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ANALYSIS OF SEQUENCE POLYMORPHISM OF *OsHKT1;5* GENE IN RICE (*Oryza sativa* L.)

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SUMMARY

In salt stress conditions, the excessive accumulation of Na^+ ion in the cytoplasm could cause ion toxicity, metabolic imbalances, and reduction in growth of plant. The ability of maintaining a low Na^+ concentrations or low Na^+/K^+ ratio in cytosol plays the important roles of plant adapt to salinity stress. In rice, the HKT transporter family has been proven to be critical for salt tolerance and the maintenance of Na^+, K^+ homeostasis under salinity conditions. In this study, we conducted the sequence polymorphisms analysis in the *OsHKT1;5* coding region in order to detect the potential allelic variants in some local rice cultivars in Viet Nam. The exon 1 region and exon 2-3 region of *OsHKT1;5* gene were amplified by PCR in two separate reactions. After direct sequencing of PCR products, the full length coding region of *OsHKT1;5* gene was obtained and compared to reference Nipponbare *OsHKT1;5* coding sequence. Accordingly, twelve nucleotide substitutions in coding sequence of *OsHKT1;5* were identified, including six non-synonymous substitutions (G382A, C418G, G551A, C994G, C1183G, A1630C) and the other six synonymous substitutions (G225A, C1038G, G1152A, G1261A, G1304C, C1608T). Further analysis revealed that six non-synonymous substitutions (G382A, C418G, G551A, C994G, C1183G, A1630C) caused the changes in amino acids (D128N, P140A, R184H, H332D, V395L, and S544R). The single nucleotide polymorphism A1630C leading substitution of S544R was newly found in the Chiem Rong cultivar. In silico analysis of protein structure and post-translational modifications indicated that amino acid substitutions had no influence on protein structure but led to changes in post-translational modifications such as phosphorylation and SUMOylation.

Keywords: OsHKT1;5 gene, polymorphism analysis, rice, salinity

INTRODUCTION

Salinity is a major abiotic stress factor that considerably affects growth, development, and crop productivity (Munns, Tester, 2008). High salinity not only affects the morphological, physiological, and biochemical processes but causes osmotic stress, ionic and nutritional imbalances in plants (Ashraf, 2004; Slama *et al.*, 2015). To survive under salinity stress, plants have developed a variety of precise response and adaptive mechanisms, including hormonal regulation, osmolyte accumulation, ion homeostasis, and tissue tolerance (Liang *et al.*, 2018). Among these, the maintenance of Na^+ balance in the cell is one of the most important strategies for the survival of plants under

saline conditions (Tester, Davenport, 2003; Almeida *et al.*, 2017).

Plant salt stress responses are physiologically and genetically complex and involve biochemical pathways and many different gene families (Smethurst *et al.,* 2008). It is well-known that High-affinity K^+ transporters (HKTs) are an essential determinant for salt tolerance and the maintenance of Na^+, K^+ homeostasis in plants (Corratgé-Faillie *et al.,* 2010). The HKT protein family is segregated into two groups based on the structure and transport characteristics. Group 1 HKT transporter has a serine at the first place of S-G-G-G motif, and they are Na^+-selective transporters. Whereas this place at the most of group 2 members was glycine, forming G-G-G-G motif, and generally exhibit Na^+-K^+ co-transport (Mäser *et al.,* 2002; Platten *et al.,* 2006). In rice, there are 9 members of the *HKT* gene family including *OsHKT1;1, OsHKT1;2, OsHKT1;3, OsHKT1;4, OsHKT1;5*, *OsHKT2;1, OsHKT2;2, OsHKT2;3*, and *OsHKT2;4*. Except for *OsHKT1;2* is pseudo gene and *OsHKT2;2* is not found in Nipponbare variety (Platten *et al.,* 2006), other functional genes encode for transporters with distinct transport activities which can be found in various tissues and/or organs (Jabnoune *et al.,* 2009). For example, OsHKT2;4 transcripts accumulated in root cells, shoots, and leaf sheaths of rice. It is proposed that OsHKT2;4 has strong K^+ permeability and can transport Mg^{2+} and Ca^{2+} (Yao *et al.,* 2010; Horie *et al.,* 2011; Almeida *et al.,* 2013). OsHKT2;1 located on the plasma membrane in root and leaf tissue, show either Na^+-K^+ co-transporter or Na^+ uniport activity depending on the concentrations of external K^+ and Na^+. In contrast, OsHKT2;2 acts as a Na^+ uniporter and expresses specifically in the root (Mäser *et al.,* 2002; Garciadeblás *et al.,* 2003; Horie *et al.,* 2007). Some other members of the rice HKT transporter family have been proven to be involved in the salt tolerance mechanism, such as OsHKT1;1, OsHKT1;4, OsHKT1;5 (Ren *et al.,* 2005; Cotsaftis *et al.,* 2012; Wang *et al.,* 2015; Suzuki *et al.,* 2016; Kobayashi *et al.,* 2017). This indicates that the HKT transporter family plays a crucial role in the studies of physiological and genetical mechanisms of salt tolerance in plants.

OsHKT1;5 is considered a member of group 1 and located on the plasma membrane in the rice root (Chen *et al.,* 2018). OsHKT1;5 transporter functions by preventing the transport of Na^+ from the root to the young leaves by Na^+ unloading from the xylem of root and young leaf sheaths and excluding Na^+ in the phloem of the basal nodes (Kobayashi *et al.,* 2017). Under salt stress, the loss of function of rice roots OsHKT1;5 can cause a massive accumulation of Na^+ ions in shoots. Therefore, it is assumed that OsHKT1;5 plays an important role in maintaining Na^+/K^+ homeostasis in shoots under salt stress (Ren *et al.,* 2005; Chen *et al.,* 2018). The *OsHKT1;5* gene was found to be highly expressed in roots than those of shoots (Shohan *et al.,* 2019). Allelic polymorphisms in the sequences of *OsHKT1;5* gene have been detected in two rice cultivars, namely Nona Bokra and Koshihikari. Accordingly, the V395L substitution present in the salt-tolerant Nona Bokra could enhance Na^+ transport activity (Ren *et al.,* 2005; Cotsaftis *et al.,* 2012).

In this study we have attempted to identify polymorphisms in the coding sequence of the *OsHKT1;5* gene and theirs influences on protein structure and modification after translation using some local rice varieties.

