EVALUATING SALT TOLERANCE OF TWENTY TRADITIONAL RICE VARIETIES FROM VIETNAM

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ABSTRACT

Due to the negative effects of climate change, sea water intrusion is causing difficulties for rice production in Vietnam. Presently, there are several Vietnam rice landraces being preserved at International Rice Research Institute (IRRI) which have not been adequately characterized to utilize as breeding materials for developing rice cultivars to cope with salinity stress. In this study, total of 20 rice landraces were evaluated for salinity tolerance in NaCl 6‰ salinized solutions in three weeks. The results showed that there are 4 out of 20 accessions presenting high salinity tolerance in term of plant growth and physiological parameters. Although known salinity tolerance-related gene was not detected, there is the apparent difference of total protein profile of highly tolerant accessions between saline and control condition. The obtained results in this work could pave the way for following studies to breed new rice cultivars adapting to climate change in Vietnam.

Keywords: Rice landraces, salt tolerance, *Saltol*, IRRI, SDS-PAGE.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is an important crop of Vietnam economy. Although it is normally grown in lowland area, rice is sensitive to salinity. Growth and development from germination to maturity of rice are seriously affected by salted condition [1]. With total of 40,000 km^2, Cuu Long Delta is the largest rice production area in the country. However, approximately 40% rice growing area is negatively affected with salinity. In next 30 years, rice growing area having salinity of 4‰ will increase up to 1,605,200 ha covering 41% total region area [2]. Thus, rice production will be harder and food security will also be a big challenge. Together with different efforts to slow down climate change, study to develop new rice varieties having higher salinity tolerance to adapt with saline soil is necessary.

Presently, rice breeding for salinity tolerance is facing several difficulties and one of them is the lacking of breeding materials. In intensive agriculture, farmers in Cuu Long Delta prefer to use high yielding rice cultivars. These cultivars have higher yield and quality but they are sensitive to salinity. Rice breeding for salinity is largely depended on rice landraces which are highly adapted to adverse conditions. So far, there are very few rice accessions which are able to grow in salinity of 6‰. In 1997, at International Rice Research Institute (IRRI), Gregorio identified *Saltol* QTL which is responsible for salinity tolerance in Pokkali, an Indian-origin rice. This rice variety has been used to improve salinity tolerance in different rice cultivars [3]. In Vietnam, when breeders have plans to use Pokkali for breeding, they normally asked IRRI for this variety [4]. The importing process is complicated and time consuming which causes delay in breeding program. At present, IRRI

is reserved more than five thousands traditional rice varieties which are not available in Vietnam rice paddy. These collections are valuable and useful for rice breeders and it is necessary to exploit this genetic collection for rice breeding to cope with climate change [5].

In this study, a total of 20 rice accessions originally from Vietnam were evaluated for salinity tolerance. The result of this study will contribute to enhance the awareness rice genetic diversity and the potential of exploiting them in rice production.

2. MATERIALS AND METHODS

2.1. Materials

Rice accessions used in this study were provided by IRRI in Philippines and shown in Table 1. These 20 accessions were collected from Vietnam, especially in Cuu Long Delta region. In addition, two accessions namely IR29 and FL478 were also included as the sensitive and tolerant control, respectively.

Table 1. List of rice accessions used in this study

Code	Accession number	Common name
1	IRGC 196	LUA THUOC
2	IRGC 197	TRANG DOC
3	IRGC 201	DOC PHUNG
4	IRGC 209	SOC NAU
5	IRGC 220	NANG QUOT
6	IRGC 229	NANG THOM
7	IRGC 5868	DOC PHUNG LUN A R 16
8	IRGC 5967	NANG TAY NHO CF 15
9	IRGC 47487	CHIEM NAM 2
10	IRGC 58697	OC NAM DINH
11	IRGC 198	MO NHAC
12	IRGC 199	NANG CHET CUC
13	IRGC 7219	BA BONG
14	IRGC 10231	DOC PHUNG LUN
15	IRGC 10865	TIEU PHAT
16	IRGC 16749	NANG SOM
17	IRGC 16783	TRANG TEP
18	IRGC 16852	LUA NGU
19	IRGC 16878	NANG SON
20	IRGC 17013	SOC NAU
21	IRGC 30412	IR29
22	IRIS 66-333787	FL478

