Designing functional markers to identify the candidate gene SRWD2 involved in salt tolerances of Vietnamese rice landraces

Huu Trung Khuat, Truong Khoa Nguyen, Thuy Diep Nguyen, Thi Thuy Tran, Thi Thanh Ha Dang, Duy Cuong Tran, Dang Khanh Tran*

Agricultural Genetics Insitute, Hanoi, Vietnam

Received 13 December 2016; accepted 15 January 2017

Abstract:

Salinity is one of the major abiotic stresses, and it limits rice production in many worldwide areas. A lot of attention has been paid to developing rice salinity tolerance to cope with climate change. This study is based on the genome sequence databases of 36 Vietnamese landraces. The candidate gene, *SRWD2,* has been predicted and identified. The salt responsive WD40 protein 2 is involved in salt tolerance yield of nine rice landraces. Specifically, nine homologous segments of the sequences were found to be components of the CSD nucleotides, which is shown to be similar to the reference genome LOC_Os02g48964.1 and LOC_Os02g48964.1 CDS, respectively. At the same time, the functional marker, *SRWD2add14,* was designed to identify the candidate gene, *SRWD2,* based on its size as 150 bp (candidate gene tolerant to salinity) and 136 bp (different sequences to compare *SRWD2*-reference genome sequence). The findings show that Mot bui do landrace carries the candidate gene, *SRWD2* (homozygote). The rice genotype should be used as a potential material for rice breeding to develop salt tolerance in rice varieties.

Keywords: *candidate gene, marker, salt tolerance, SRWD2.*

Classification number: *3.1*

Introduction

Saline tolerance of rice is derived from the genes that limit the rate of salt uptake from the soil, and transport the salt throughout the plant. This adjusts the ionic and osmotic balance of cells in roots and shoots, and regulates leaf developments and the onset of senescence [1]. Salinity is a key abiotic constraint that devastates crop production in the world. One-fifth of irrigated, arable lands in the world have been reported to be adversly affected by high soil salinity. According to a report by FAO [2], over 800 million ha of land worldwide are severly affected by salt and approximately 20% of those areas are irrigated. In Asia, 21.5 million ha of land are being influenced by salinity, and are estimated to cause a loss of up to 50% of fertile land by the 21st mid-century [3]. In Vietnam, huge rice growing areas are being affected by salinity intrusion over 1 million ha, equal with 3% of total areas of this country, causing the economic loss by salt intrusion in 2005 to be up to 45 million USD [4]. To date, approximately 600,000 ha are being severely affected by drought and saline intrusion in the early year of 2016, causing economic losses up to 15 trillion VND in rice only (≈670 million USD) [5].

To enhance the salt tolerance of crops, included rice, the plants undergo a variety of changes, from physiological adaptations to gene expression. Hence, it requires new genetic sources of this tolerance and more efficient techniques, to evaluate the salt tolerant germplasm. Advanced, powerful molecular tools, including the complete genome sequence of rice, were created in 2005 and made available [6]. These tools often facilitate the plants to find new tolerant genetic resouces. Numerous QTLs/genes, candidate genes involved in salt tolerance in rice such as *Salt1, RSS1, SCK*1, *Saltol* etc have been identified [1, 7]. *SRWD* (Salt responsive *WD40* protein) genes form a novel *WD40* subfamily, and were found to be involved in rice salt tolerance. The *SRWD* proteins consists of five *WD40*-motif repeats. They demonstrated no sequence similarities with other previously known proteins. *SRWD2* is located on rice's chromosome 2 and is the size of 446 amino acids. Huang, et al. (2008) [8] predicted the *SRWD2* gene has two motifs: *WD40-3* and *WD40-4*.

*Corresponding author: Email: khanhkonkuk@gmail.com

The SRWD2 is highly expressed in the ovary, embryo, endosperm and seed [8]. Based on the genome sequence database of 36 Vietnamese rice landraces, the objectives of this study are to evaluate the genetic similarities (homologous segments of DNA and proteins), then to design functional markers, which will identify accurate candidate gene *SRWD2* to further molecular breeding program to develop salt tolerance rice genotypes.

Materials and methods

Materials

- The database of 36 sequenced Vietnamese rice landrace genomes are shown in Table 1 and on the Internet at: www.riceagi.org.vn.

Methods

- Nucleotides were predicted and analyzed via the software NextGENe_V2.3, which detected SNPs/InDels accordingly.

- Phylogenetics and nucleotide alignment were recorded by the MEGA 6.0 Windows (http://www.megasoftware.net/mega.php, http://www.softgenetics.com/NextGENe.html; http://rice.plantbiology.msu.edu/cgi-in/ORF_infopage.cgi?orf=LOC_Os02g48964).