MATERIALS AND METHODS

Rice materials

Seeds of 16 rice (*Oryza sativa* L.) cultivars including Man 1, Man 2, Chiem Rong, Cham, Tep Lai, Chiem Den, Nep Cuc, Pokkali, Nep Vai, Ngoi, Re Nuoc, Cham Bien, Nep Non Tre, Nep Oc, Chiem Cu, and Dau An Do were provided by Vietnam National University of Agriculture (Hanoi, Vietnam). The seedlings were grown in soil, then the leaves were collected and stored at -80^{o}C for further analysis.

Extraction of DNA

The DNA extraction was conducted by using

the CTAB method. About 200 mg leaf powder was mixed with 500 µL CTAB buffer 2x and incubated at 65°C for 15 min. Then 500 µL CI 24:1 (chloroform: isoamyl alcohol) was added and centrifuged at 14 000 rpm at 4°C for 15 min. The collected supernatant was transferred into a new 1.5 µL tube, and the DNA was precipitated by cold isopropanol for 15 min. The DNA pellet was collected by centrifuging at 10 000 rpm at 4°C for 5 min and washed with 70% ethanol to remove excess salt. After drying at room temperature, the pellet was dissolved in Tris-EDTA buffer and kept at -20°C. The quality and quantity of extracted DNA checked on 1% agarose gel electrophoresis in TAE buffer.

Primer design and amplification of *OsHKT1;5* gene by PCR

The exon 1 region and exon 2-3 region of the *OsHKT1;5* gene were amplified separately from genomic DNA by PCR technique using two separate primer pairs named OsHKT1;5-1 and OsHKT1;5-2, respectively. The specific primers were designed by the Primer-BLAST program on the NCBI webpage. The list of PCR primers was shown in Table 1.

Each PCR reaction contained a volume of 50 µL including 1 µL genomic DNA (10-20 ng), 5 µL dNTPs (2 mM), 5 µL DreamTaq buffer (10x), 0.4 µL DreamTaq polymerase (5 U), 1.5 µL of OsHKT1;5-Fw and OsHKT1;5-RV primer each and 35.2 µL H2O. The PCR reaction was amplified under the following conditions: initial denaturation at 94°C for 5 min, 35 cycles of denaturing at 94°C for 30 s, 57°C (HKT1.5-1 and HKT1.5-2 primers) for 30 s, and extension at 72°C for 2 min, and 72°C for 7 min. Then, 5 µL of PCR products were run on 1% agarose gel for 30 min at a constant 90 V in TAE buffer (1X). Next, the PCR products were purified and sequenced by the First BASE DNA sequencing service (Singapore).

Table 1. Primers used for PCR amplification of *OsHKT1;5* coding sequence

Primer name			Primer sequences		Amplicon size (bp)
OsHKT1.5-1	FW	5'-	GCCCTTGGTGCAATAGCTTTC	-3'	1639
	RV	5'-	AAAATATGTCCCAGGCCAGAGTA	-3'	
OsHKT1.5-2	FW	5'-	AACACCGAATGAAGTCAACATCG	-3'	1460
	RV	5'-	AGGAGTTTTAGGAGGGGAGACC	-3'	

Sequence analysis

Gene sequences were analyzed using Bioedit (Hall, 1999) and Multalin webserver (Corpet, 1988). The amino acid sequences were predicted from nucleotide sequences by using the Expasy webserver (http://web.expasy.org/translate/). The coding sequences of *OsHKT1;5* of all cultivars were compared to those of Nipponbare.

Evaluation of putative changes at protein level

To evaluate the effects of amino acid substitutions on protein properties, several bioinformatics tools were used. Transmembrane protein structure prediction based on Phyre 2 webserver (Kelley *et al.*, 2015). The 3D structure model of OsHKT1;5 protein was predicted by SWISS-MODEL webserver (Waterhouse *et al.*, 2018). The UCSF Chimera v1.15 was used to visualize the 3-D protein structure (Pettersen *et al.*, 2004). To analyze the protein putative post-translational modifications, the MusiteDeep webserver was used for phosphorylation site prediction (Wang *et al.*, 2020) and SUMOplot™ (http://www.abgent.com/sumoplot) was applied to analyze the possible SUMOylation sites.

RESULTS AND DISCUSSION

Amplification of *OsHKT1;5* gene from sixteen rice cultivars

OsHKT1;5 gene sequences were amplified by PCR technique using specific primer pairs and

genomic DNA as a template (Figure 1). It could be seen that the PCR products were specific with corrected length as designed. Thus, the PCR products of *OsHKT1;5* gene were successfully amplified in all 16 investigated rice cultivars (Figure 1).

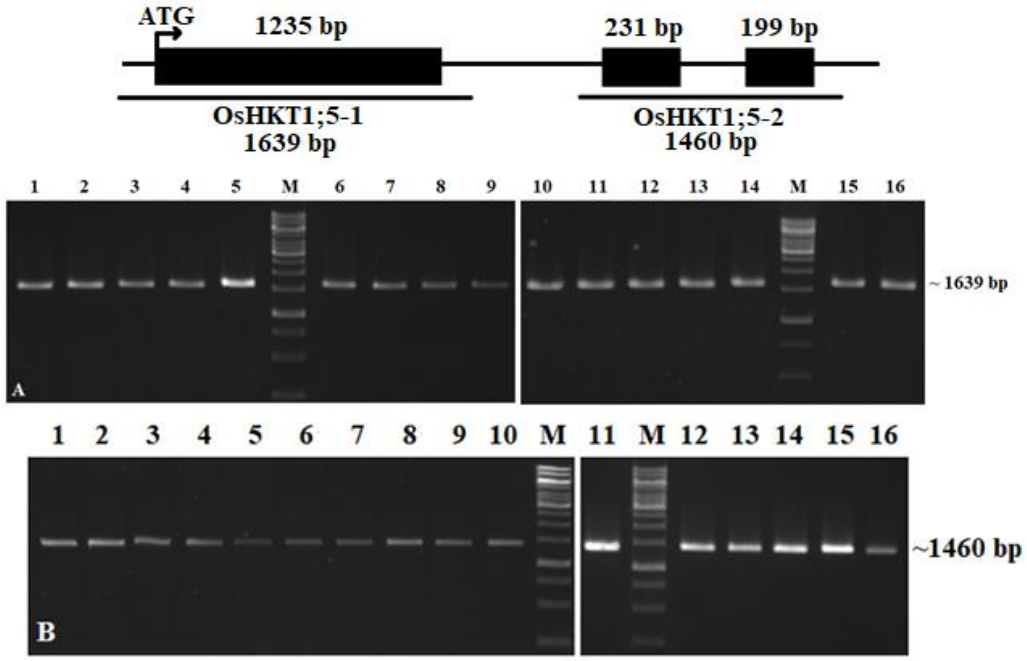
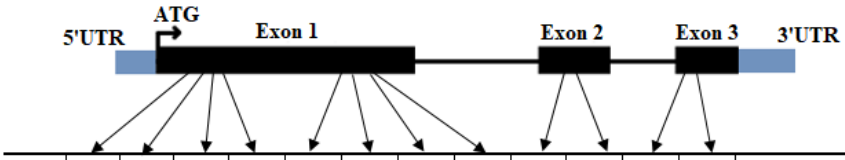


