VNU Journal of Science: Natural Sciences and Technology, Vol. 32, No. 1S (2016) 253-257

# Analysis of DNA Methylation Status of the *OsSOS1* Gene under Salt Stress in Rice

Pham Quynh Hoa, Nguyen Dinh Thanh, Do Thi Phuc*

*Faculty of Biology, VNU University of Science, 334 Nguyen Trai, Hanoi, Vietnam*

Received 02 June 2016
Revised 02 August 2016; Accepted 09 Septeber 2016

**Abstract:** OsSOS1 is a $Na^+/H^+$ transporter presented on cellular membranes which plays a crucial role in salt response in plant through SOS pathway. DNA methylation is an inherited and reversible epigenetic modification which affects directly on the regulation of gene expression, and therefore might effect on salt tolerance in plant. In this study, McrBC – an endonuclease which is sensitive to methylcytosine – was used to detect DNA methylation status in some regions of the *OsSOS1* gene in two rice cultivars: a salt sensitive cultivar Nipponbare and a salt tolerant cultivar Pokkali under saline condition. McrBC-PCR revealed two hypermethylation regions in the middle of *OsSOS1* gene while the other two showed hypomethylation. Furthermore, the methylation status was the same in shoot and in root tissues, and had no significant change under salt condition compared to control condition in both investigated cultivars. Result of bisulfite sequencing showed that the hypermethylation only occurred in CpG sites but not CHG and CHH contexts.

*Keywords:* DNA methylation, *OsSOS1*, rice.

## 1. Introduction

DNA methylation is one of epigenetic modifications which plays a significant role in regulation of gene expression, genomic imprinting and transposon silencing for protecting the genome [1, 2]. Unlike in animal cells, DNA methylation in plant cells occurs not only restricts at symmetric CG context but also CHH and CHG contexts (where H = A, T, or C) with lower percentage, about 1.7% and 6.7%, respectively [3]. DNA methylation with high percentage is mainly found in heterochromatin where transposons and repeated sequences [4]. While DNA methylation focuses on 5' region of the genes in animals, in plant DNA methylation often occurs within gene body and only 5% of genes are methylated within their promoters [4]. DNA methylation often links with gene expression inhibition. SOS1 is a $Na^+/H^+$efflux transporter located within cellular membranes and regulated by protein kinase complex SOS2-SOS3 in famous SOS pathway [5]. SOS1 is primarily expressed at the root tip epidermis and in xylem parenchyma at the xylem-symplast boundary throughout the plant [6]. In *A.thaliana*, mutants lacking SOS1 transporter showed extremely sensitive to salt stress and had various defects of $Na^+$ efflux [7, 8]. When ectopic expressed in *S.cerevisia* membranes, OsSOS1 acted as a $Na^+/H^+$

* Corresponding author. Tel.: 84-4-38584748
Email: phucthido@vnu.edu.vn

transporter and reduced $Na^+$ concentration in cytosol [9]. Overexpression of *OsSOS1* in sos1 mutants of Arabidopsis restored the salt tolerance for this mutant line and suppressed the growth defections [9]. After 3 hours of salt treatment, expression level of *OsSOS1* in the root raised about 2-fold and reached maximal 6-fold after 15 hours while it decreased in leave tissue [9].

Rice is an important crop plant, however, its yield and cultural areas are severely affected by salinization and climate change. There is a wide collection of rice cultivars and some of them display salt tolerance at a certain level while the others are highly susceptible. Studies about the salt response mechanism have been based on genomic, transcriptomic and proteomic approaches. Recently, epigenetic modifications such as DNA methylation are also concerned since they are involved in regulation of gene expression, reversible due to environment condition and also inherited [10, 11]. In this study, we analyzed the DNA methylation status of *OsSOS1* gene in rice salt sensitive cultivar Nipponbare and salt tolerant cultivar Pokkali under saline stress in both shoot and root tissues. McrBC-PCR was used to screen for hypermethylated regions and followed by bisulfate sequencing to investigate exact methylation site at single base-pair.

## 2. Materials and method

*Plant materials and DNA isolation*: Seeds of salt sensitive cultivar Nipponbare and salt tolerant cultivar Pokkali were germinated on wet tissue in the dark for 24 hours. The one-leaf seedlings were transferred into Yoshida hydroponic culture [12] and moved into growth chamber with 12h dark/12h light cycle, light strength 500 µE $m^{-2}$ $s^{-1}$, day/night thermoperiod of 26°C /22°C.The salt treatment was applied on 14$^{th}$ day after germination at concentration 100 mM of NaCl, and the one without treatment was served as control. The shoot and root samples were collected after 3 hours of treatment and ground into fine powder in liquid nitrogen. The total DNA isolation was performed by CTAB method [13], and the DNA was stored in TE buffer at -20°C. Quantity and quality of the DNA was estimated by gel electrophoresis at 1% agarose and OD 260/280 by NanoDrop.

