitou and Nei 1987). Motif distribution pattern was detected for these genes with MEME 4.10.02 software, and an overlay was produced with the NJ tree as given in Figure 2.

Molecular diversity at Nramp's locus

For extensive analysis, the 27 Nramp's allelic DNA sequences of six different plant, including monocots and dicots were retrieved from the NCBI database. High level of sequence variations were observed among them. The highest polymorphic site (0.793) was identified in *Oryza sativa* followed by *Zea mays* (0.749) and lowest (0.487) in *Arabidopsis thaliana* was identified among plant species (Table 1). The highest nucleotide diversity (0.478) was identified in *Oryza sativa* and lowest (0.310) in *Arabidopsis thaliana* among species (Table 1). D statistics (Tajima's D test) was estimated across the accessions for evolutionary analysis of Nramp genes. This statistics do not reveal deviation from neutrality test and showed positive values of Tajima's D test for both, monocots and dicots. Among all monocot and dicot species, highest D test value (2.817) was obtained in *O. sativa* while it was found minimum (1.771) in case of *A. thaliana*. This study indicates that higher sequence variability for Nramp alleles exist in monocots (*O. sativa* and *Z. Mays*) because polymorphic site and nucleotide diversity were found highest in *O. Sativa* and *Z. Mays* among studied species.

Phylogenetic analysis of Nramp's genes

The phylogenetic tree was constructed by MEGA version 6.0 and arbitrarily divided into four main clusters, namely I, II, III and IV. The cluster I contained four dicot (*A. thaliana; AtNramp2, 3, 5 & 6; G. max; GmNramp-2like & 3like; B. Juncea; BjNramp4.1 & 5 and C. arietinum; CaNramp-2like & 3*) species while cluster II included two monocots (*O. Sativa; OsNramp2 &7 and Z. Mays; ZmNramp3 & 6 like*) species. The cluster I comprised only dicot while cluster II contained only monocots and separated with the highest bootstrap value (100%) from each other, suggesting that Nramp genes diverged during the split of monocot and dicot in the course of phylogenetic development. Cluster III contained two dicot species (*G.max*; GmNramp-1like & 6 like and *C. arietinum; Canramp6*) and Cluster IV comprised two monocots (*O. Sativa; Osnramp1, 4, 5 & 6 and Z. Mays; ZmNramp-1 like, 4like & 5*) species while one monocot (OsNramp3) and two dicot (CaNramp-5like and GmNramp-5like) species were unclustered (Fig. 1) proposing that some genomic forces (insertion, deletion etc.) may affect the gene structure of Nramp's family.

Table 1. Molecular evolutionary parameters and neutrality test from different plant/crop species.

Plant Species	*m*	n	*S*	p_s	Θ	π	*D*
Oryza sativa	7	1700	1350	0.793	0.323	0.478	2.817
Arabidopsis thaliana	4	1517	740	0.487	0.265	0.310	1.771
Glycine max	5	1700	1212	0.712	0.342	0.445	2.304
Zea mays	5	1314	985	0.749	0.359	0.472	2.389
Cicer arietinum	4	1807	1250	0.691	0.377	0.464	2.433

Abbreviations: m = number of sequences, n = total number of sites, S = Number of segregating sites, $p_s = S/n$, $\Theta = p_s/a_1$, π = nucleotide diversity, and D is the Tajima test statistic.

Comparative analysis & Homologs

Four homologues were found for bread wheat (*O. Sativa; Ta-OsNramp2 &7 and Z. Mays; Ta-ZmNramp3 & 6 like*) when using each of the six plant (different alleles of Nramps from 4 dicot and 2 monocot) family of NRAMP copies as queries. Those BLAST hits were considered significant with bit score ≥100 and E-value $\leq e^{-20}$ as shown in Supplement Table 1 and used for constructions of phylogenetic tree. All bread wheat homologues were grouped in NRAMP phylogenetic relationship (Fig. 2), and indicate both species specificity genome specificity and can be assigned one of the three genome progenitor (A, B & D) of bread wheat.