Figure 1. PCR amplification of *OsHKT1;5 gene*. A: PCR products of 1639 bp using primer pair OsHKT1;5-1; B: PCR product of 1460 bp using primer pair OsHKT1;5-2. M: 1 kb ladder marker; Lanes 1 to 16: PCR products from Man 1, Man 2, Chiem Rong, Cham, Tep Lai, Chiem Den, Nep Cuc, Pokkali, Nep Vai, Ngoi, Re Nuoc, Cham Bien, Nep Non Tre, Nep Oc, Chiem Cu, and Dau An Do, respectively

Polymorphisms in the *OsHKT1;5* coding sequence

To investigate the polymorphisms in the *OsHKT1;5* coding sequences, the PCR products of *OsHKT1;5* gene were sequenced. The coding sequences of *OsHKT1;5* of all 16 rice cultivars were deposited in NCBI GenBank with accession numbers as Man 1- MW822154, Tep Lai -MW822153, Re Nuoc -MW822151, Nep Vai -MW822150, Ngoi- MW822152, Man 2- MW822155, Nep Non Tre- MW822156, Nep Oc- MW822157, Pokkali- MW822158, Cham Bien- MW822159, Cham- MW822160, Chiem Den- MW822161, Chiem Cu- MW822162, Dau An Do- MW822163, Chiem Rong- MW822164, Nep Cuc- MW822166. Then, the obtained sequences were compared with sequence of the reference Nipponbare cultivar (Appendix figure 1). In total, there were 12 nucleotide substitutions in the *OsHKT1;5* coding sequence (Figure 2). Amongst 12 identified single nucleotide polymorphisms (SNPs), six were non-synonymous substitutions (G382A, C418G, G551A, C994G, C1183G, A1630C) and the other six were synonymous substitutions (G225A, C1038G, G1152A, G1261A, G1304C, C1608T). The four nucleotide substitutions G382A, C418G, G551A, and C994G were also reported in the study of Nguyen *et al*., 2019 using 22 high yielding rice cultivars. The non-synonymous substitutions G382A, C418G, G551A, C994G, C1183G, A1630C caused the amino acid changes, including D128N, P140A, R184H, H332D, V395L, and S544R, respectively (Table 2).



Site	225	382	418	551	994	1038	1152	1183	1261	1304	1608	1630	Cultivars
	G	G	C	G	C	C	G	C	G	G	C	A	Nipponbare, Nep Cuc, Ngoi Nep Vai, Re Nuoc
	G	**A**	**G**	G	C	C	G	C	G	G	C	A	Tep lai
	A	G	C	G	**G**	**G**	G	**G**	G	**C**	C	A	Man 1, Man 2
	G	G	**G**	**A**	**G**	C	**A**	**G**	G	**C**	C	A	Nep Non Tre, Nep Oc, Pokkali, Cham Bien, Cham
	G	**A**	**G**	G	**G**	C	G	**G**	G	**C**	C	A	Chiem Den, Chiem Cu, Dau An Do
	G	G	**G**	**A**	**G**	C	**A**	**G**	**A**	**C**	**T**	**C**	Chiem Rong

Figure 2. Schematic illustration of *OsHKT1;5* gene and polymorphism sites in the coding region. Bold letters represent the nucleotide polymorphisms in rice cultivars

To illustrate the putative consequences of nucleotide variations on protein properties, the structural model of OsHKT1;5 protein was predicted and positions of these amino acid polymorphisms on protein domains were modeled by using PHYRE2 webserver. It showed that OsHKT1;5 contains 10 transmembrane domains (TMD), and four amino acid sites Ser76, Gly 264, Gly391, Gly495 forming the sodium selective filter (Figure 3A, Figure 3B). This model is in agreement with study by Cotsaftis *et al.* (2012). The two substituted amino sites (L3595V, S544R) were shown to locate in the extracellular region, and four substituted amino sites (D128N P140A, H184R, and H332D) locate in the cytoplasmic region. The H332D is positioned in inner loop between TMD 5 and TMD 6, while L395V is located on the outer loop between TMD 6 and TMD 7 (Figure 3A). These sites are near the entrance of the filter pore. Thus, it was proposed that these substitutions might help enhance salt tolerance of tolerant rice cultivars (Ren *et al.*, 2005; Cotsaftis *et al.*, 2012; Shohan *et al.*, 2019). Our finding of five substitutions (D128N, P140A, H184R, H332D and L395V) were the same with the previous report of Negrão *et al.* (2013). However, the polymorphism site S544R in Chiem Rong cultivar was newly found in this study.

Previous studies indicated that amino acid alterations could affect the post-translational modifications which involved in the process of activation or suppression of the protein's activity (Negrão *et al.*, 2013; Friso, Wijk, 2015; Barker, Rinehart, 2018). The post-translational modifications are the important factors that can be regulated to influence the salinity tolerance of plants (Roy *et al.*, 2014). Our recent study showed that two substitutions (F179S, N258S) of OsHKT1;1 could lead to the post-translational modifications (phosphorylation, ubiquitination) in salt-tolerant cultivars Cham-Bien, Chiem-Rong, Nuoc-Man-1, Pokkali, Nuoc-Man-2; while the other substitution L94F caused SUMOylation modification was found only in salt-sensitive cultivar IR29 (Do *et al.*, 2020). Therefore, the post-translational modifications

of OsHKT1;5 protein were investigated. The results showed that while H332D substitution could enhance the putative phosphorylation of Tyrosine residue at position 331Y, S544R substitution led to the loss of phosphorylated Serine residue at 544. The L395V substitution increased the probability of the SUMOylation from 394 to 398 (V**V**DLS). Accordingly, the putative phosphorylation and SUMOylation were found in cultivars Man 1, Man 2, Dau An Do, Chiem Rong, Nep Non Tre, Nep Oc, Pokkali, Cham Bien, Chiem Den, and Chiem Cu. In contrast, the lack of phosphorylated Serine at 544 was found only in Chiem Rong cultivar. The remaining three substitutions have no effect on post-translational modifications of protein (Table 2). It might be interesting to further investigate the relationship between post-translational modifications of OsHKT1;5 protein with salt tolerant levels of different rice cultivars. Therefore, we plan to classify the salt tolerant levels of all different rice cultivars in order to elucidate the roles of post-translational modifications of OsHKT1;5 protein in regulation of its activity and consequence to salt tolerant ability of plants.