*McrBC-PCR*: 1µg of total DNA was digested with 20 unit of McrBC in 50 µL reaction volume for 30 min and 5 hours at 37°C. The reaction that did not contain McrBC was used as control. After treatment, equal amounts of McrBC-treated DNA and non McrBC-treated DNA were used for PCR amplification using 4 specific primer pairs designed along 14-kb-in-length of *OsSOS1* gene (SOS1-a, SOS1-b, SOS1-c, and SOS1-d as shown in Table 1). The 25-µL PCR reaction mixture contained Dream*Taq* Buffer (1X) including 1.5 mM of $Mg^{2+}$, 0.2 mM of dNTP mixture, 0.32 µM of each primer and 1U of Dream*Taq* DNA polymerase (Thermo Fisher). The PCR thermocycle consisted 5 min of pre-denaturation at 94°C, 35 cycles of (94°C for 15s, Tm for 20s, 72°C for 30s) (with Tm is optimal annealing temperature for each primer pairs), and 5 min at 72°C for prolonged extension step. The PCR products were separated on 2% agarose gel in TAE buffer under 90 V of voltage for 30 minutes and visualized under UV.

*Bisulfite sequencing*: 500 ng of genomic DNA was treated with sodium bisulfite using EpiJETBisulfite Conversion Kit (Thermo Fisher) by long protocol according to the manufacturer's instructions. 1 µL of treated DNA was used as template for Hot Start PCR using HotStarTaq Master Mix Kit (Qiagen) with two bisulfite primer pairs (Bi-SOS1-b, Bi-SOS1-c as shown in Table 1). The thermocycle was 94°C for 15 min, 40 cycles of (94°C for 15s, 56°C for 30s, 72°C for 20s), 72°C for 5 min. After visualized on 2% agarose gel stained with Ethidium Bromide, the PCR products were purified using Gel Purification Kit (Thermo Fisher) and sent to directly sequencing at 1$^{st}$ Base Company (Singapore).

Table 1. List of specific primers for McrBC-PCR and bisulfite sequencing.

| Primer name | Sequence |
|---|---|
| SOS1-a | Fw:GCCTTGGATTTTCCACTTGC<br>Rv:AGAGCGGGTAGAAAAACAGC |
| SOS1-b | Fw:GCTGACATGGACATTCTGGA<br>Rv:ATGCCACCAGTGAAGAACAC |
| SOS1-c | Fw:AGGTGTCCAAGCTGCTTACT<br>Rv:ACCAAGAATATGTTCCACCCAC |
| SOS1-d | Fw:GGGCGAAGAAATTGAACTTGAG<br>Rv:TCAATGACGCACTCCTTTGC |
| Bi-SOS1-b | Fw:TGGTTTGGATTTGAAAGAAGTTATAAT<br>Rv: ACCACCAATAAAAAACACAAACTAC |
| Bi-SOS1-c | Fw:TTTAAGAATTATGTGATTGGAAGGG<br>Rv:TTTACCAAAAAAATCATATAACTACC |

## 3. Result and discussion

### *3.1. Methylation status of the SOS1 gene under saline condition*

To investigate the methylation status of the *OsSOS1* gene, the McrBC-PCR approach was applied. The equal amount of genomic DNA was digested with McrBC - an endonuclease sensitive to methylcytosine – followed by PCR amplification. McrBC was an endonuclease that cut DNA containing methylcytosine on one or both strands but not unmethylated DNA. In addition, havingmore flexible cleavage sites than other methylation-sensitive restricted enzymes such as HpaII or MspI makes McrBC become one of the most suitable restricted enzymes for determining DNA methylation status.

As shown in Fig. 1, the result showed that the two *SOS1* gene regions corresponding to fragment b and c were hypermethylated, while the ones corresponding to fragment a and d displayed hypomethylation (Fig. 1). In addition, the methylation status was unchanged under saline condition compared to control condition in both shoot (Fig. 1A) and root (Fig. 1B) of two contrasting cultivars Nipponbare and Pokkali, and also not depended on time of exposure to salt stress (after 30 min and 5 hrs of salt treatment) (Fig. 1).