Fig 1.An unrooted neighbor-joining (NJ)-based tree of the NRAMP family in selected plants. The tree was generated using MEGA version 6.0 after sequence alignment. Bootstrap values are indicated (1,000 replicates).

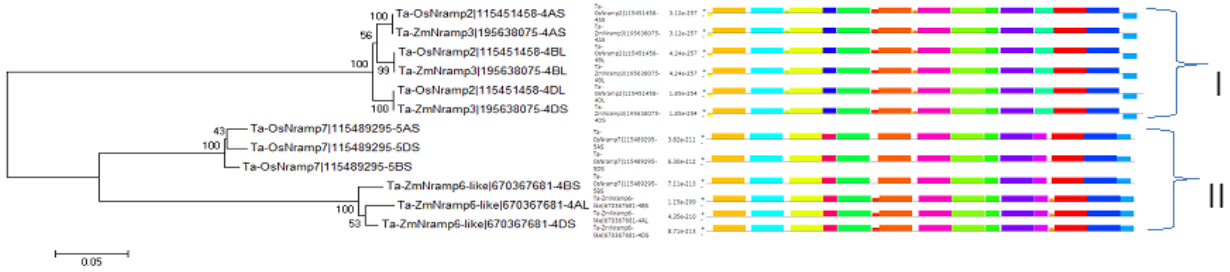

Fig 2. An unrooted, neighbor-joining (NJ) based tree of the NRAMP family (homolog sequences from *T. aestivum* database) in selected plants. MEME4.10.2 was applied to show that different subgroups were distinguished by the motif distribution, which is consistent with the phylogenetic subgroups obtained by MEGA version 6.0 after sequence alignment. Bootstrap values are indicated (1,000 replicates).

DISCUSSION

The availability of the whole genome and abundant genetic and genomic resources of model crops viz. *Oryza sativa & Arabidopsis thaliana* with high-syntenic relationships with other plant genomes makes it a better option for comparative genome analysis (Han and Zhang 2008; Li et al. 2011; Nakagami et al. 2010). We have downloaded their DNA sequences from the National Centre for Biotechnology Information (NCBI). We included the representative six plant genomes comprised four dicots (*Arabidopsis thaliana, Glycine max, Brassica juncea and Cicer aierentinum*) and two monocots (*Zea maysand Oryza sativa*), in this study for comparative analysis and identification of homologs in bread wheat.

Many studies have shown that there is a wide variation in grain Fe and Zn concentrations in wild relatives of modern wheat and the concentrations found can significantly exceed those found in modern elite cultivars (Cakmak et al., 2000; Monasterio and Graham, 2000). This natural variation can be utilized to biofortify wheat for Fe and Zn, such as has been achieved using the transcription factor NAM-B1 (Uauy et al., 2006) which was originally identified for increasing protein content in wild emmer (*Triticum turgidum* ssp *dicoccoides*). In near isogenic lines the presence of NAM-B1 increased Fe and Zn grain concentrations by 18 and 12%, respectively, (Distelfeld et al., 2007). This gene is being widely used in breeding programes across several continents (Kumar et al., 2011; Randhawa et al., 2013; Tabbita et al., 2013). To keep view above said, this study was pertaining to understanding of the Nramps relationship between and among several genomic backgrounds (monocot, dicot and legumes).

In BLAST searches, the E-values are lowest (closer to 0) for BLAST hits with a high degree of homology to the query sequence and they increase as BLAST hits are detected with lower similarity. This work should provide a basis for further experimental molecular and genomic studies of metal homeostasis and tolerance in photosynthetic plants. Genomic and phylogenetic information gained from these sequenced plant species will greatly accelerate the bio-fortification in wheat.

