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Polymorphic analysis of the *OsHKT1;5* exon 1 gene region on seasonal rice varieties with salt tolerant capacity

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Abstract. *Nguyen TP, La HTG, Do TK, Tran DG. 2023. Polymorphic analysis of the OsHKT1;5 exon 1 gene region on seasonal rice varieties with salt tolerant capacity. Biodiversitas 24: 4159-4165.* In recent years, saline intrusion has caused serious damage to agricultural production in the Mekong Delta; therefore, research on salinity-tolerant rice varieties is necessary. This study was carried out to screen the salt tolerance capacity of 38 seasonal rice varieties in four concentrations of NaCl supplements, including 4‰, 6‰, 8‰, and 10‰ in comparison with IR28 and Pokkali checks according to IRRI (2021) methods. Eight rice cultivars in four levels of salinity tolerance (two varieties of each level) were selected to evaluate the nucleotide sequence polymorphism in the coding region of *OsHKT1;5* exon 1, compared with that of IR28 and Pokkali check cultivars and Nipponbare referential variety. The results of salt tolerance screening showed that the salt concentration and the rate of reduction of plant height and root length were positively correlated. Nucleotide sequence polymorphism analysis of *OsHKT1;5* exon 1 indicated six nucleotide substitutions (SNP) at positions 382, 418, 551, 994, 1119, and 1152. Further analysis revealed that five SNPs were detected with three missense mutations (P60A, R63H, and H255D) in salt tolerant check variety (Pokkali), three to four SNPs with one to two missense mutations H255D or P60A and H255D were observed in salt moderate tolerant varieties, while salt-sensitive varieties and sensitive check variety IR28 were observed with two SNPs and one missense mutation (H255D).

Keywords: *OsHKT1;5* gene, phenotypic screening, seasonal rice, SNP marker

INTRODUCTION

The Mekong Delta is considered one of the most affected places by climate change, in which saline intrusion has caused great consequences, especially on agricultural production in the whole region (Loc et al. 2021). Among abiotic stresses, salinity intrusion is recognized as one of the most serious hazards that seriously threaten crop production. High salt concentration affects seed germination, growth, and crop yield (Hussain et al. 2017); rice is considered one of the most sensitive species to salinity (Hussain et al. 2017; Kakar et al. 2019). According to Liu et al. (2019), the cause of damage to rice plants in saline environments is due to the excessive accumulation of Na^+ ions, which is directly toxic to plants, making Cl^- become the dominant anion in plants. In a saline environment, the accumulation of Na^+ is more than K^+, and excess Na^+ leads to homeostasis imbalance. When Na^+ excess is present in the roots, it competes with K^+, especially for low-affinity K^+ channels, resulting in a low K^+/Na^+ ratio in the cytoplasm. In addition, the accumulation of Na^+ ions in parts of the reproductive organs inhibits photosynthesis and starch transport, thereby reducing rice yield.

The mechanism of salt tolerance in rice has been reported in many studies as a complex mechanism controlled by many genes (Reddy et al. 2017). Many transport channels on plant cell membranes play a key role in salt stress tolerance mechanisms, particularly the Na^+ and K^+ channels involved in salt tolerance (Zhang et al. 2018). It is well-known that High-affinity K^+ transporters (HKTs) are essential determinants for salt tolerance and maintaining Na+ and K^+ homeostasis in rice plants (Rubio et al. 2019). The HKT protein family is segregated into two groups based on structure and transport characteristics. Group 1 HKT transporter has a serine at the first place of the S-G-G-G motif and is Na^+ selective transporter. This place at most of group 2 members was glycine, forming G-G-G-G motif and generally exhibiting Na^+-K^+ co-transport (Corratgé-Faillie et al. 2010). In rice, there are nine members of the HKTgene family, except *OsHKT1;2* is a pseudogene, 8 OsHKT genes were haplotype including *OsHKT1;1, OsHKT1;2, OsHKT1;3, OsHKT1;4, OsHKT1;5, OsHKT2;1, OsHKT2;3,* and *OsHKT2;4*, in which, three tolerant haplotypes were identified, one for the *HKT1;5* gene and two for the *HKT 2;3* gene (Mishra et al. 2016). A study by Shohan et al. (2019) showed that the *OsHKT1;5* gene encodes a protein that transports Na^+ ions from root to shoot, which is the key characteristic in salt tolerance of rice. In another study ((Natsuko et al. 2017), a novel function of *OsHKT1;5* was reported in mediating Na^+ exclusion in the phloem to prevent Na^+ transfer to young leaf blades. The *OsHKT1;5* is about 4,487 bp in length, in which the coding region is 1,665 bp, consisting of 3 exon regions with their length of 1,235 bp for exon 1, 231 bp for exon 2 and 199 bp for exon 3 (www.rapdb.dna.affrc.go.jp). Therefore, exon 1 was advantageous for sequencing and