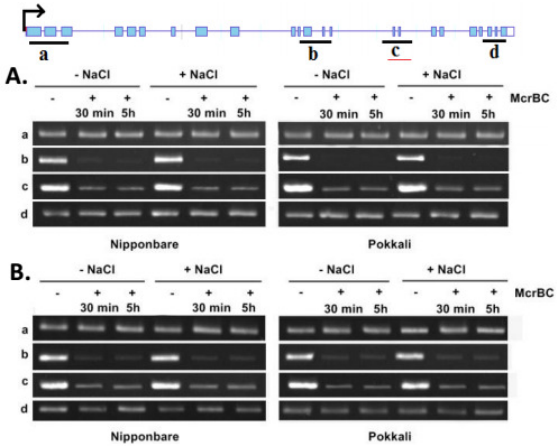


Figure 1. McrBC-PCR analysis of DNA methylation on *OsSOS1* in shoot (A) and root (B) tissue of two rice cultivars Nipponbare and Pokkali. – NaCl: control; + NaCl: salt treatment; McrBC: (-) no enzyme digestion, (+) 30 min: treated for 30 min; (+) 5h: treated for 5 hours with enzyme; a, b, c, d: 4 regions in *SOS1* corresponding to amplifying regions of primers named SOS1-a, SOS1-b, SOS1-c, and SOS1-d as shown in Table 1, respectively.

### *3.2. Determination of exact methylation site*

As methylation can occur at CHG, CHH and CpG sites in plants, thus we further analyzed on exact methylation sites of Cytosines in hypermethylated region b and c by the bisulfite sequencing. Genomic DNA was treated with sodium bisulfite and used as template for Hot Start PCR using bisulfite

primers (Table 1). The bisulfite modification converts the unmethylated Cytosines into Uracils which display as Timins in PCR and sequencing while methylcytosines unchanged. The result of Hot Start PCR amplification from bisulfite-treated DNA was shown in Fig. 2.

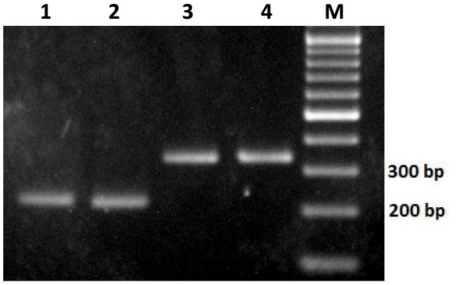


Figure 2. Bisulfite PCR amplification products. Lane 1: from region b of Nipponbare under control condition. Lane 2: from region b of Nipponbare under saline condition. Lane 3: from region c of Nipponbare under control condition. Lane 4: from region c of Nipponbare under saline condition. Lane M: 100 bp ladder (Thermo Fisher).

The sequencing results from bisultife PCR products indicated that methylcytosines were observed only at CpG sites of region b and c, while no methylcytosine was found at CHG and CHH sites in neither region b nor region c. Thus, hypermethylation was only occurred at CpG sites.

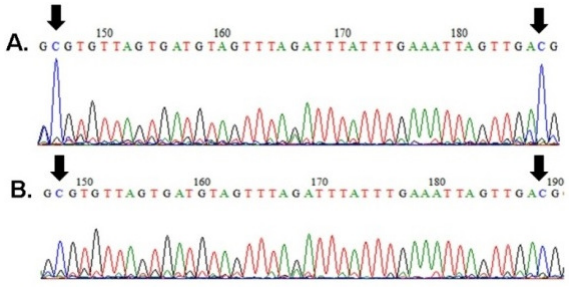


Figure 3. Partial of bisulfite sequencing results of region b in non-treated (A) and salt-treated (B) samples of cultivar Nipponbare. The arrows indicate methylcytosine sites.

## 4. Conclusion

We determined hypermethylation in the middle regions (b and c) and hypomethylation in 5' and 3' regions (a, d) regions of the *OsSOS1* gene by using McrBC-PCR method. The methylation presented in two middle regions (b, c) was only restricted at CpG sites but not CHG and CHH contexts. The methylation status of the *OsSOS1* gene was similar between shoot and root tissues under salt and non-salt conditions. In addition, there was no significant different in methylation status between the salt sensitive cultivar Nipponbare and the salt tolerant cultivar Pokkali. Thus, DNA methylation of *SOS1* might not play roles in regulation of *SOS1* gene expression in response to salt stress in rice.

## Acknowledgements

This work was financially supported by the TWAS (project code 14_216RG/BIO/AS_I to Phuc Thi Do).