Table 2. Putative effect of nucleotide polymorphisms present in the *OsHKT1;5* coding region

Site	Substitution type		Effect on post-translational modification	Cultivars
225	G/A	Synonymous		Man 1, Man 2
382	G/A	Non- Synonymous **(D128N)**		Tep Lai, Chiem Den, Chiem Cu, Dau An Do
418	C/G	Non- Synonymous **(P140A)**		Tep Lai, Nep Non Tre, Dau An Do, Chiem Rong, Nep Oc, Pokkali, Cham Bien, Cham, Chiem Den, Chiem Cu
551	G/A	Non- Synonymous **(R184H)**		Nep Non Tre, Cham, Pokkali, Cham Bien, Nep Oc, Chiem Rong
994	C/G	Non- Synonymous **(H332D)**	Phosphorylation at 331Y	Man 1, Man 2, Dau An Do, Chiem Rong, Nep Non Tre, Nep Oc, Pokkali, Cham Bien, Cham, Chiem Den, Chiem Cu
1038	C/G	Synonymous		Man 1, Man 2
1152	G/A	Synonymous		Nep Non Tre, Cham, Pokkali, Cham Bien, Nep Oc, Chiem Rong
1183	C/G	Non- Synonymous **(L395V)**	Sumoylation from 394 to 398 (V**V**DLS)	Man 1, Man 2, Dau An Do, Chiem Rong, Nep Non Tre, Nep Oc, Pokkali, Cham Bien, Cham, Chiem Den, Chiem Cu
1261	G/A	Synonymous		Chiem Rong
1304	G/C	Synonymous		Man 1, Man 2, Dau An Do, Chiem Rong, Nep Non Tre, Nep Oc, Pokkali, Cham Bien, Cham, Chiem Den, Chiem Cu
1608	C/T	Synonymous		
1630	A/C	Non- Synonymous **(S544R)**	The absence of phosphorylation at 544S	Chiem Rong

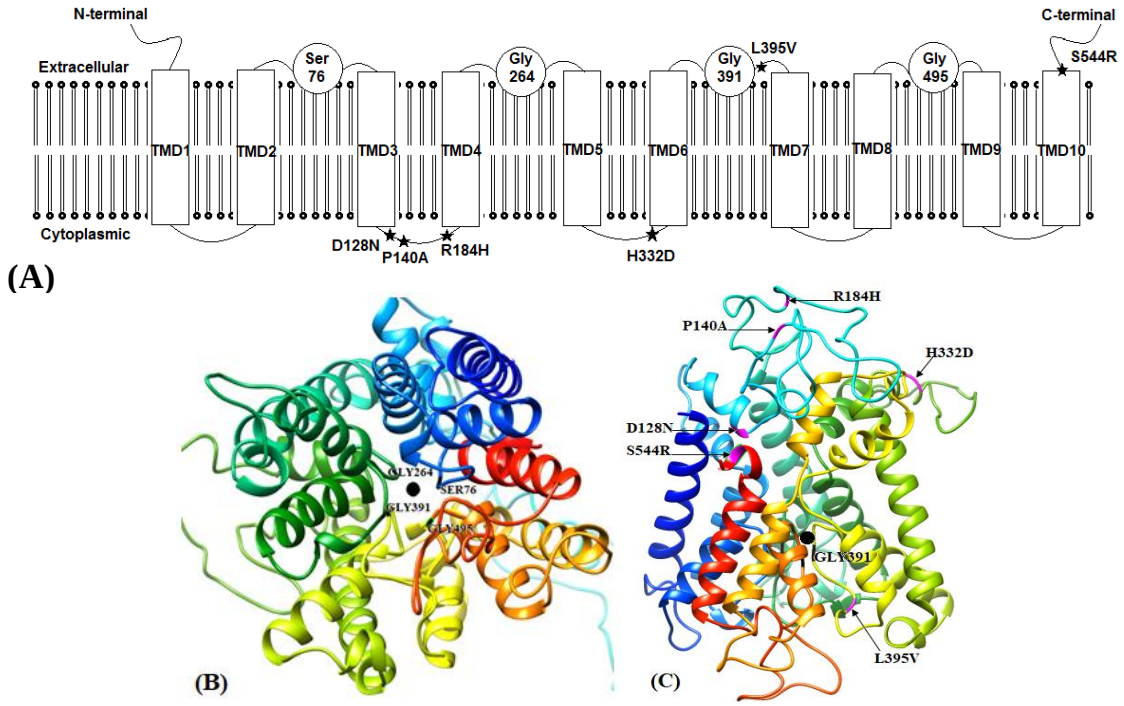


Figure 3. Modelling of OsHKT1;5 protein structure. A: The 2D model with motif of four transmembrane–pore loop–transmembrane domain. Asterisks indicate positions of substituted amino acids. B: 3D model of the OsHKT1;5 transporter from the top/from the side. The Ser-Gly-Gly-Gly motif forms the ion filter pore. **C**: Visualization from the side of protein model, showing positions of six amino acid variants.

CONCLUSION

In conclusion, we have succeeded in amplification and sequencing of *OsHKT1;5* gene in 16 rice cultivars. We could detect 12 polymorphisms in the coding sequence, including six synonymous substitutions and six non-synonymous substitutions which lead to changes in amino acid residues of OsHKT1;5 protein. Notably, the A1630C substitution is newly found in Chiem Rong cultivar. *In silico* analysis revealed that the SNPs in the coding sequence had no impact on protein structure but affected post-translational modifications of protein.