contained enough information to detect SNPs. Landraces of traditional season rice were considered useful gene resources for abiotic stress tolerance, for instance, N22 for drought resistance trait (Vikram et al. 2016), flood tolerance contributed by FR13A, and Pokkali served as a parent for salinity resistance (Singh et al. 2016). This study focuses on analyzing the sequence polymorphisms of the exon 1 region of *OsHKT1;5* gene in landraces of seasonal rice varieties, thereby showing the change in protein structure and finding out the rules of salt tolerance mechanism for rice breeding.

MATERIALS AND METHODS

Materials

The experiment used 40 rice varieties, consisting of 38 seasonal rice varieties and IR28 and Pokkali varieties. The two latter varieties were used as controls (sensitive and tolerant varieties, respectively). These rice varieties were provided by the Cuu Long Delta Rice Research Institute, Tan Thanh, Thoi Lai, and Can Tho (Table 1).

Salt tolerance screening methods

Rice varieties were screened for salt tolerance in Yoshida solution (IRRI. 1976) according to the method of IRRI (2021) and evaluated as SES (standard evaluation system) of IRRI (2013) (Table 2). The present study was carried out in a completely randomized design with three replicates, including four treatments of NaCl concentrations including 0‰, 4‰, 8‰ and 10‰. During the experiment, the treatments were always maintained at pH=5, monitored every other day, adjusted pH and appropriate salt concentration, and the medium was renewed every seven days. The time to evaluate salt tolerance was when the standard sensitive check variety (IR28) died completely.

Investigation of nucleotides sequence polymorphism in *OsHKT1;5* gene

DNA extraction

Rice seedlings were cultured on petri dishes for about seven days until 10-15 cm tall, the rice leaves were collected, and DNA extraction was done according to the modified CTAB procedure (Aboul-Maaty and Oraby 2019).

OsHKT1;5 gene sequencing

Eight varieties of seasonal rice identified in four levels of salt tolerance (two varieties in each level) according to IRRI (2013) were selected, along with Pokkali and IR28, which were used for DNA extraction and target gene sequencing. Good-quality DNA samples were PCR-amplified with primers designed by Pha et al. (2019) to amplify the exon 1 region of the *OsHKT1;5* gene fragment. The primer pair sequences were given as F: 5'GGACCTGATTCTTCAC GTCGG3'; R: 5'GAGCACCCATCTCACCGGAG3'. The expected amplified product length is 1000 bps.

Table 1. The list of 40 seasonal rice varieties used

Code	Varieties name	Code	Varieties name	Code	Varieties name	Code	Varieties name
1	IR28 (sensitive check)	11	Lun sua	21	Thom mua	31	Mot bui lun Ca Mau
2	Pokkali (tolerance check)	12	Doc Phung	22	Tet ran	32	Mot bui trang
3	Ba bong man	13	Mong chim den	23	Trang bo cau	33	Nang quot bien
4	Lun phen	14	Nam tai	24	Trang tron	34	Nep sua
5	Lun do	15	Mong chim roi	25	Nang thom	35	Soi lun
6	Lun phen hat nho	16	Nang cum	26	Mot bui cao	36	Ngoc nu
7	Lun man	17	Ba bui lun	27	Lun can do	37	Tra long
8	Lun vang	18	Mot bui	28	Lun can trang	38	Ba bui
9	Lun phet	19	Mot bui do Ca Mau	29	Lun pheu	39	Nang quot bien 1
10	Lun hen	20	Nang gao trang	30	Mot bui lun	40	Bo liep

Table 2. The standard evaluation system for salt tolerance in rice (IRRI 2013)

Scale	Description	Tolerance
1	Normal growth, only the old leaves show white tips, while No symptoms on young leaves	Highly tolerant
3	Close to Normal growth; only leaf tips look burned, and a few older leaves partially become whitish	Tolerant
5	Growth is severely retarded; most old leaves are severely injured, and few young leaves elongating	Moderately tolerant
7	Complete cessation of growth most leaves dried, only a few young leaves still green	Sensitive
9	Almost all plants are dead or dying	Highly sensitive

The composition of each amplification reaction (Polymerase chain reaction - PCR) includes 10 µL master buffer 5X, 0.5 µL Taq Polymerase (5 unit/ 1µL); 2 µL primer R; 2 µL primer F (10 pmol each primer) and 2 µL DNA (~50ng). Subsequently, sterile double distilled water was added to reach 50 µL. The amplification reaction was carried out at 94°C for 2 minutes, then repeated 30 cycles with the following steps: denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 45 seconds, a final extension period at 72°C for 7 minutes, and a storage period maintained at 4°C. The amplified PCR products were subjected to gen electrophoresis on 2% agarose gel in 1X TBE buffer and imaged with Biorad UV 2000 gel imager. The sufficient-quality PCR product was sent to Phu Sa Biochemistry Co., Ltd., Can Tho City, for sequencing.