A clear correlation between the motif pattern and the NJ phylogenetic tree (those BLAST hits were considered significant with bit score ≥100 and E-value $\leq e^{-20}$ as shown in Supplement Table 1 and used for constructions of phylogenetic tree as shown in Fig. 2) can be found, where each group or subgroup of tree is essentially sharing the same motif pattern. Many motifs are more conserved and appeared in almost all groups or subgroups, except the ones at the middle portion of the tree. These conserved motifs could be the essential elements determining the Nramp family's common molecular function among different plant species. Six of 20 Nramp genes lack many motifs and might not be having the close evolutionary relations with other groups. The motif distribution revealed that the genes having the same motifs determined by MEME usually evolved from gene expansion within the same group or cluster whether they belong to higher or lower species (Di et al. 2010). It can be explained that the ancestor genes with various motif structure seem to appear early in the evolution and then the same structure was maintained by the recent genes through the evolution. In the present study, similar motif distribution points toward the conservation of the Nramp genes throughout all of the groups and subgroups in the phylogenetic tree (Figure 2).

In the present study four homologues for bread wheat (*O. Sativa; Ta-OsNramp2 &7 and Z. Mays; Ta-ZmNramp3 & 6 like*) were found most significant (BLAST hits were considered most significant with bit score ≥400 and E-value $\leq e^{-162/0}$as shown in Supplement Table 1) among different Nramp alleles. The present investigation opens the new opportunitiesthat can complement natural variation and genome-wide association, and lead to faster improvements in Fe and Zn grain content in bread wheat in near future. This study indicates that higher sequence variability for Nramp alleles exist in both monocots (*O. Sativa* and *Z. Mays*) because polymorphic site, nucleotide diversity and positive Tajima's D test were found highest in *O. Sativa* and *Z. Mays* among studied species. One of the key findings of this study is based on phylogenetic analysis of four wheat homologs helps in the understanding of the variability present in Nramps gene family in the different genomic background of bread wheat (ABD genome) that can be employed in the selection of better alleles for management of divalent metal (Fe & Zn) transport in wheat improvement programmes.

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Supplementary table

Table 1. New genomic resources enable identification of NRAMP homologs in wheat (*Triticum aestivum* L.).

S. No.	NRAMP (LOCUS ID)	Wheat homolog	Genome	Wheat's homolog Arm	Wheat Sequence[a]	E-value	Score (bits)
1	OsNramp1|115471432	Ta-OsNramp1	A	4AS, 5AL & 7AL	4AS_5926812	$2e^{-59}$	239
			B	4BL, 4BS, 5BL & 7BL	7BL_6744498	$1e^{-61}$	246
			D	2DL, 4DL, 7DL & 7DS	7DL_3317468	$2e^{-64}$	255
2	OsNramp2|115451458	Ta-OsNramp2	A	1AS, 4AL, 4AS, 5AS & 6AS	4AS_5952279	0.0	854
			B	4BL, 4BS, 5BS & 7BS	4BL_7000373	0.0	827
			D	4DL, 4DS & 5DS	4DL_14450878	0.0	839
3	OsNramp3|297725180	Ta-OsNramp3	A	4AS, 5AL & 7AL	7AL_4392690	$3e^{-70}$	275
			B	4BS, 5BL, 6BL & 7BL	7BL_6748183	$1e^{-75}$	293
			D	2DL, 3DL, 4DL, 6DL & DL	7DL_3360602	$2e^{-77}$	298
4	OsNramp4|115444028	Ta-OsNramp4	A	4AS & 6AS	6AS_4346871	$4e^{-61}$	244
			B	4BL, 4BS, 6BS 7 7BL	6BS_2318478	$2e^{-65}$	259
			D	4DL & 7DL	7DL_3317468	$3e^{-30}$	141
5	OsNramp5|115471426	Ta-OsNramp5	A	4AS, 5AL, 6AS & 7AL	4AS_5926812	$3e^{-75}$	291
			B	4BL, 4BS, 5BL, 6BS & 7BL	4BL_7037107	$4e^{-86}$	327
			D	2DL, 4DL, 5DL, 7DL & 7DS	4DL_14404139	$1e^{-87}$	333
6	OsNramp6|115436797	Ta-OsNramp6	A	3AS & 4AS	3AS_3396100	$1e^{-55}$	226
			B	3B, 4BS & 4BL	3B_10682934	$3e^{-51}$	212
			D	3DL & 4DL	3DL_6945789	$5e^{-54}$	221
7	OsNramp7|115489295	Ta-OsNramp7	A	1AS, 4AL, 4AS, 5AS & 6AS	5AS_1501999	0.0	764
			B	4BL, 4BS, 5BS & 7BS	5BS_2288821	0.0	749
			D	4DL, 4DS & 5DS	5DS_2767814	0.0	758