- Primer design based on the difference of rice genome sequence was due to Primer 3.0 (http://primer3plus.com/web_3.0.0/primer3web_input.htm).

Method to test candidate gene resistance:

+ Leaf samples of each of the rice landraces were collected and DNA was extracted using a modified CTAP method [9].

+ PCR reactions were perfomed by Veriti 96-well Thermal cycler. Total volume was 15 µl, included: 5 µl DNA; 0.15 µM primer; 0.2 mM dNTPs; 1 X Buffer PCR; 2.5 mM $MgCl_2$ and 0.25 Taq polymerase.

Table 1. List of genome sequence of 36 Vietnamese rice landraces.

No.	Name of landrace	No.	Name of landrace
1	Tam xoan Bac Ninh	19	Coi ba dat
2	Tam xoan Hai Hau	20	OM5629
3	Te nuong	21	Nep bo hong Hai Duong
4	Nang thom Cho Dao	22	Tan ngan
5	Thom lai	23	Ba cho K'te
6	Nep man	24	Blao sinh sai
7	Chiem do	25	Nang quot bien
8	Lua ngoi	26	Tep Thai Binh
9	Mot bui do	27	Khau dien lu
10	Nang co do 2	28	Nep meo nuong
11	Ble te lo	29	Toc lun
12	Chiem nho Bac Ninh 2	30	Hom rau
13	Nep lun	31	Nep ong tao
14	Khau mac buoc	32	OM3536
15	OM6377	33	Khau lien
16	Chan thom	34	Lua goc do
17	Xuong ga	35	Chiem da
18	Khau giang	36	IS1.2

+ PCR products were analyzed by electrophoresis on 6% gel polyacrylamide. The gels were stained in 0.5 mg/ml ethidium bromide and were documented using Alpha Imager 1220 (Alpha Innotech, CA, USA).

Results and discussion

Prediction and analysis of nucleotide in CDS (Coding DNA Sequence) and amino acids of candidate gen SRWD2

Based on the genome sequences of 36 rice landraces, our reseach was conducted to find homologous sequences of those landraces and compare them to the *SRWD2* (Salt responsive *WD40* protein 2) which had the code name LOC_Os02g48964 (Rice Genome Annotation Project release 5.6). The results show that nine sequenced segments of nine genome sequences of rice landraces have shown to have the equivalent of the length of the reference one which involves the salt tolerance of *SRWD2,* including Te nuong, Nep meo nuong, Hom rau, Xuong ga, Nang quot bien, Nep bo hong Hai Duong, OM3536, IS1.2 and Mot bui do. Homologous segment were similar to the candidate gene *SRWD2* of nine rice landrace-genome sequences with the similarity of percentage of C, A, G and the reference genome. However, the number of nucleotides has revealed difference by 7171 to 7194 nucleotides (Table 2).

Table 2. The statistical number and proportion of the candidate gene SRWD2 nucleotide in rice genome sequence.

Rice landrace and reference gene	% Nucleotide of each landrace				Total Nucleotide
	T(U)	*C*	*A*	*G*	
SRWD2 (LOC_Os02g48964)	26.6	22.3	31.1	20.1	7193.0
Te nuong	26.6	22.3	31.1	20.1	7179.0
Nep meo nuong	26.5	22.3	31.1	20.1	7179.0
Hom rau	26.6	22.3	31.1	20.1	7179.0
Xuong ga	26.5	22.3	31.1	20.1	7180.0
Nang quot bien	26.5	22.3	31.1	20.1	7180.0
Nep bo hong Hai Duong	26.6	22.3	31.1	20.1	7171.0
OM3536	26.6	22.3	31.1	20.1	7180.0
IS1.2	26.5	22.3	31.1	20.1	7180.0
Mot bui do	26.6	22.3	31.1	20.1	7194.0
Average	*26.6*	*22.3*	*31.1*	*20.1*	*7181.5*

Table 3. The number and percentage of CDS nucleotides of candidate gene SRWD2 in rice genome sequence landraces.

Rice landrace and reference gene	T(U)	C	A	G	Total
LOC Os02g48964.1	28.8	25.2	26.5	19.5	1341.0
LOC Os02g48964.1 CDS	28.8	25.2	26.5	19.5	1341.0
Te nuong	28.8	25.2	26.5	19.5	1341.0
Nep meo nuong	28.8	25.2	26.5	19.5	1341.0
Hom rau	28.8	25.2	26.5	19.5	1341.0
Xuong ga	28.6	25.4	26.3	19.7	1341.0
Nang quot bien	28.6	25.4	26.3	19.7	1341.0
Nep bo hong Hai Duong	28.7	25.3	26.4	19.6	1341.0
OM3536	28.7	25.3	26.4	19.6	1341.0
IS1.2	28.6	25.4	26.3	19.7	1341.0
Mot bui do	28.6	25.4	26.5	19.5	1341.0
Average	*28.7*	*25.3*	*26.5*	*19.6*	*1341.0*

