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Study on Polymorphisms of *OsHKT2;4* Gene in some Vietnamese Rice

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Abstract: Salt stress leads to serious inhibiting of the growth and development of plants. The selection and generation of plants resistant to salinity is effective and sustainable way to overcome the saline soil. It has been proven that the HKT transporter is involved in salinity tolerance in plants. *OsHKT2;4* gene is a member of class II of HKT gene family in rice encoding protein which mediates Na^+-K^+ cotransport and at high Na^+ concentration preferred Na^+ -selective transport. In this study, the natural variations in *OsHKT2;4* gene sequence was investigated in some Vietnamese rice cultivars. The full length of gene sequence was amplified by PCR, followed by direct sequencing of PCR product. Analysis of obtained sequences among cultivars revealed the 11 single nucleotide polymorphisms, consisting of 8 sites in exons and 3 sites in intron. Further analysis showed that these 8 substitutions in exons were non-synonymous which caused changes in amino acids at signal peptide (S11F and N17T), at the loop (T66V, T84I, S133L, S342N) and the transmembranes (V53M, L253F).

Keywords: Genetic polymorphism, HKT, rice, *OsHKT2;4*.

1. Introduction

Specific plant membrane proteins HKTs (High-affinity potassium transporters) transport ions across the cell membrane. The members of HKT protein family were found in many plant species and had been shown to play roles in salinity tolerance [1-5]. Based on the ability to transport specific ions, HKT proteins are divided into two groups: the Na^+ carrier selection group (group I) and the Na^+ - K^+ symport group (group II) [6].

The number of HKT gene members varies between species [2, 3]. In rice (*Oryza sativa*), there are 9 genes coding HKT proteins, dividing in two groups: group I consists of *OsHKT1;1*, *OsHKT1;2*, *OsHKT1;3*, *OsHKT1;4*, *OsHKT1;5* and group II consists of *OsHKT2;1*, *OsHKT2;2*, *OsHKT2;3*, *OsHKT2;4* [7-10].

The *OsHKT2;4* gene has been determined to express in vasculature of root cells, leaf sheaths, mesophyll cells and the base of stem. It is proposed that OsHKT2;4 plays possible role in K^+ transporter involved in both nutritional K^+ uptake and long distance K^+ transport, and also can transport Na^+, Ca^+, Mg^+ [11].

Analysis of genetic polymorphisms in *HKT* genes recovered the functions of genes involved in the salt adaption mechanisms of the plant [12, 13]. Study on *OsHKT1;5* in two rice

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cultivars differing in salt tolerance showed that in the coding region the four amino acid substitutions were linked to the functional variation of these two alleles [12]. Recently, it showed that the V395L substitution present in Nona Bokra could directly affect the Na^+ transport rates [13].

In this study, we analyzed the presence of natural variation in the sequence of *OsHKT2;4* gene in Vietnamese rice cultivars. Several single nucleotide polymorphisms were identified.

2. Materials and methods

Plant material: In this research, we used 12 rice cultivars which were provided by Vietnam National University of Agriculture, including Nep vai, Chanh trui, Ngoi, Hom rau, Nep cuc, Re nuoc, Cuom dang 2, Nep non tre, Te tep, Nep oc, Nuoc man 2 and Chiem cu.

DNA extraction: Frozen leaf samples were ground to powder using a Mixer mill (Retsch, Germany) for 1.5 min at 25 Hz. DNA extraction was performed using CTAB as described by Doyle and Doyle [14]. Concentration of DNA was quantified by determination of OD_{260} using Nanodrop Spectrophotometer (Nanodrop Technologies, USA).

PCR and DNA sequencing: The *OsHKT2;4* gene was amplified by PCR using a specific primer pair, including OsHKT2;4-Fw (5'-ATGCTCCAGTGCTATCGATTGGT-3') and OsHKT2;4-Rv (5'-CTTGTGGTTGCTTGGCCTGAG-3'). PCR reaction mixtures consisted of 1 µl DNA (50-100 ng); 5µl 2mM dNTPs; 5µl 10x Taq Buffer; 2µl OsHKT2;4-Fw primer (10 pmol); 2µl OsHKT2;4-Rv primer (10 pmol); 0.4µl 5U Taq polymerase and 34.6µl H_2O. The PCR reaction was performed with thermocycle of 94°C for 7 minutes; 35 cycles (94°C for 30 seconds, 56°C for 20 seconds, 72°C for 60 seconds) and 72°C for 5 minutes. The products were examined by electrophoresis with 1% agarose gel and stained with ethidium bromide.