2.2. Methods

2.2.1. Evaluation plant growth parameters

After soaking three days in water, germinated rice seeds were grown in Yoshida nutrients solution [22] until 14 days and then transferred to saline solution with NaCl 6‰ in three weeks. The control treatment was kept in non NaCl condition during experiment period. Salinity tolerance of 22 rice accessions was evaluated using the modified IRRI standard evaluation scoring (SES) system based on visual symptoms of salt injury at the seedling stage [6]. Visual symptoms were evaluated with scores ranging from 1 to 9, with 1 being the most tolerant and 9 the most sensitive as shown in Table 2. Physiological parameters such as dry biomass and survival rate were also conducted as this instruction. Experiments were laid out as Random Complete Block Design with 3 replications (20 rice seedlings per replication). Data were analyzed with Statgraphics Centurion XVI.

Table 2. Modified IRRI standard evaluation scoring (SES) system based on the visual symptoms of salt stress injury of rice at seedling stage

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded, most leaves are rolled; few elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Sensitive
9	Almost all plants dead or dying	Highly sensitive

2.2.2. Identification of salinity tolerance gene

DNA was extracted from rice leaf by using CTAB protocol. Quality and quantity of obtained DNA were identified by Nanodrop spectrophotometer (Thermo Scientific NanoDrop 2000, USA). DNA was then diluted with deinonized water to the concentration of 35 ng/µL and kept at -80 ^{o}C until use. PCR reactions were performed with total volume of 25 µL containing 1 µL DNA, 0.5 µL of every primer (10 nM); 22.5 µL Supper Mix (Invitrogen, USA) and water make up to final volume. PCR profiles were programmed as follows: initial denaturation at 94 ºC for 3 min, followed by 35 cycles of denaturation at 94 ºC for 15 sec, annealing at 60 ºC for 1 min and elongation at 72 ^{o}C for 2 min, and a final extension step of 7 min at 72 ºC. The PCR cocktails were run using a SureCycler 8800 Thermal Cycler (Agilent, USA). The 2 µL of PCR products together with 5 µL of 10X loading dye (bromphenol) were loaded into 1.5% agarose gel and run at 100 volts for 30 minutes. A 1 kb DNA ladder was used as the size standard. Gels were stained with ethidium bromide 0.5 µg/mL visualized under UV by using gel documentation system Quantum - ST4 3000 (Montreal - Biotech, Canada). Two primer pairs were used to identify the present of *Saltol* relating to salinity tolerance of rice (Table 3).

Table 3. Primer used to identify the presence of *Saltol*

Primer	Sequence	Position on Chromosome 1 (Mb)
RM493	GTACGTAAACGCGGAAGGTGACG RV-5'-CGACGTACGAGATGCCGATCC	12,3
AP3206f	GCAAGAATTAATCCATGTGAAAGA AGTGCAGGATCTGCCATGA	11,2

2.2.3. Evaluation the changing in protein profile

Total protein from rice was isolated as suggested by Banti *et al*.[7]. Approximately 50 mg of leaf and stem of rice was finely ground in liquid nitrogen with mortar and pestle. One mL of isolation buffer was then added and mixed carefully. Samples were then centrifuged with cool centrifuge at speed of 12,000 g in 10 minutes. The supernatant was transferred to new tube and kept at -80 ^{o}C until use. Protein concentration was identified by using BCA protein Assay (Pierce Thermo Scientific, USA). Protein profile was analyzed by SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) with concentration of stacking gel at 4% and separating gel at 12.5% by using gel electrophoresis apparatus (Biorad, USA).