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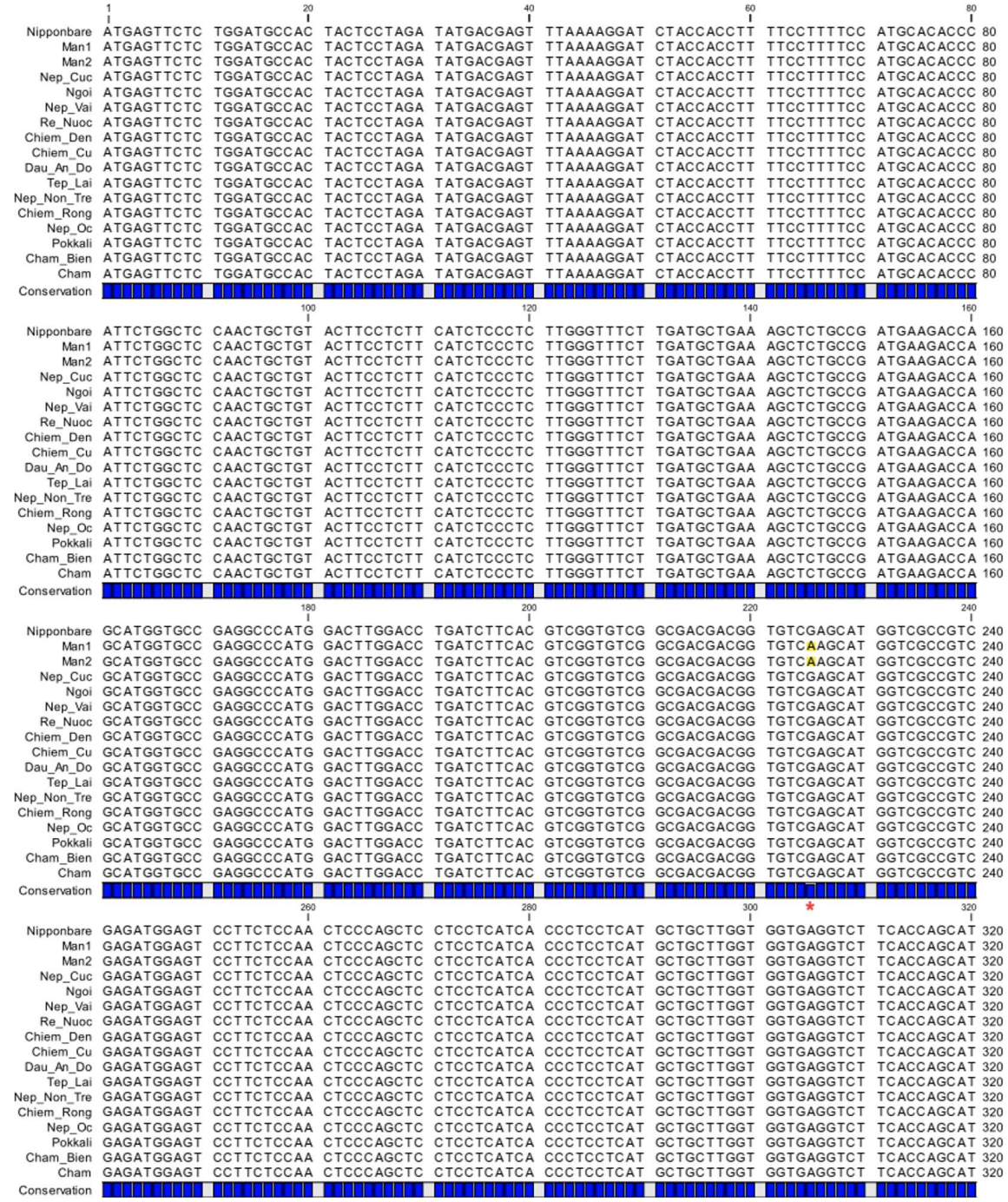
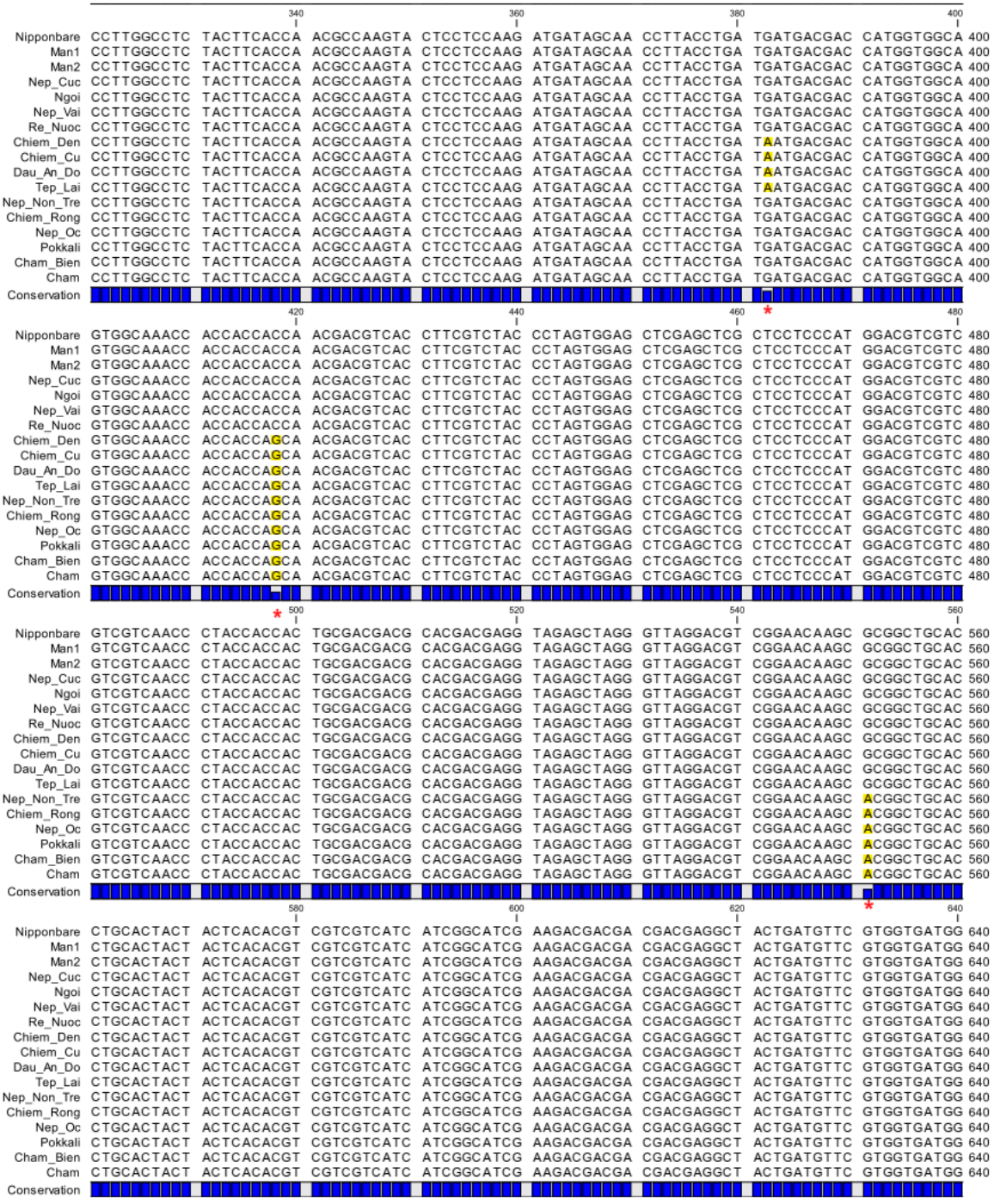
Appendix figure 1. Multiple sequence alignment of OsHKT1;5 gene coding sequences of 16 studied rice cultivars and reference Nipponbare cultivar. Red asterisk represents polymorphic site and yellow colour highlights polymorphic nucleotide