Data analysis

From the *OsHKT1;5* gene sequence data of rice varieties, Bioedit software was used to compare their sequences to find out SNPs markers. These SNPs markers were used to deduce the amino acids sequence in the corresponding protein. Therefore, the differences in protein among the surveyed rice varieties would be distinguished and compared with the salt-tolerant and sensitive check varieties.

RESULTS AND DISCUSSION

Salt tolerance evaluation of rice varieties

The results of salt tolerance screening (Figure 1, Figure 2, and Table 3) showed that all 40 rice varieties grew healthy in the 0‰ treatment. These mean that good seed quality, a high germination rate, and a suitable nutrient medium for rice were performed. In the treatment with a salt concentration of 4‰, after ten days of handling, almost all rice varieties grew normally, but in the following days, some of them showed signs of salt sensitivity (growth reduction, leaf color change). After a 30-day treatment, the standard sensitive IR28 check variety was completely dead, and the salt tolerance of rice varieties was evaluated. Whereby the majority of rice varieties were moderate to high salt tolerance (scales 3-5), accounting for 67.5% (27/40 varieties); a quarter of salt-sensitive varieties were found (scale 7), accounting for 25% (10/40 varieties), and rarely high salt-sensitive varieties were observed (scale 9), accounting for 7.5% (3/340 varieties). Lun Hen and Bo Liep were two rice varieties that were highly sensitive in 4‰ treatment, equivalent to IR28. Fourteen high salt-tolerant varieties were observed: Pokkali, Lun Man, Lun Vang, Lun Phet, Lun Sua, Doc Phung, Nang Cum, Luan Can Do, Lun Can Trang, Lun Pheu, Mot Bui Lun Ca Mau, Nang Quot Bien, Soi Lun, and Tra Long.

In treatments with added NaCl concentrations of 6‰, 8‰, and 10‰, most rice varieties had a remarkable salt sensitiveness after ten days of handling. In the 6‰ salt treatment, after 22 days of salt treatment (when the sensitive variety IR28 has completely died), the salt tolerance of the rice varieties was scored and distributed relatively even in three levels of salt tolerance. The number of varieties with moderate to high salt tolerance was about one-third of total rice varieties (13/40 varieties), accounting for 32.5%; similarly, the salt-sensitive and highly sensitive varieties were evenly distributed with 35% and 32.5%, respectively (14/40 and 13/40 varieties). Five varieties were found to have high salt tolerance, including Pokkali, Doc Phung, Lun Can Do, Lun Can Trang, and Lun Pheu (Table 3).

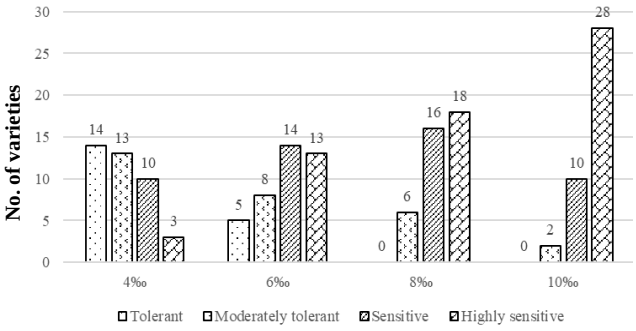

Figure 2. Salt tolerance evaluation of 40 rice varieties in Yoshida medium supplement with NaCl concentrations of 4 ‰, 6‰, 8‰, and 10‰ when IR28 completely died