The PCR products were purified and conducted DNA sequencing by 1st Base Company, Singapore using ABI PRISM 3730xl Genetic Analyzer system (Applied Biosystems, USA).

Data analysis: The specific primers were designed by Primer-BLAST in NCBI webpage. Gene sequences were analyzed using Bioedit and Clustal Omega. The 3D model of OsHKT2;4 protein was predicted and analyzed by Phyre 2.

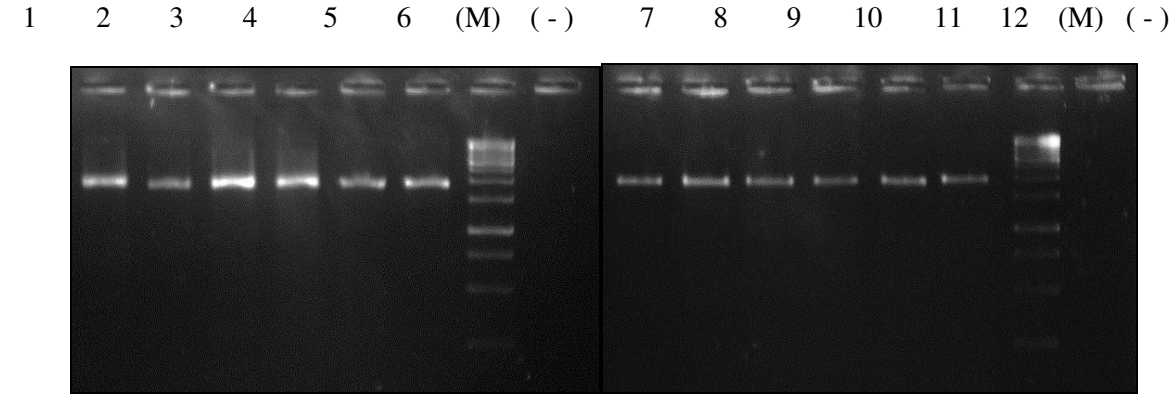

Figure 1. Electrophoresis of PCR amplification of the *OsHKT2;4* gene.
M: 1kb marker, (-) negative control, lane 1-12: PCR products from Nep vai, Chanh trui, Ngoi, Hom rau, Nep cuc, Re nuoc, Cuom dang 2, Nep non tre, Te tep, Nep oc, Nuoc man 2 and Chiem cu, respectively .

3. Results and discussion

3.1. Amplifiction of OsHKT2;4 gene by PCR

The rice plants were grown in soil for 2 weeks, then the leaves were collected for genomic DNA extraction. The extracted DNA was used as template in PCR reaction for amplification of *OsHKT2;4* gene (Fig. 1). As shown in Fig. 1, the PCR products were specific with corrected size (expected PCR product length of 1998 bp). Therefore, the *OsHKT2;4* gene was successfully amplified in all 12 investigated rice cultivars.

3.2. Polymorphism in nucleotide sequence of OsHKT2;4 gene

To investigate the polymorphism in nucleotide sequence of *OsHKT2;4* gene, the amplified PCR products were purified and sequenced. In total, we could detect 11 nucleotide substitutions, including 8 substitutions in exon regions and 3 substitutions in intron regions (Table 1). As shown in Table 1, all 8 substitutions in exon regions were non-synonymous that lead to amino acid changes. Based on these polymorphisms, the 12 rice cultivars were divided into 2 groups: Group 1 consists of cultivars having no polymorphism (Nep vai, Re nuoc, Nep cuc, Hom rau, Ngoi, Chanh trui) and Group 2 includes the rice cultivars having all 11 nucleotide substitutions (Cuom dang 2, Nep non tre, Nuoc man dang 2, Chiem cu, Nep oc, Te tep).