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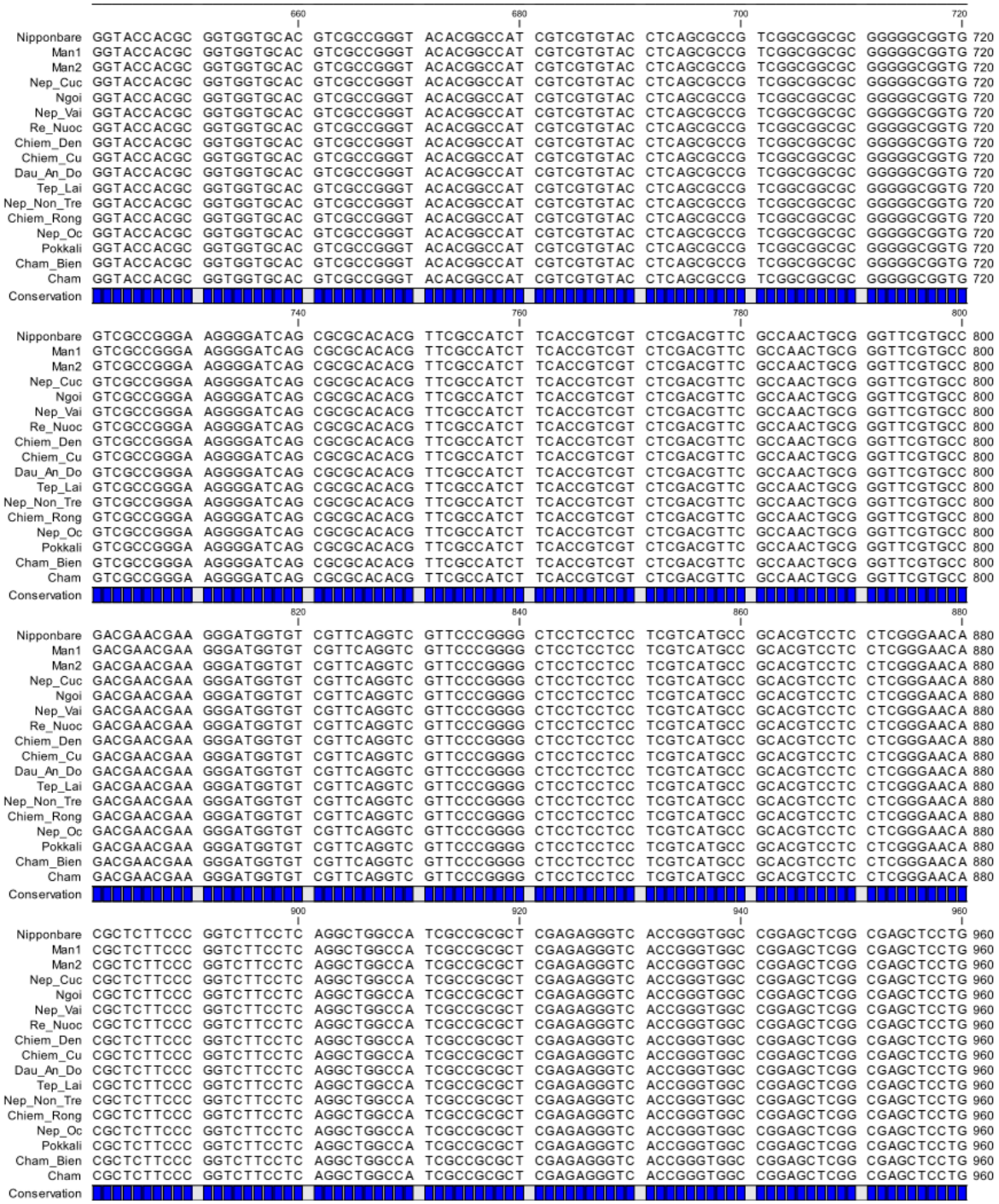
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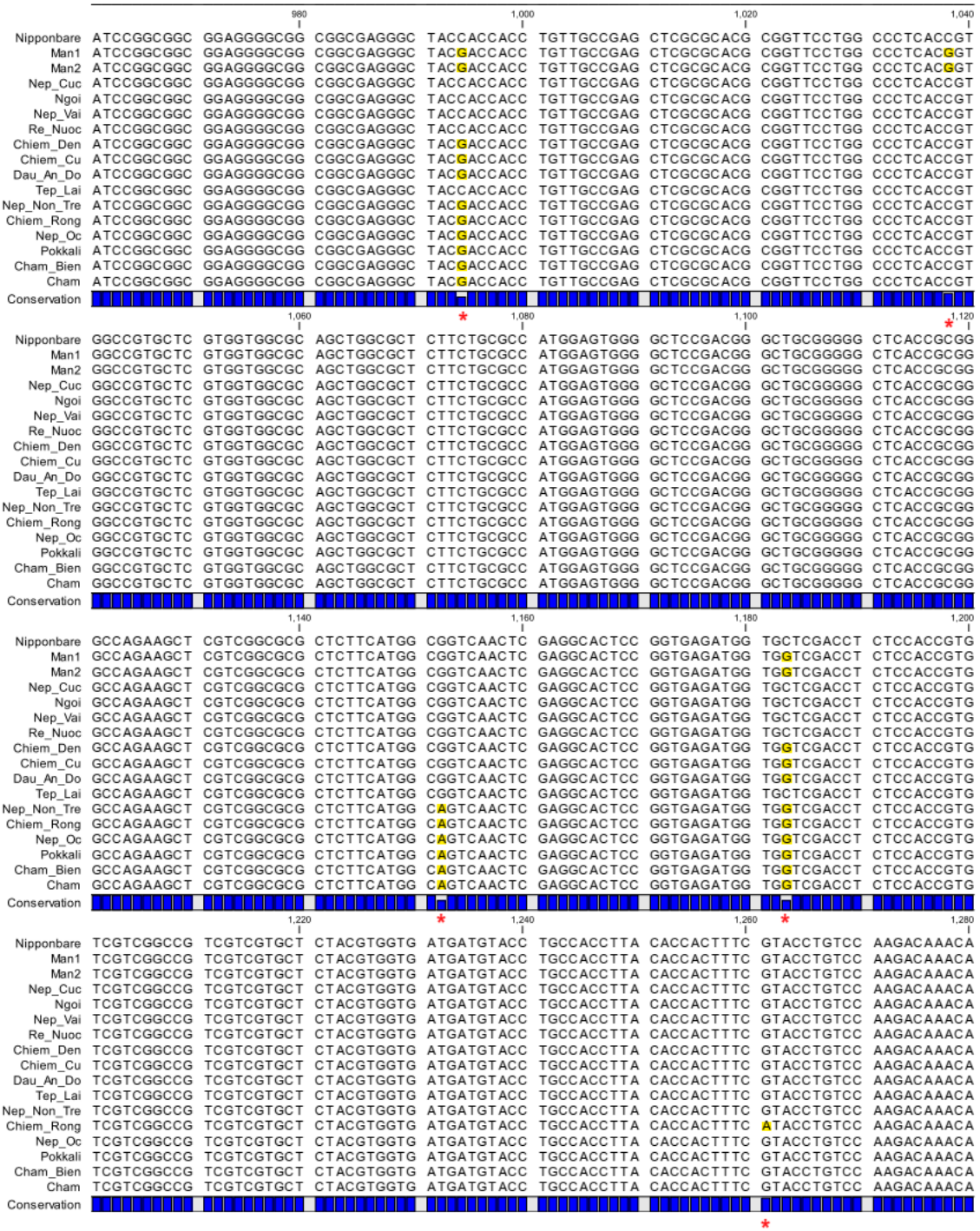
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Ngoi GGCCGTGCTC GTGGTGGCGC AGCTGGCGCT CTTCTGCGCC ATGGAGTGGG GCTCCGACGG GCTGCGGGGG CTCACCGCGG 1120
Nep_Vai GGCCGTGCTC GTGGTGGCGC AGCTGGCGCT CTTCTGCGCC ATGGAGTGGG GCTCCGACGG GCTGCGGGGG CTCACCGCGG 1120
Re_Nuoc GGCCGTGCTC GTGGTGGCGC AGCTGGCGCT CTTCTGCGCC ATGGAGTGGG GCTCCGACGG GCTGCGGGGG CTCACCGCGG 1120
Chiem_Den GGCCGTGCTC GTGGTGGCGC AGCTGGCGCT CTTCTGCGCC ATGGAGTGGG GCTCCGACGG GCTGCGGGGG CTCACCGCGG 1120
Chiem_Cu GGCCGTGCTC GTGGTGGCGC AGCTGGCGCT CTTCTGCGCC ATGGAGTGGG GCTCCGACGG GCTGCGGGGG CTCACCGCGG 1120
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Pokkali GGCCGTGCTC GTGGTGGCGC AGCTGGCGCT CTTCTGCGCC ATGGAGTGGG GCTCCGACGG GCTGCGGGGG CTCACCGCGG 1120
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Conservation
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Nep_Non_Tre GCCAGAAGCT CGTCGGCGCG CTCTTCATGG CAGTCAACTC GAGGCACTCC GGTGAGATGG TGGTCGACCT CTCCACCGTG 1200
Chiem_Rong GCCAGAAGCT CGTCGGCGCG CTCTTCATGG CAGTCAACTC GAGGCACTCC GGTGAGATGG TGGTCGACCT CTCCACCGTG 1200
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Conservation
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Dau_An_Do TCGTCGGCCG TCGTCGTGCT CTACGTGGTG ATGATGTACC TGCCACCTTA CACCACTTTC GTACCTGTCC AAGACAAACA 1280