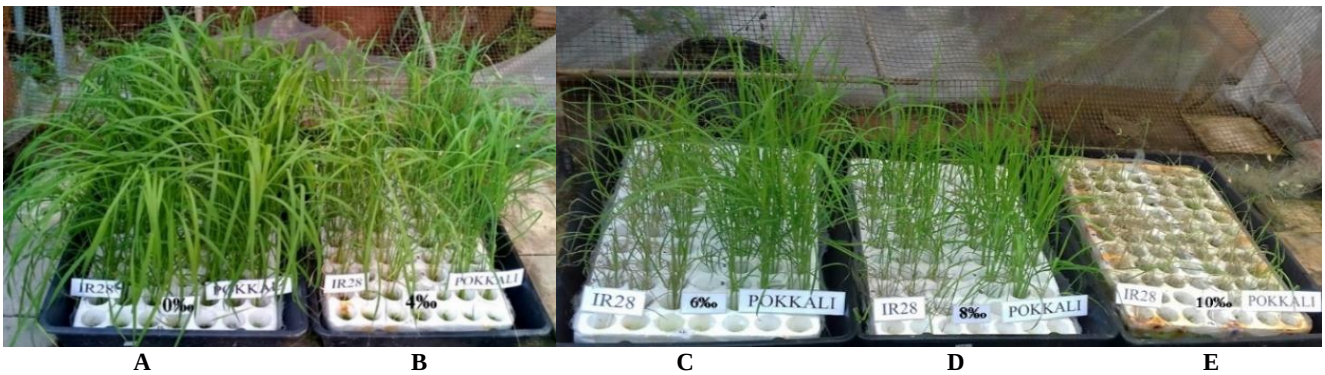

Figure 1. Salt tolerance screening of 40 rice varieties in Yoshida medium supplemented with NaCl concentrations of: A. 0‰, B. 4 ‰, C. 6‰, D. 8‰, and E. 10‰ at 16 days after treatment

In the two remaining treatments with 8‰ and 10‰ NaCl concentrations (assessment after 19 and 13 days of salt addition), most rice varieties were sensitive and highly sensitive (scale 7-9), with 34 and 38 per 40 varieties, respectively, accounting for 85% and 95%. Especially in the treatment of 10‰, twenty-eight per forty rice varieties (70.0%) showed a highly sensitive response (scale 9), and all the plants died in the saline environment (Table 3). Six rice varieties (Pokkali, Doc Phung, Lun Can Do, Lun Can Trang, Lun Pheu, and Mot Bui Lun) exhibited moderate salt tolerance in 8‰ treatment, while only two rice varieties (Pokkali and Doc Phung) could do the same in 10‰ treatment.

The salt tolerance of testing rice varieties decreased gradually over the concentrations, in which the majority of moderate and high tolerance was observed (grades 3, 5) in the 4‰ and less tolerance in the 6‰ NaCl concentration treatment. Sensitive response increased in the 8‰ and 10‰ salt treatments with a predominant number of sensitive and high sensitive (scale 7, 9). This result is similar to the study of Thai and Dung (2013), which showed that the number of varieties with high tolerance was very small. Whereby only 24/244 and 16/244 rice varieties were observed with high tolerance at 4‰ salt concentration and 6‰ NaCl concentration, respectively (accounting for 10% and 6.5%); the moderately tolerant varieties were reported at 100/244 and 34/244 rice varieties in 4‰ salt concentration and 6‰ NaCl concentration, respectively. Minh et al. (2016) announced that 19 out of 36 tested rice varieties exhibited moderate to high salt tolerance at 6‰ salinity. Also, in a 6‰ salt concentration medium, research by Rubel et al. (2014) and Bhowmik et al. (2009) reported that 12/27 rice varieties were tolerant, and 7/11 were moderate to high salt tolerances, respectively. This finding is consistent with the breeding of new rice varieties for salt tolerance at 4‰ to 6‰ salt concentrations (OM5629, OM9921, OM6677, OM10252).

Table 3. Salt tolerant evaluation of 40 rice varieties in Yoshida medium supplement with NaCl concentrations of 0‰, 4 ‰, 6‰, 8‰, 10‰ according to SES of IRRI (2013)