3. RESULTS AND DISCUSSION

3.1. Variation in salinity tolerance of rice genotypes

Rice is highly sensitive to salinity at seedling stage. If plant is saline stressed at this stage the plant growth will be delayed leading the yield reduction. Salinity tolerance of rice at seedling stage is evaluated through several parameters such as stress symptom and growth retardation. After three weeks under saline stress, there was large variation in salinity tolerance among different rice genotypes. Different genotypes showed different levels of salt injury. Symptoms such as leaf rolling, formation of new leaves, brownish and whitish leaf tips were more evident on the first and second leaves. Stunted growth and cessation of growth and dying of seedlings in the sensitive lines were also observed. Some rice genotypes showed high salt sensitivity similar to control check IR29 such as Soc Nau, Oc Nam Dinh, Ba Bong, Nang Son. There were also several highly tolerant phenotypes such as Doc Phung, Nang Thom, Doc Phung Lun and Tieu Phat. These phenotypes show the tolerance as good as tolerant check FL478 (Figure 1) which is the most tolerant genotype and currently used in different breeding programs to improve salinity of rice [8, 9]. Previous studies also reported the usefulness of using rice landraces as breeding material for different stresses such as salinity, submergence, drought and plant pathogens [10-12].

In Vietnam, Le and Tran [13] surveyed 224 different rice genotypes for salinity tolerance. The result revealed that all newly breed cultivars are sensitive, whereas rice landraces showed higher tolerance such as Mot Bui Do, Cu Lu 2, Mot Bui Do Dia 1, Muoi Luyen, Nam Vang 1, Nang Quot Diem và Tet Hanh Lun. However, most of these genotypes are not ready for use in rice breeding programs because these genotypes just showed intermediate tolerance. In our study, the genotypes namely Doc Phung, Nang Thom, Doc Phung Lun and Tieu Phat showed relatively high tolerance which can be considered as potential candidates for further characterization and apply to breeding programs.

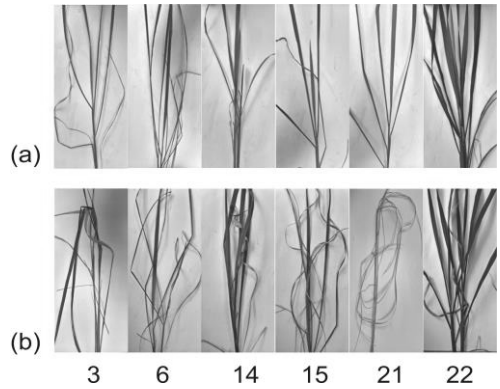

Figure 1. Visual symptom of some rice landraces after three weeks under control (a) and salinity stressed with NaCl at concentration of 6‰ (b). (The phenotype numbers are corresponding with Table 1).

Table 4. Salt tolerance of rice landraces after 3 weeks under salined stress with NaCl 6‰

No.	Code	Common name	Dried biomass (g)	Survival rate (%)	SES
1	IRGC 196	LUA THUOC	0,73^{b}	61,4^{ab}	5,6^{bc}
2	IRGC 197	TRANG DOC	0,37^{c}	44,6^{c}	4,1^{ab}
3	IRGC 201	DOC PHUNG	1,64^{a}	74,4^{a}	2,3^{a}
4	IRGC 209	SOC NAU	0,21^{cd}	11,5^{de}	8,6^{de}
5	IRGC 220	NANG QUOT	1,54^{ab}	64,8^{ab}	3,5^{ab}
6	IRGC 229	NANG THOM	1,66^{a}	79,2^{a}	2,2^{a}
7	IRGC 5868	DOC PHUNG LUN AR 16	1,35^{ab}	59,5^{bc}	3,7^{ab}
8	IRGC 5967	NANG TAY NHO CF 15	0,16^{de}	37,4^{cd}	6,9^{cd}
9	IRGC 47487	CHIEM NAM 2	0,14^{de}	21,5^{de}	7,8^{d}
10	IRGC 58697	OC NAM DINH	0,21^{cd}	45,3^{c}	8,3^{de}
11	IRGC 198	MO NHAC	0,78^{b}	49,6^{bc}	5,9^{c}
12	IRGC 199	NANG CHET CUC	0,49^{bc}	63,1^{ab}	5,0^{bc}
13	IRGC 7219	BA BONG	0,42^{c}	32,6^{cd}	8,4^{de}
14	IRGC 10231	DOC PHUNG LUN	1,89^{a}	80,7^{a}	1,9^{a}
15	IRGC 10865	TIEU PHAT	2,03^{a}	75,5^{a}	2,3^{a}
16	IRGC 16749	NANG SOM	0,67^{bc}	39,6^{cd}	4,5^{b}
17	IRGC 16783	TRANG TEP	0,13^{de}	40,6^{cd}	7,5^{cd}
18	IRGC 16852	LUA NGU	0,59^{bc}	51,2^{bc}	4,9^{bc}
19	IRGC 16878	NANG SON	0,4^{bc}	9,4^{e}	8,8^{de}
20	IRGC 17013	SOC NAU	0,83^{b}	39,5^{cd}	4,9^{bc}
21	IRGC 30412	IR29	0,09^{e}	5,6^{f}	8,7^{de}
22	IRIS 66-333787	FL478	2,13^{a}	91,2^{a}	1,6^{a}