The results of the alignment of the CDS (coding DNA sequence) of the candidate gene SRWD2 showed that all nine rice landraces have had similar numbers of nucleotides to compare with the published reference genome LOC_Os02g48964.1 and LOC_Os02g48964.1 CDS. Similarly, the amino acid sequence alignments of nine rice landraces have demonstrated that the three landraces viz. Xuong ga, Nang quot bien, and IS1.2 have attained more than one amino acid as compared to published reference genome LOC_Os02g48964.1 and LOC_Os02g48964.1 CDS (446 amino acids) (Table 3).

Designing a marker to identify the candidate gen SRWD2 involved in salt tolerance

According to software analyses, the resulting prediction of comparing the gene sequences of the candidate gene *SRWD2* from 36 rice genome sequences, indicated the frequency of single nucleotide polymorphisms (SNPs) and segment insertion, and deletion (InDels) among the landraces. They were surprisingly high. The study notes that 14 nucleotide sequence insertions have been found in some rice landraces, which are similar to the published SRWD2 gene (Fig. 1).

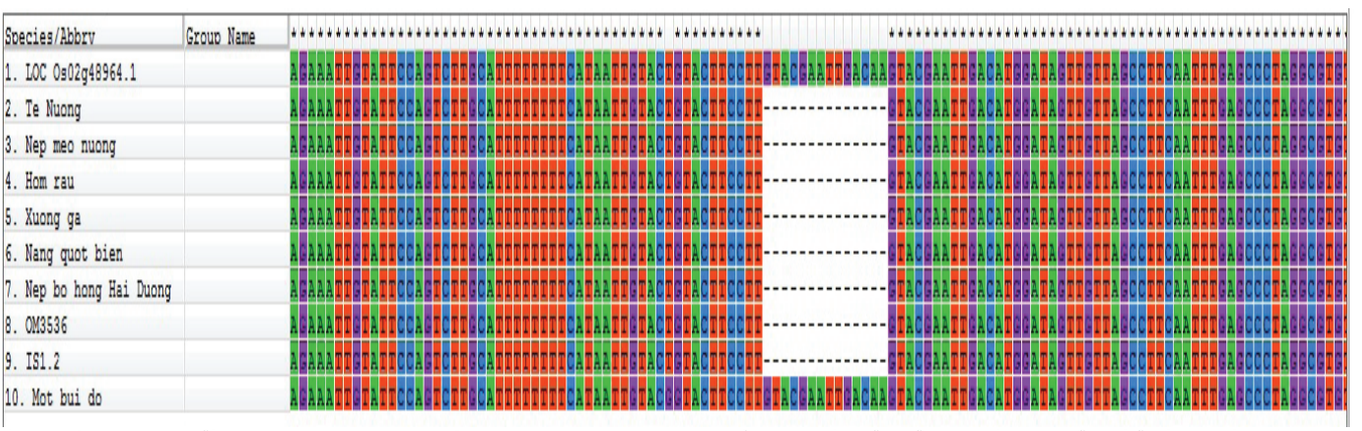

Fig. 1. Sequence alignment to compare SRWD2 gene segments of some rice landraces (14 nucleotide insertions).