Code (1)	Varieties name (2)	0‰ (3)	4‰ (4)	6‰ (5)	8‰ (6)	10‰ (7)	Average of (4) to (7)
2	Pokkali**	1	3	3	5	5	4.0
12	Doc phung	1	3	3	5	5	4.0
28	Lun can trang	1	3	3	5	7	4.5
29	Lun pheu	1	3	3	5	7	4.5
27	Lun can do	1	3	3	5	9	5.0
9	Lun phet	1	3	5	7	7	5.5
11	Lun sua	1	3	5	7	7	5.5
35	Soi lun	1	3	5	7	7	5.5
7	Lun man	1	3	5	7	9	6.0
8	Lun vang	1	3	5	7	9	6.0
33	Nang quot bien	1	3	5	7	9	6.0
16	Nang cum	1	3	7	7	7	6.0
31	Mot bui lun Ca Mau	1	3	7	7	7	6.0
37	Tra long	1	3	7	7	7	6.0
30	Mot bui lun	1	5	5	5	9	6.0
32	Mot bui trang	1	5	5	7	9	6.5
13	Mong chim den	1	5	7	7	7	6.5
24	Trang tron	1	5	7	7	7	6.5
5	Lun do	1	5	7	7	9	7.0
6	Lun phen hat nho	1	5	7	7	9	7.0
22	Tet ran	1	5	7	7	9	7.0
39	Nang quot bien 1	1	5	7	7	9	7.0
20	Nang gao trang	1	5	7	9	9	7.5
25	Nang thom	1	5	7	9	9	7.5
26	Mot bui cao	1	5	7	9	9	7.5
21	Thom mua	1	5	9	9	9	8.0
36	Ngoc nu	1	5	9	9	9	8.0
3	Ba bong man	1	7	7	9	9	8.0
23	Trang bo cau	1	7	7	9	9	8.0
4	Lun phen	1	7	9	9	9	8.5
14	Nam tai	1	7	9	9	9	8.5
15	Mong chim roi	1	7	9	9	9	8.5
17	Ba bui lun	1	7	9	9	9	8.5
18	Mot bui	1	7	9	9	9	8.5
19	Mot bui do Ca Mau	1	7	9	9	9	8.5
34	Nep sua	1	7	9	9	9	8.5
38	Ba bui	1	7	9	9	9	8.5
40	Bo liep	1	9	9	9	9	9.0
10	Lun hen	1	9	9	9	9	9.0
1	IR28*	1	9	9	9	9	9.0
	Average	1.0	5.1	6.8	7.6	8.3	

Among the 40 rice varieties screened, three varieties, Lun Can Trang, Doc Phung and Lun Pheu, showed high tolerance across treatments, nearly equivalence to the Pokkali rice variety. This result is consistent with the fact that the rice variety of Doc Phung has been used as a standard variety for salt tolerance instead of Pokkali (Duy and Lien 2022). Meanwhile, the two rice varieties, Bo Liep and Lun Hen were evaluated to have the most salt sensitiveness, equivalent to IR28 scored a scale of 9 in all treatments with salt addition.

Exon 1 region of *OsHKT1;5* gene amplification

The results of the salt tolerance evaluation of 40 rice varieties in this study (Table 3) showed that their salt tolerance levels were different in the gradual salt concentrations of 4‰, 6‰, 8‰, 10‰, and compared with the control (0‰), which at 6‰ salt concentration, there was a relatively uniform distribution of salt tolerance scales according to the SES evaluation of IRRI (2013), with 4 scales of salt tolerance that included 6 varieties evaluated as scale 3, seven varieties in scale 5, fourteen varieties in scale 7, and 13 varieties observed as scale 9 (Figure 2). Furthermore, in the Mekong Delta rice production, a salinity of 6‰ is the highest limit that can be planned for rice cultivation. Therefore, this concentration was used as the main factor in the responses of rice varieties to other salt concentrations to select rice varieties with different tolerance levels to investigate the *OsHKT1;5* gene polymorphism (Table 4).

Specifically, eight rice varieties corresponding to 4 scales of salt tolerance (Table 4), Lun Pheu and Doc Phung in scale 3, Mot Bui Trang and Nang Quot Bien in scale 5, Lun Do, and Lun Phen Hat Nho in scale 7, and Bo Liep, Ngoc Nu in scale 9, along with two check rice varieties, IR28 and Pokkali were selected and subjected to DNA extraction (Table 4) and PCR amplification with specific primers and sequences to find out SNP marker for evaluation of the *OsHKT1;5* gene polymorphism.

The results of exon 1 sequencing of 10 rice varieties showed a stable signal region of 938 bp length, extending from the 230th nucleotide position to the 1168th nucleotide position. The target DNA fragments were amplified and sequenced, which resulted in 6 nucleotide substitutions (SNPs) when referenced with the Nipponbare rice sequence in the database at the nucleotide positions of 382nd, 418th, 551st, 994th, 1119th and 1152nd. In total, ten varieties were sequenced; there was one variety with five nucleotide substitutions (Pokkali), accounting for 10%; another variety with four nucleotide substitutions, accounting for 10% (Nang Quot Bien), three varieties were recorded with 3 SNP markers accounting for 30% (Lun pheu, Doc Phung, Mot Bui Trang), and the remaining five varieties observed with 2 SNP markers (Lun Do, Lun Phen Hat Nho, Bo Liep, Lun Hen, and IR28), accounting for 50% (Table 5).