## References

[1] R.A. Martienssen, V Colot, DNA methylation and epigenetic inheritance in plants and filamentous fungi, Science 293 (2001) 1070

[2] J.A. Law, S.E Jacobsen, Establishing, maintaining and modifying DNA methylation patterns in plants and animals, Nat. Rev. Genet. 11 (2010) 204

[3] SJ Cokus, S. Feng, X.Zhang, Z.Chen, B.Merriman, C.D.Haudenschild, Shotgun bisulphite sequencing of the Arabidopsis genome reveals DNA methylation patterning. *Nature* 452 (2008) 215

[4] X.Zhang, J.Yazaki, A.Sundaresan , S.Cokus, S.W.Chan, H.Chen, I.R.Henderson, P.Shinn, M. Pellegrini, S.E.Jacobsen, J.R.Ecker , Genome-wide high-resolution mapping and functional analysis of DNA methylation in Arabidopsis. *Cell* 126 (2006) 1189

[5] Q.S. Qiu, Y. Guo, M. A. Dietrich, K. S.Schumaker and J. K.Zhu ,Regulation of

SOS1, a plasma membrane Na+/H+ exchanger in Arabidopsis thaliana, by SOS2 and SOS3. ProcNatlAcadSci U S A 99 (2002) 8436

[6] H.Shi, F.J.Qhintero, J.M.Pardo, J.K.Zhu, The Putative Plasma Membrane Na+/H+ Antiporter SOS1 Controls Long-Distance Na+ Transport in Plants, Plant Cell 14 (2002) 465

[7] Q.S.Qiu, Y.Guo, M.A.Dietrich, K.S. Schumaker, J.K.Zhu, Regulation of SOS1, a plasma membrane Na+/H+ exchanger in Arabidopsis thaliana, by SOS2 and SOS3. ProcNatlAcadSci USA 99 (2002) 8436

[8] H.Shi, F.J.Quintero, J.M.Pardo J.K. Zhu, The putative plasma membrane $Na^+/H^+$antiporter SOS1 controls long-distance $Na^+$ transport in plants. Plant Cell 14 (2002) 465

[9] J. Martínez-Atienza, X.Jiang, B.Garciadeblas, I.Mendoza , J.K. Zhu, J.M. Pardo and F.J. Quintero, Conservation of the Salt Overly Sensitive Pathway in Rice, Plant Physology 143 (2007) 1001

[10] R. Garg, V.V.S. Chevala, R. Shankar and M. Jain, Divergent DNA methylation patterns associated with gene expression in rice cultivars with contrasting drought and salinity stress response, Scientific Reports 5 (2015) 14922.

[11] W.Wang, F. Huang, Q. Qui, X. Zhao, Z. Li, B. Fu, Comparative analysis of DNA methylation changes in two rice genotypes under salt stress and subsequent recovery. Biochem Biophys Res Commun 465(4) (2015) 790

[12] Yoshida et al, Laboratory manual for physiology studies of rice (1976).

[13] Pham Quynh Hoa, Tran Xuan An, Tran Thi Thuy Anh, Hoang Hai Yen, Nguyen Thi Hong Van, Tang Thi Hanh, Phuc Thi Do, Investigation of polymorphisms in the coding region of OsHKT1 gene in relation to salinity in rice, Rice Science (2016) in press.

# Khảo sát tình trạng Methyl hóa của gen *OsSOS1* ở một số giống lúa trong điều kiện mặn

Phạm Quỳnh Hoa, Nguyễn Đình Thanh, Đỗ Thị Phúc

*Khoa Sinh học, Trường Đại học Khoa học Tự nhiên, ĐHQGHN, 334 Nguyễn Trãi, Hà Nội, Việt Nam*

**Tóm tắt:** *OsSOS1* là protein vận chuyển $Na^+/H^+$ trên màng tế bào đóng vai trò quan trọng trong quá trình đáp ứng stress mặn ở thực vật thông qua con đường SOS. Sự methyl hóa ADN là một biến đổi di truyền ngoại gen có liên quan đến điều hòa biểu hiện gen. Do vậy có thể ảnh hưởng đến tính kháng mặn ở cây trồng. Trong nghiên cứu này, chúng tôi sử dụng enzym McrBC - một enzym cắt giới hạn nhạy cảm với cytosine bị methyl hóa - để xác định tình trạng methyl hóa trên một số vùng của gen *OsSOS1* ở hai giống lúa: giống mẫn cảm mặn Nipponbare và giống kháng mặn Pokkali trong điều kiện xử lý mặn. Kết quả McrBC-PCR cho thấy hai vùng ở phần giữa gen *OsSOS1* bị methyl hóa cao trong khi các vùng nằm gần đầu 3' và 5' của gen ít bị methyl hóa. Tình trạng methyl hóa là tương tự giữa mô lá và rễ ở cả hai giống lúa trong điều kiện xử lý mặn khi so sánh với điều kiện bình thường. Bên cạnh đó, kết quả phân tích giải trình tự bisulfite cho thấy sự methyl hóa cao chỉ xảy ra ở các vị trí CpG mà không mở rộng ở các vị trí CHG và CHH.

*Từ khóa:* Methyl hóa ADN, gen *OsSOS1*, lúa.