Table 1. Polymorphism in the OsHKT2;4 sequence

Nucleotide polymorphism		Amino Acid polymorphism	
site	substitution	Site	substitution
50	C/T	11	S > F
68	A/C	17	N > T
175	G/A	53	V > M
214	A/G	66	T > V
416	C/T	84	T > I
663	C/T	133	S > L
775	C/T	253	L > F
1043	G/A	342	S> N
1185	Addition of C	Intron	
1189	G/A	Intron	
1510	G/A	Intron	

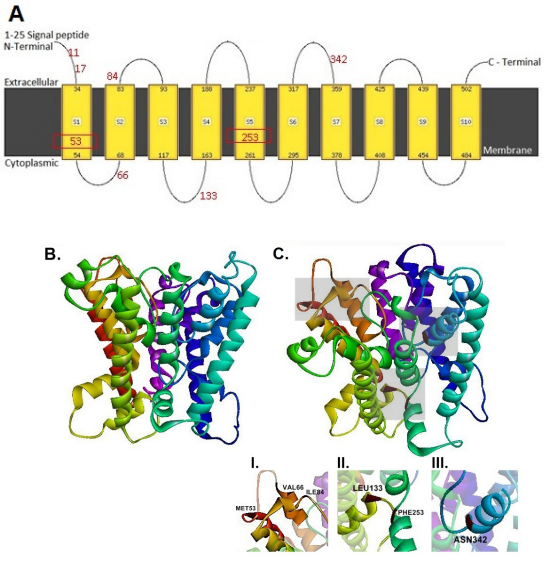

Figure 2. Topological model (A) and 3D ribbon model (B, C) of rice OsHKT2;4 protein. A: The numbers in red indicated the position of substituted amino acids. B: 3D model of original amino acid sequence, C: 3D model of substituted amino acid sequence.

3.3. Prediction of potential subsequence change caused by nucleotide substitution on protein structure

The protein structure of OsHKT2;4 transporter was predicted by using PHYRE2 webserver. Among eight non-synonymous, two were placed in the signal peptide (S11F, N17T), two were in transmembrane (V53M, L253F) and four were in the loop (T66V, T84I, S133L, S342N) (Fig. 2A, C). The predicted 3D protein structure of changed amino acid sequence was similar to the 3D protein model of original amino acid sequence (Fig. 2B, C). However, the two substitutions V53M, L253F might cause affect on protein folding. Thus, further investigation on the subsequent influence of these changes on protein functions should be experimentally carried out.

4. Conclusion

We have succeeded in amplification and sequencing of OsHKT2;4 gene of 12 rice cultivars. We could detect 11 single nucleotide polymorphisms, out of them 8 ones in exon regions and other 3 ones in intron region occurring in 6 rice cultivars. These 8 nucleotide substitutions are non-synonymous leading changes in amino acids which were placed at signal peptide (S11F and N17T), at the loop (T66V, T84I, S133L, S342N) and the transmembranes (V53M, L253F). These changed amino acid should be further analyzed to determine the influence on protein functions.

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Nghiên cứu đa hình gen *OsHKT2;4* ở một số giống lúa Việt Nam

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Tóm tắt: Đất nhiễm mặn gây ức chế nghiêm trọng đến sự tăng trưởng và phát triển của thực vật. Việc chọn tạo ra các giống cây trồng có khả năng chịu mặn là hướng đi hiệu quả và bền vững để khắc phục tình trạng đất nhiễm mặn. Các nghiên cứu chỉ ra rằng kênh vận chuyển HKT tham gia vào khả năng chống chịu mặn của cây trồng. Gen *OsHKT2;4* là một thành viên của lớp II thuộc họ gen HKT ở lúa mã hóa cho protein có khả năng đồng vận chuyển Na+ - K+ và vận chuyển Na+ khi ion này ở nồng độ cao. Trong nghiên cứu này, sự đa hình của gen *OsHKT2;4* được xem xét ở một số giống lúa Việt Nam. Toàn bộ chiều dài gen được nhân bản sử dụng phương pháp PCR, sau đó sản phẩm PCR được giải trình tự trực tiếp. Phân tích kết quả trình tự gen thu được giữa các giống lúa cho thấy có 11 vị trí đa hình, trong đó có 8 vị trí xảy ra ở vùng exon và 3 vị trí ở vùng intron. Tiếp tục phân tích sâu hơn cho thấy cả 8 đa hình ở vùng exon đều là đa hình sai nghĩa, dẫn đến sự thay đổi axit amin tại đoạn tín hiệu dẫn ((S11F và N17T), tại cấu trúc loop (T66V, T84I, S133L, S342N) và tại vùng xuyên màng (V53M, L253F).

Từ khóa: Đa hình di truyền, HKT, lúa, OsHKT2;4.