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Chiem_Rong TCGTCGGCCG TCGTCGTGCT CTACGTGGTG ATGATGTACC TGCCACCTTA CACCACTTTC ATACCTGTCC AAGACAAACA 1280
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Pokkali TCGTCGGCCG TCGTCGTGCT CTACGTGGTG ATGATGTACC TGCCACCTTA CACCACTTTC GTACCTGTCC AAGACAAACA 1280
Cham_Bien TCGTCGGCCG TCGTCGTGCT CTACGTGGTG ATGATGTACC TGCCACCTTA CACCACTTTC GTACCTGTCC AAGACAAACA 1280
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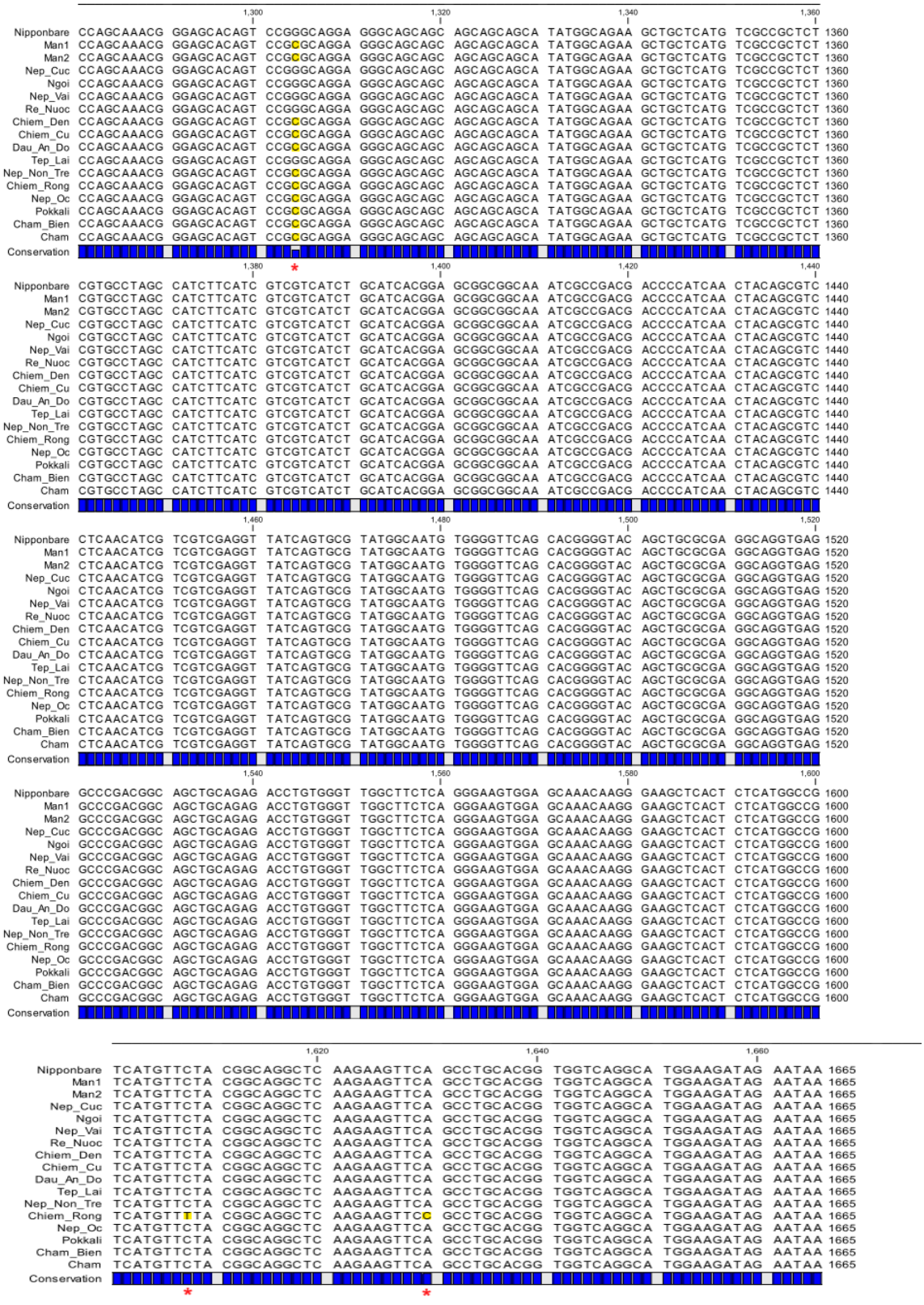
Conservation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Nep_Cuc CCAGCAAACG GGAGCACAGT CCGGGCAGGA GGGCAGCAGC AGCAGCAGCA TATGGCAGAA GCTGCTCATG TCGCCGCTCT 1360
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Nep_Vai CCAGCAAACG GGAGCACAGT CCGGGCAGGA GGGCAGCAGC AGCAGCAGCA TATGGCAGAA GCTGCTCATG TCGCCGCTCT 1360
Re_Nuoc CCAGCAAACG GGAGCACAGT CCGGGCAGGA GGGCAGCAGC AGCAGCAGCA TATGGCAGAA GCTGCTCATG TCGCCGCTCT 1360
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Tep_Lai CCAGCAAACG GGAGCACAGT CCGGGCAGGA GGGCAGCAGC AGCAGCAGCA TATGGCAGAA GCTGCTCATG TCGCCGCTCT 1360
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Conservation
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Nep_Cuc CGTGCCTAGC CATCTTCATC GTCGTCATCT GCATCACGGA GCGGCGGCAA ATCGCCGACG ACCCCATCAA CTACAGCGTC 1440
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Nep_Vai CGTGCCTAGC CATCTTCATC GTCGTCATCT GCATCACGGA GCGGCGGCAA ATCGCCGACG ACCCCATCAA CTACAGCGTC 1440
Re_Nuoc CGTGCCTAGC CATCTTCATC GTCGTCATCT GCATCACGGA GCGGCGGCAA ATCGCCGACG ACCCCATCAA CTACAGCGTC 1440