8	AtNramp2|30694026	Ta-AtNramp2	A	1AS, 4AS, 4AL, 5AS & 6AS	5AS_1501999	$7e^{-113}$	416
			B	4BS, 4BL & 5BS	5BS_2288821	$5e^{-108}$	399
			D	4DS, 4DL & 5DS	5DS_2767814	$2e^{-114}$	421
9	AtNramp3|145360236	Ta-AtNramp3	A	4AS, 4AL & 5AS	5AS_1501999	$2e^{-93}$	351
			B	4BS, 4BL & 5BS	5BS_2288821	$1e^{-97}$	365
			D	4DS, 4DL & 5DS	5DS_2767814	$1e^{-102}$	381
10	AtNramp5|18415172	Ta-AtNramp5	A	1AS, 4AS, 4AL & 5AS	5AS_1501999	$9e^{-81}$	309
			B	4BS, 4BL & 5BS	5BS_2288821	$9e^{-81}$	309
			D	4DS, 4DL & 5DS	5DS_2767814	$9e^{-87}$	331
11	AtNramp6|280595546.1	Ta-AtNramp6	A	1AS, 4AS, 4AL & 5AS	5AS_1501999	$3e^{-88}$	335
			B	4BS, 4BL & 5BS	5BS_2288821	$6e^{-90}$	340
			D	4DS, 4DL & 5DS	5DS_2767814	$9e^{-88}$	333
12	GmNramp1-like|571464530	Ta-GmNramp1-like	A	4AS & 7AL	7AL_4392690	$3e^{-18}$	102
			B	4BL & 7BL	7BL_6748183	$1e^{-16}$	96.9
			D	7 DL	7DL_3360602	$1e^{-18}$	104
13	GmNramp2-like|571525143	Ta-GmNramp2-like	A	1AS, 4AS, 4AL, 5AS & 6AS	5AS_1501999	$1e^{-67}$	266
			B	4BS, 4BL, 5BS & 7BS	5BS_2288821	$7e^{-71}$	277
			D	4DS, 4DL & 5DS	5DS_2767814	$6e^{-72}$	280
14	GmNramp3-like|571487378	Ta-GmNramp3-like	A	1AS, 4AS, 4AL, 5AS & 6AS	5AS_1501999	$2e^{-96}$	361
			B	4BS, 4BL & 5BS	5BS_2288821	$1e^{-99}$	372
			D	4DS, 4DL & 5DS	5DS_2767814	$2e^{-105}$	385