Table 4. Saline-tolerant rice varieties at different scales in 6‰ salt concentration and selected varieties for gene sequencing

Scales	Variety name	Selected for sequencing
Controls	Pokkali (standard tolerance) IR28 (standard sensitive)	Pokkali (standard tolerance) IR28 (standard sensitive)
Scale 3	Lun Can Do, Lun Can Trang, Lun Pheu, Mot Bui Lun, Doc Phung	Lun Pheu, Doc Phung
Scale 5	Lun Man, Lun Vang, Lun Phet, Lun Sua, Mot Bui Trang, Nang Quot Bien, Soi Lun	Mot Bui Trang, Nang Quot Bien
Scale 7	Ba Bong Man, Lun Do, Lun Phen Hat Nho, Mot Bui Lun Ca Mau, Tra Long, Nang Quot Bien 1, Mong Chim Den, Nang Cum, Nang Gao Trang, Tet Ran, Trang Bo Cau, Trang Tron, Nang Thom, Mot Bui Cao	Lun Do, Lun Phen Hat Nho
Scale 9	Bo Liep, Lun Phen, Lun Hen, Nep Sua, Ngoc Nu, Ba Bui, Nam Tai, Mong Chim Roi, Ba Bui Lun, Mot Bui, Mot Bui Do Ca Mau, Thom Mua	Bo Liep, Lun Hen

Table 5. Polymorphism of exon 1 region of *OsHKT1;5* gene

SNP position	382	418	551	994	1119	1152	Varieties with nucleotides change
Amino acid substitutions	SS	P60A	R63H	H255D	SS	SS	
Nipponbare	G	C	G	C	G	G	
	-	G	A	G	C	A	Pokkali
	A	G	-	G	C	-	Nang Quot Bien
	-	-	-	G	C	A	Lun Pheu, Doc Phung, Mot Bui Trang
	-	-	-	G	C	-	Bo Liep, Lun Do, Lun Phen Hat Nho, Lun Hen, IR28

∗Note: SS: synonymous substitutions

Among 6 SNP markers, three SNPs led to nonsynonymous mutations (missense variant), and three other SNPs gave synonymous substitutions. Three nonsynonymous substitutions occurred at the 418th, 551st, and 994th nucleotide positions. Whereby, at the 418th position, C nucleotide was replaced by G (C418G), corresponding Proline amino acid (at the 60th position) was substituted by Alanine (P60A). This replacement was found in the Nang Quot Bien and Pokkali rice varieties. At the 551st nucleotide position, G nucleotide was replaced by A nucleotide (G551A), distorted Arginine amino acid to Histidine at the 63rd position (R63H, available in the Pokkali variety). The replacement of C nucleotide by G, led to a change in the Histidine amino acid to Aspartate at the 255th position (H255D, found in all ten varieties). The nucleotide substitutions at the 382nd, 1119th, and 1152nd positions indicated that the nucleotide change did not change the amino acid profile on exon 1 (Table 5). This can be explained by the fact that the genetic code is degenerate; multiple sets of genetic code can encode an amino acid.

The results of this research are similar to that reported by Pha et al. (2019), surveying the exon 1 region of the *OsHKT1;5* gene polymorphism in 22 high-yielding rice varieties, in which five nucleotide substitutions were found leading to 5 amino acid changes on the coding region. However, the rate of SNP found in this study is lower than that of Negrão et al. (2013) on 392 rice varieties, in which 29 SNPs were found in the *OsHKT1;5* gene; or the study of Mishra et al. (2016) on 299 wild rice accessions collected in India, 45 SNP markers were detected, including 8 SNPs in the coding region and 37 SNPs in the non-coding region.

The results of sequencing the exon 1 region of the *OsHKT1;5* gene is consistent with the results of phenotypic salt tolerance screening of 40 rice varieties in this research. Specifically, 5 SNP markers on exon 1 region of *OsHKT1;5* gene were found in the salt tolerance check Pokkali variety, including C418G, G551A, C994G, G1119C, and G1152A, in which three missense mutations P60A, R63H, and H255D were observed. The rice varieties Lun Pheu, Doc Phung, Mot Bui Trang, and Nang Quot Bien were respectively observed with salt tolerance of scales 3 and 5 in 6‰ salinity (two varieties each scale), which were detected with 3 SNP markers (C994G, G1119C, and G1152A) for Lun Pheu, Doc Phung, Mot Bui Trang, and 4 SNPs (G382A, C418G, C994G, and G1119C) for Nang Quot Bien rice varieties, in which two missense mutations P60A and H255D were observed in Nang Quot Bien variety, while only one missense mutations H255D was detected in the other three varieties. The rice varieties Bo Liep, Lun Do, Lun Phen Hat Nho, and Lun Hen were evaluated at sensitiveness, and high sensitiveness (scale 7 and scale 9) were found with two nucleotide differences on exon 1 region of the *OsHKT1;5* gene (C994G and G1119C) with only one missense mutation (H255D), equivalent to that of check sensitive IR28 variety. This result strengthened the hypothesis that the nucleotide changes in the genotype (SNP) compared with the original gene sequence can help rice plants better adapt to the adverse environment (salinity stress), increasing the viability of plants in general and rice in particular. On the other hand, research of Quynh-Hoa et al. (2016) and Do et al. (2016) indicated that it is quite difficult to point out the relationship between nucleotide polymorphism in the exon 1 region of *OsHKT1*, and *OsHKT1;2* genes and the salt tolerance level of the investigated rice cultivars. It might be helpful to explore the nucleotide polymorphism in *the OsHKT1;5* gene, which regulates gene expression.