Chiem_Den CGTGCCTAGC CATCTTCATC GTCGTCATCT GCATCACGGA GCGGCGGCAA ATCGCCGACG ACCCCATCAA CTACAGCGTC 1440
Chiem_Cu CGTGCCTAGC CATCTTCATC GTCGTCATCT GCATCACGGA GCGGCGGCAA ATCGCCGACG ACCCCATCAA CTACAGCGTC 1440
Dau_An_Do CGTGCCTAGC CATCTTCATC GTCGTCATCT GCATCACGGA GCGGCGGCAA ATCGCCGACG ACCCCATCAA CTACAGCGTC 1440
Tep_Lai CGTGCCTAGC CATCTTCATC GTCGTCATCT GCATCACGGA GCGGCGGCAA ATCGCCGACG ACCCCATCAA CTACAGCGTC 1440
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Chiem_Rong CGTGCCTAGC CATCTTCATC GTCGTCATCT GCATCACGGA GCGGCGGCAA ATCGCCGACG ACCCCATCAA CTACAGCGTC 1440
Nep_Oc CGTGCCTAGC CATCTTCATC GTCGTCATCT GCATCACGGA GCGGCGGCAA ATCGCCGACG ACCCCATCAA CTACAGCGTC 1440
Pokkali CGTGCCTAGC CATCTTCATC GTCGTCATCT GCATCACGGA GCGGCGGCAA ATCGCCGACG ACCCCATCAA CTACAGCGTC 1440
Cham_Bien CGTGCCTAGC CATCTTCATC GTCGTCATCT GCATCACGGA GCGGCGGCAA ATCGCCGACG ACCCCATCAA CTACAGCGTC 1440
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Conservation
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Dau_An_Do CTCAACATCG TCGTCGAGGT TATCAGTGCG TATGGCAATG TGGGGTTCAG CACGGGGTAC AGCTGCGCGA GGCAGGTGAG 1520
Tep_Lai CTCAACATCG TCGTCGAGGT TATCAGTGCG TATGGCAATG TGGGGTTCAG CACGGGGTAC AGCTGCGCGA GGCAGGTGAG 1520
Nep_Non_Tre CTCAACATCG TCGTCGAGGT TATCAGTGCG TATGGCAATG TGGGGTTCAG CACGGGGTAC AGCTGCGCGA GGCAGGTGAG 1520
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Conservation
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Conservation
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