15	GmNramp5-like|356515920	Ta-GmNramp5-like	A	4AS, 5AL, 6AS & 7AL	Absent	-	-
			B	4BS, 4BL & 5BL, 6BS & 7BL	4BL_6972619	$8e^{-26}$	127
			D	4DL, 5DL & 7DL	7DL_3317468	$1e^{-22}$	116
16	GmNramp6-like|571503944	Ta-GmNramp6-like	A	4AS & 7AL	7AL_4434087	$6e^{-21}$	111
			B	7BL	7BL_6748182	$2e^{-21}$	113
			D	7DL	7DL_3333302	$4e^{-23}$	118
17	ZmNramp1-like|670378217	Ta-ZmNramp1-like	A	3AS, 5AL, 6AS & 7AL	4AS_592681	$1e^{-55}$	226
			B	3B, 4BS, 4BL, 5BL & 6BS	7BL_67333398	$4e^{-55}$	224
			D	2DL, 3DL & 5DL	7DL_3317468	$2e^{-58}$	235
18	ZmNramp3|195 638075	Ta-ZmNramp3	A	1AS, 4AS, 4AL, 5AS & 6AS	4AS_5952279	0.0	814
			B	4BS, 4BL, 5BS & 7BS	4BL_7000373	0.0	791
			D	4DS, 4DL, 5DS 7 7DL	4DL_14450878	0.0	809
19	ZmNramp4-like|670379926	Ta-ZmNramp4-like	A	3AS	3AS_3396100	$1e^{-43}$	187
			B	3B	3B_10682934	$3e^{-38}$	168
			D	3DL	3DL_6945789	$1e^{-42}$	183
20	ZmNramp5|670 422714	Ta-ZmNramp5	A	4AS, 5AL, 6AS & 7AL	4AS_592681	$1e^{-85}$	325
			B	4BS, 4BL, 5BL, 6BS & 7BL	4BL_7037107	$2e^{-89}$	338
			D	2DL, 4DL, 5DL, 7DL	4DL_144041	$7e^{-95}$	356
21	ZmNramp6-like|670367681	Ta-ZmNramp6-like	A	1AS, 4AS, 4AL, 5AS, 5AL & 6AS	4AL_7173573	$2e^{-165}$	590
			B	4BS, 4BL, 5BS & 7BS	4BS_3944622	$3e^{-177}$	630
			D	4DS, 4DL & 5DS	4DS_2292562	$1e^{-162}$	581

22	BjNramp-4.1|300379522	Ta-BjNramp-4.1	A	1AS, 4AS, 4AL, 5AS & 6AS	5AS_1501999	$5e^{-97}$	363
			B	4BS, 4BL, 5BS, 7BS & 7BL	5BS_2288821	$3e^{-94}$	354
			D	4DS, 4DL, 5DS 7 7DL	5DS_2767814	$6e^{-96}$	360
23	BjNramp5|689262301	Ta-BjNramp5	A	1AS, 4AS, 4AL, 5AS & 6AS	5AS_1501999	$4e^{-93}$	351
			B	4BS, 5BS & 5BL	5BS_2288821	$2e^{-90}$	342
			D	4DS, 4DL & 5DS	5DS_2767814	$4e^{-94}$	354
24	CaNramp2-like|828328994	Ta-CaNramp2-like	A	1AS, 4AS, 4AL, 5AS & 6AS	5AS_1501999	$1e^{-86}$	329
			B	4BS, 4BL & 5BS	5BS_2288821	$2e^{-89}$	328
			D	4DS, 4DL & 5DS	5DS_2767814	$6e^{-98}$	343
25	CaNramp3|828331152	Ta-CaNramp3	A	1AS, 4AS, 4AL & 5AS	5AS_1501999	$9e^{-95}$	356
			B	4BS, 4BL & 5BS	5BS_2288821	$8e^{-102}$	379
			D	4DS, 4DL & 5DS	5DS_2767814	$7e^{-103}$	383
26	CaNramp5-like|828315981	Ta-CaNramp5-like	A	4AS & 7AL	4AS_5926812	$5e^{-15}$	91.5
			B	4BS, 4BL & 7BL	4BS_8497176	$2e^{-15}$	89.7
			D	4DL & 7DL	4DL_1440413	$2e^{-14}$	89.7
27	CaNramp6|828289233	Ta-CaNramp6	A	4AS	4AS_592681	$4e^{-09}$	71.6
			B	Absent	-	-	-
			D	Absent	-	-	-

[a]International wheat genome sequencing consortium chromosome-arm survey sequences are available at Ensembl Plants (Ensembl; http://plants.ensembl.org/index.html) and at Unité de Recherche Génomique Info (URGI; http://www.wheatgenome.org/Tools-and-Resources)

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