(Different letters in the same column indicate statistical significant different at $p < 0.05$)

In observed physiological parameters such as dried biomass, survival rate and standard evaluation scoring, there was the corelation between dried biomass and survival rate under salined condition. The phenotypes with low survival rate showed low dried biomass (Table 4). This result is in agreement with previous studies explaining that the plant with higher biomass will have higher tolerance due to dilution mechnism to reduce salt concentration in stem and leaves [14]. In 2004, Peng and Ismail also reported that rice seedling with higher biomass is preferable characteristics to tolerate salinity because salinity problem is often happen at the beginning of raining season when rice are being sown [15]. However, this phenotype is usually ignored in breeding program due to its drawback which cause rice vulnerable to lodging.

Investigatigation to identify salintiy tolerance genotypes are carrying out in different countries. In 2014, after screening 33 rice landraces in West Bangal, India, Ali and colleagues found four rice genotypes which were able to used as breeding material for breeding programs [16]. In Bangladesh, another major rice producer, also focused on exploiting genetic resources to find tolerance rice landracs. In 2015, after surveying 86 rice landraces, research group led by Emon found 11 landraces with high tolerance [17].

3.2. The absence of *Saltol* gene in salinity tolerance genotypes

Saltol is DNA fragment located on short arm of chromosome 1 and largely responsible for salinity tolerance of salinity check genotype FL478 [3]. Using two primer pairs specific to this gene, we did not detect the present of *Saltol* FL478 (Figure 2). Thus, we initially suggest that the high tolerant rice found in this study possess unknown mechanism to cope with salinity condition and different with *Saltol* as previous publishcations [3, 8].

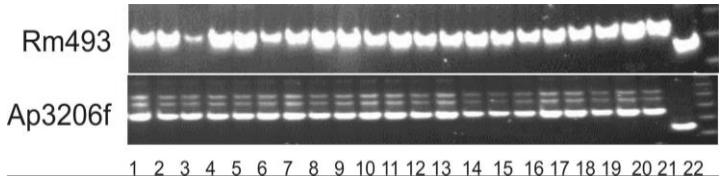

Figure 2. Screening *Saltol* in rice landraces with RM493 AP3206f primers
(The genotype numbers are corresponding with Table 1).

There were several studies successful in using molecular markers to identify the present of *Saltol* in rice landraces [13, 16, 17]. It is possible that the tolerant mechanism of rice landraces in our study is dependent on unknown loci that requires further investigation to localize these DNA regions for future studies.

3.3. The changing of protein profile under saline condition

Application of SDS-PAGE in analyzing protein profile has been proven as a useful method to identify preferable agronomical characteristics in rice. In Cuu Long Delta, Nguyen and colleagues found specific protein bands responsible for grain quality such as α-glutelin, β-glutelin and pro-glutelin [18]. These are valuable proteins which is important in rice embryo and good for human health. Protein responsible for aromatic feature was also identified by this technique in 2007 [19].