The mechanism of plant adaptation in general, particularly rice in the salt stress environment, is very complex, involving physiological characteristics, metabolic processes and the interaction of many genes on chromosomes. In addition, the tolerance to salinity may change according to the plant growth stages. The results of this study are useful in future research on the influence of SNP changes on the salt tolerance of rice for the new breeding approach.

In conclusion, forty rice varieties were screened in Yoshida nutrient medium supplemented with salt concentrations (4‰, 6‰, 8‰, 10‰), showing that salt concentrations affected the growth and development of all rice varieties surveyed from the 6‰ salinity. Among 40 rice varieties surveyed, Doc Phung and Lun Pheu varieties have high salt tolerance at salt concentrations of 4‰ - 6‰, equivalent to Pokkali tolerant varieties. The results of polymorphism analysis of exon 1 region of *OsHKT1;5* gene of 8 rice varieties distributed in four scales of salt tolerance along with the standard IR28 and Pokkali varieties indicated that there were six single nucleotide polymorphisms detected at the 382nd, 418th, 551st, 994th, 1119th, and 1152nd positions. Among these 6 SNP markers, three SNPs resulted in synonymous substitutions, and the other three SNPs were missense mutations (P60A, R63H and H255D). These mutations may contribute to the salt tolerance of rice varieties.

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REFERENCES

Aboul-Maaty NA, Oraby HA. 2019. Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bull Natl Res Cent 43: 25. DOI: 10.1186/s42269-019-0066-1.

Bhowmik U, Kibria MG, Rhaman MS, Murata Y, Hoque MA. 2009. Screening of rice genotypes for salt tolerance by physiological and biochemical characters. Plant Sci Today 8: 770-775. DOI: 10.14719/pst.2021.8.3.1098.

Corratgé-Faillie C, Jabnoune M, Zimmermann S, Véry AA, Fizames C, Sentenac H. 2010. Potassium and sodium transport in non-animal cells: The Trk/Ktr/HKT transporter family. Cell Mol Life Sci 67 (15): 2511-32. DOI: 10.1007/s00018-010-0317-7.

Do PT, Hoang YH, Le MQ, Tang HT, Nguyen DH. 2016. OsHKT1;3 gene sequence polymorphisms and expression profile in rice (*Oryza sativa* L.). Afr J Agric Res 13: 2659-2667. DOI: 10.5897/ajar2018.13549.

Duy PVK, Lien QTA. 2022. Evaluation of salt-tolerant traditional rice varieties in Mekong Delta, Vietnam. Research Square. DOI: 10.21203/rs.3.rs-1499963/v1.

Hussain S, Zhang J-H, Zhong C, Zhu L-F, Cao X-C, Yu S-M, Bohr JA, Hu JJ, Jin Q-Y. 2017. Effects of salt stress on rice growth, development characteristics, and the regulating ways: A review. J Integr Agric 16: 2357-2374. DOI: 10.1016/S2095-3119(16)61608-8.

IRRI [International Rice Research Institute]. 1976. Laboratory Manual for Physiological Studies of Rice. International Rice Research Institute. https://books.google.com.vn/books?id=CakaugAACAAJ.

IRRI [International Rice Research Institute]. 2013. Standard evaluation system for rice. P.O. Box 933, 1099. Manila; Philippines. 2013. IRRI. SES Standard evaluation system for Rice | RICEPEDIA

IRRI [International Rice Research Institute]. 2021. Phenotyping protocols for abiotic stress tolerance in rice. International Rice Research Institute. Los Baños, Philippines. https://books.google.co.uk/books?id=dwMlEAAAQBAJ

Kakar N, Jumaa SH, Redoña ED, Marilyn L. Warburton, Reddy KR. 2019. Evaluating rice for salinity using pot-culture provides a systematic tolerance assessment at the seedling stage. Rice 12: 57. DOI: 10.1186/s12284-019-0317-7.