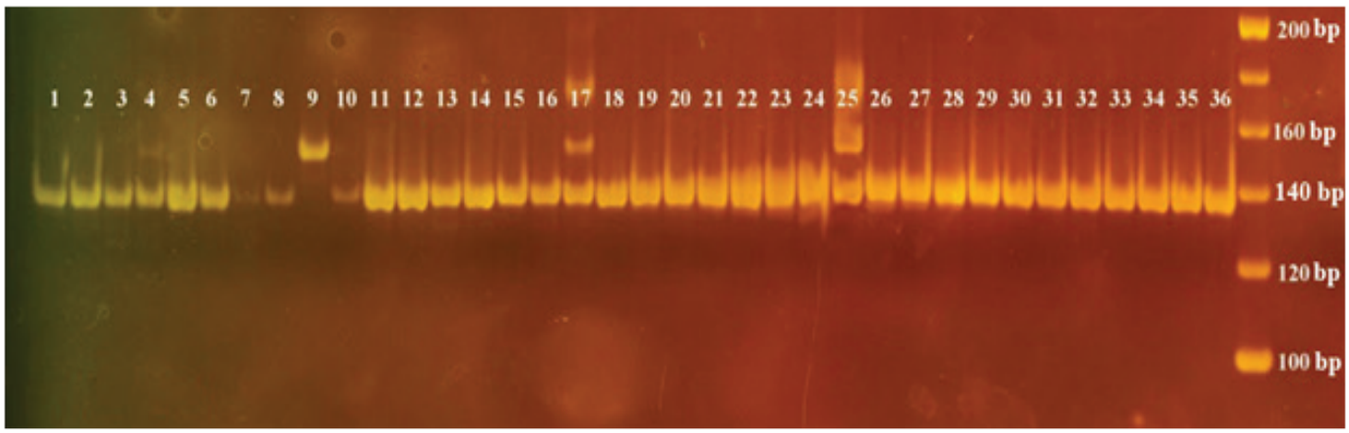

Fig. 2. SRWD2add14 electrophothesis to identify SRWD2 gene in Vietnamese rice landraces.
Band 1-36: genome sequenced rice landrace (Table 1), band 37: marker O'Range ruler 200 bp.

Based on the differences found in the *SRWD2* gene segment sequences between rice landraces, the software Primer 3.0 was used to design primers namely *SRWD2 del14* with the sequence *SRWD2add14* as F: 5'-CATAATTGTACGGTACTTCCTT-3', R: 5'-TGCTGAAGTAACATGTGTATGG-3' (Fig. 2).

It is possible to amplify the candidate gene segment by 150 bp with rice landraces carrying the candidate genes involved in salt tolerance, which showed similarity to the reference gene and 136 bp in the rice landraces with different sequences to compare with the published sequence segment.

To examine the candidate gene *SRWD2* in 36 rice genome sequenced landraces by use of the designed primers showed that the appearance of band 150 bp in four rice landraces. However, only Mot bui do carrying the candidate gene *SRWD2* in homozygote was found, while other rice landraces were shown to be heterozygous including Nang thom Cho Dao, Xuong ga and Nang quot bien, which are carrying the candidate gene *SRWD2* due to attributable to both 150 and 136 bp bands. Therefore, the designed primers exhibited the polymorphism of the candidate gene *SRWD2* and should be used to determine the presence of the candidate gene in the parental generations, progenies for rice breeding program to develop rice varieties tolerant to salinity.

Conclusions

The candidate gene *SRWD2* from nine rice genome sequenced landraces including Te nuong, Nep meo nuong, Hom rau, Xuong ga, Nang quot bien, Nep bo hong Hai Duong, OM3536, IS1.2 and Mot bui do, which exhibited CSD nucleotides similar to the reference gene LOC_Os02g48964.1 and LOC_Os02g48964.1 CDS.

The primer SSLP (*SRWD2add14*) has been sucessfully designed to determine the candidate gen *SRWD2* involving in salt tolerance for rice breeding program.

Rice landrace Mot bui do carrying candidate gen *SRWD2* was identified. This landrace should be used as the donor in salt tolerance rice breeding.

REFERENCES

[1] R. Munns (2005), "Genes and salt tolerance: bringing them together", *New Phytol*, **167**, pp.645-663.

[2] Food and Agriculture Organization (FAO) (2011), *Report of salt affected agriculture*, http://www.fao.org/ag/agl/agll/spush/.

[3] R. Nazar, N. Iqbal, A. Masood, S. Syeed, N.A. Khan (2011), "Understanding the significance of sulfur in improving salinity tolerance in plants", *Environ Experiment Botany*, **70**, pp.80-87.

[4] L.H. Ham, T.D. Khanh (2015), Applying molecular breeding for rice improvement, *Agriculture* Publishing, Vietnam, pp.25-40.

[5] L.H. Anh, H.T. Hue, N.K. Quoc, L.T. Nghia, K.H. Trung, T.N. Trung, D.H. Trang, T.D. Xuan, T.D. Khanh (2016), "Effect of salt on growth of rice landraces in Vietnam", *Inter Let Natl Sci*, **59**, pp.72-81.

[6] S.A. Goff, D. Ricke, T.H. Lan, et al. (2005), "A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*)", *Science*, **296**, pp.92-100.

[7] S. Negrao, B. Courtois, N. Ahmadi, I. Abreu, N. Saibo, M.M. Oliveira (2011), "Recent updates on salinity stress in rice: From physiological to molecular responses", *Critical Reviews in Plant Sciences*, **30**, pp.329-377.

[8] J. Huang, M.M. Wang, Y.M. Bao, S.J. Sun, L.J. Pan, H.S. Zhang (2008), "SRWD: A novel WD40 protein subfamily regulated by salt stress in rice (*Oryza sativa* L.)", *Gene*, **424**, pp.71-79.

[9] O.P. Obara, S. Kako (1998), "Genetic diversity and identification of cymbidium cultivars as measured by random amplified polymorphic DNA (RAPD) markers", *Euphytica*, **99**, pp.95-101.