In this study, after screening 4 rice landraces which is highly salinity tolerant consisting of Doc Phung, Nang Thom, Doc Phung Lun and Tieu Phat (corresponding to number 3, 6, 14 and 15 in Table 1) we found the changing of protein profile in different growing conditions (Figure 3). In saline condition, protein content is more abundant than in control condition without stress (Figure 4b). The notable change can be easily observed at 22 kDa

(lines indicated with asterisks). This result is in agreement with previous study in 2005, where Kong-ngern and his colleagues suggested the increase of protein expression at 22 kDa in salinity tress when studied the salinity tolerant rice in Thailand [20]. Moreover, this research also found the up expression of different proteins with molecular weight ranging from 45 to 97.4 kDa. This result is similar with our findings. More recently, in 2015, another study in Cuu Long Delta also found the accumulation of rice protein having molecular weight from 115.8 to 135 kDa under salt stress [21].

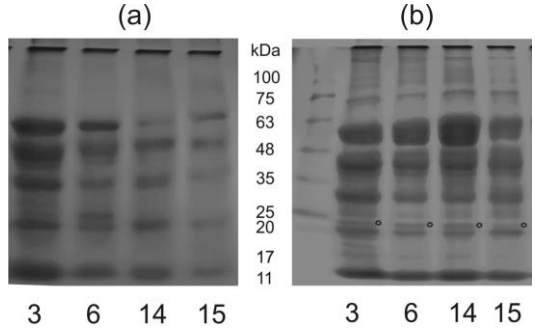

Figure 3. The change of protein profiles of for rice landraces after three weeks in control (a) and NaCl 6‰ (b). (The genotype numbers are corresponding with Table 1).

4. CONCLUSIONS

After screening 20 rice landraces originally from Vietnam, four genotypes namely Doc Phung, Nang Thom, Doc Phung Lun and Tieu Phat showed high salinity tolerance. These landraces will be potential candidates for breeding new salinity tolerant rice cultivars. Furthermore, these genotypes could be further investigated to reveal tolerant mechanism and identify genes relating to salinity tolerance. This will be useful for rice breeding through marker-assisted selection methods.

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TÓM TẮT

ĐÁNH GIÁ KHẢ NĂNG CHỊU MẶN CỦA 20 GIỐNG LÚA ĐỊA PHƯƠNG CỦA VIỆT NAM

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Do tác động của biến đổi khí hậu, hiện trạng canh tác lúa ở nước ta đang gặp nhiều khó khăn do quá trình xâm nhập mặn. Hiện tại, Viện nghiên cứu lúa quốc tế IRRI đang lưu giữ một số lượng lớn các giống lúa địa phương của nước ta; tuy nhiên việc đánh giá và khai thác tiềm năng của các giống lúa này để phục vụ công tác chọn tạo các giống lúa chịu mặn vẫn chưa được chú ý đúng mức. Trong nghiên cứu này 20 giống lúa địa phương được đánh giá về khả năng chống chịu với điều kiện mặn ở nồng độ muối NaCl 6‰ trong ba tuần. Kết quả cho thấy 4 trong 20 giống lúa có khả năng chịu mặn cao thông qua đánh giá các chỉ tiêu sinh trưởng và sinh lý. Nghiên cứu không phát hiện ra sự có mặt của các gene liên quan đến khả năng chịu mặn; tuy nhiên có sự biến đổi khác nhau về hàm lượng protein tổng số ở các cây giống lúa. Nghiên cứu này là bước đầu để tiến hành khai thác tiềm năng chịu ngập trong quần thể lúa địa phương để tạo ra những giống lúa mới có khả năng thích nghi với hậu quả của biến đổi khí hậu đang diễn ra nhanh ở nước ta.

Từ khóa: Lúa địa phương, chịu mặn, *Saltol,* IRRI, SDS-PAGE.