Liu J, Shabala S, Shabala L, Zhou M, Meinke H, Venkataraman, Chen Z, Zeng F, Zhao Q. 2019. Tissue-specific regulation of Na+ and K+ transporters explains genotypic differences in salinity stress tolerance in rice. Front Plant Sci 10: 1361. DOI: 10.3389/fpls.2019.01361.

Loc HH, Binh DV, Park E, Shrestha S, Dung TD, Son VH, Truc NHT, Mai NP, Seijger C. 2021. Intensifying saline water intrusion and drought in the Mekong Delta: from physical evidence to policy outlooks. Sci Total Environ 757: 143919. DOI: 10.1016/j.scitotenv.2020.143919.

Minh PT, Thuc LV, Dung TN. 2016. Application of SSR technique for the identification of markers linked to salinity tolerance and planthopers in F1 and BC1F1 rice generation. Can Tho University J Sci Agriculture 3: 160-169. DOI: 10.22144/ctu.jsi.2016.084. [Vietnamese]

Mishra S, Singh B, Panda K, Singh BP, Singh N, Misra P, Rai V, Singh NK. 2016a. Association of SNP haplotypes of HKT family genes with salt tolerance in indian wild rice germplasm. Rice (N Y) 9: 15. DOI: 10.1186/s12284-016-0083-8.

Natsuko I, Kobayashi, Naoki Y, Hiroki Y, Kaoru O, Hiroki U, Alex C, Keitaro T, Hideo M, Miho F, Tomoki H, Mohammad Al N, Sergey S, Gynheung A, Jian F, Tomoaki H. 2017. *OsHKT1;5* mediates Na+ exclusion in the vasculature to protect leaf blades and reproductive tissues from salt toxicity in rice. Plant J 91: 657-670. DOI: 10.1111/tpj.13595.

Negrão S, Almadanim MC, Pires IS, Abreu IA, Maroco J, Courtois B, Gregorio GB, Mcnally KL, Oliveira MM. 2013. New allelic variants found in key rice salt-tolerance genes: An association study. Plant Biotechnol J 11: 87-100. DOI: 10.1111/pbi.12010.

Pha NT, Phung NT, Truc NTN, Khang DT, Gioi TD. 2019. Investigation of polymorphisms of exon 1 region of OsHKT1; 5 gene in high yielding rice. Intl J Genet Mol Biol 11: 1-5. DOI: 10.5897/IJGMB2019.0175

Quynh-Hoa P, Xuan-An T, Nha-Trang NT, Thuy-Anh TT, Hai-Yen H, Hong-Van NT, Thi-Hanh T, Thi-Phuc D. 2016. Investigation of polymorphisms in coding region of OsHKT1 in relation to salinity in rice. Rice Sci 23: 334-338. DOI: 10.1016/j.rsci.2016.05.004.

Reddy INBL, Kim B-K, Yoon I-S, Kim K-H, Taek-Ryoun Kwon T-R. 2017. Salt tolerance in rice: Focus on mechanisms and approaches. Rice Sci 24: 123-144. DOI: 10.1016/j.rsci.2016.09.004.

Rubel M, Hassan L, Islam M, Robin A, Alam MJ. 2014. Evaluation of rice genotypes under salt stress at the seedling and reproductive stages using phenotypic and molecular markers. Pak J Bot 46: 423-432.

Rubio F, Nieves-Cordones M, Horie T, Shabala S. 2019. Doing 'business as usual' comes with a cost: Evaluating energy cost of maintaining plant intracellular K+ homeostasis under saline conditions. New Phytol 225: 1097-1104. DOI: 10.1111/nph.15852.

Shohan MUS, Sinha S, Nabila F, Dastidar S, Seraj Z. 2019. HKT1;5 transporter gene expression and association of amino acid substitutions with salt tolerance across rice genotypes. Front Plant Sci 10: 1420. DOI: 10.3389/fpls.2019.01420.

Thai LX, Dung TN. 2013. Selecting rice varieties tolerant to salinity in the Mekong Delta of Vietnam. Can Tho Univ J Sci Part B Agric Fisheries Biotechnol 28: 79-85.

Vikram P, Kadam S, Singh BP, Lee YJ, JK, Singh S, Singh ON, Swamy BPM, Thiyagarajan K, Singh S, Singh NK. 2016. Genetic diversity analysis reveals importance of green revolution gene (Sd1 locus) for drought tolerance in rice. Agric Res 5: 1-12. DOI: 10.1007/s40003-015-0199-x.

Zhang Y, Fang J, Wu X, Dong L. 2018. Na+/K+ balance and transport regulatory mechanisms in weedy and cultivated rice (*Oryza sativa* L.) under salt stress. BMC Plant Biol 18: 375. DOI: 10.1186/s12870-018-1586-9.