RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
50 (3) 344-359, 2018

DEVELOPMENT OF RICE GENOTYPES TOLERANT TO SALINITY STRESS IN THE MEKONG DELTA, VIETNAM USING MARKER-ASSISTED SELECTION

NGUYEN THI LANG[1*], NGUYEN TRONG PHUOC[1], BIEN VAN KHOA[1] and BUI CHI BUU[2 *]

[1]High Agricultural Technology Research Institute for Mekong Delta, Vietnam
[2]Institute of Agricultural Sciences for Southern Vietnam, Ho Chi Minh City, Vietnam
*Corresponding authors' email addresses: ntlang.prof@gmail.com, buu.bc@iasvn.org

SUMMARY

Salinity stress is a major limitation to rice (*Oryza sativa* L.) yields and its stability, especially in the Mekong delta, Vietnam. The purpose of this experiment was to develop rice varieties tolerant to salinity on the basis of molecular markers.Two hundred fifty three BC_2F_2 rice lines of the OM7347/OM5629 were evaluated at seedling stage in the green house of CLRRI. Molecular markers associated with salt tolerance QTLs were identified by using 416 polymorphic SSR markers. QTLs associated with stress tolerance at EC = 8 dS/m at seedling stage, detected from the BC_2F_2 population, were located on chromosomes 1 and 3. Three QTLs were identified within the intervals of RM3532-RM10694, RM3740-RM5336, and RM11125-RM9 at map positions of 4.4, 4.5, and 18 cM on chromosome 1, respectively. Two QTLs were located within the intervals of RM3867-RM6959 and RM6876-RM4425 at map positions of 4.5 and 18.0 cM on chromosome 3. Three QTLs in the regions of RM1324-RM2412, RM1185-RM24, and RM1282-RM2560 on chromosome 1, and one QTL of RM453-RM511 on chromosome 12, were associated with salt tolerance at reproductive stage (EC = 4 dS/m). In addition, three advanced backcross populations were developed as BC_2F_2 of OM6162/Pokkali (100 lines), BC_3F_2 of OMCS2000/Pokkali (50 lines), BC_3F_2 population of OM1490/Pokkali (53 lines). Their phenotypes were evaluated at seedling and reproductive stages. Marker-assisted selection was applied to identify promising lines using the markers RM3252 and RM223.

Key words: Reproductive stage, salinity, *SalTol*, seedling stage, SSR, QTL

Key findings: Marker assisted selection was performed in rice backcross populations to select for genes/QTLs associated with salinity tolerance.

Manuscript received: May 14, 2017; Decision on manuscript: September 5, 2017; Accepted: June 18, 2018.

Communicating Editor: Bertrand Collard

INTRODUCTION

Currently, salinity is considered to be a major abiotic stress in rice, which reduces productivity in affected areas. The Mekong Delta is the largest rice production region of Vietnam that constitutes 55% of the total (4.3 out of 7.9 million planted paddy area) (Buu *et al*., 1997). Rice areas affected by drought and salinity intrusion rapidly increased from 139,000 ha in mid-March 2016 to 224,552 ha by mid-April 2016, with the damage value of $US 360 million (Buu, 2017). In Asia, 12 million ha of land area is thought to be salinity affected (Kumar *et al.,* 2015). Changes in rainfall patterns, dissimilar distribution of rainfall in different areas as well as during the development of rice plant, and intrusion of saltwater into the inland during 2016 Winter-Spring season were considered as the most serious *El Nino* event after hundred years with roughly one million tons of paddy lost. Hence, it is necessary to improve rice salt tolerance in the affected areas.

Lang *et al.* (2001c) reported that salt tolerant genes tagging based on SSR markers with advanced backcross populations (BC_2F_2) of IR64/Cheng Hui 448, IR64/OM1706, and IR64/FR13A derived alleles nearly located on chromosome 1 while in the population of IR68552-55-3-2/OM1706, the alleles are linked with RM223 on chromosome 8.

The major quantitative trait locus (QTL) for salinity tolerance (*Saltol*) was mapped on chromosome 1 and chromosome 8 (Lang, 1999; Lang, 2001). RM223 linked to salt tolerance gene at the distance of 6.3 cM on chromosome 8 at vegetative stage under EC = 10 $dS.m^{-1}$ from F_3 population of IR28/Doc Phung (Lang *et al.*, 1999).

Bonilla *et al.* (2002) mapped *Saltol* locus linked to major QTLs for Na^+ and K^+ uptake and Na^+/K^+ ratio on chromosome 1 explaining 64.3% phenotypic variance. Ammar *et al.* (2009) reported 25 QTLs for salt ion concentrations (Na^+, K^+ and Cl^- measured in the leaf tissues at the reproductive stage) on rice chromosomes 1, 2, 3, and 8. Pandit *et al.* (2010) reported eight QTLs for salt ion concentration on rice chromosomes 1, 8, and 12, and Cheng *et al.* (2012) reported 12 QTLs for salt ion concentration on rice chromosomes 1, 2, 3, 4, 7, and 11, respectively.

Kumar *et al.* (2015) applied genome wide association study (GWAS) to identify loci controlling salinity tolerance in rice. A custom-designed array based on 6,000 single nucleotide polymorphisms (SNPs) in as many stress-responsive genes, distributed at an average physical interval of < 100 kb on 12 rice chromosomes, was used to genotype 220 rice accessions using Infinium high-throughput assay. They identified 20 SNPs (loci) significantly associated with Na^+/K^+ ratio, and 44 SNPs with other traits observed under stress condition. The region harbouring *Saltol* on chromosome 1 is known to control salinity tolerance at seedling stage (Kumar *et al.,* 2015).

QTL mapping is carried out by genotyping a large number of individuals that are progeny of a bi-parental cross, the process is labor-intensive, time-consuming and costly (Salvi and Tuberosa 2005). High-throughput genotyping platforms based on SNP arrays and next generation sequencing (NGS) technologies have evolved very fast

during the last decade (Tiwari *et al.,* 2016).

Nounjan *et al.* (2016) identified two chromosome segment substitution lines of Khao Dawk Mali 105 (KDML 105) rice that carry QTLs for drought tolerance located on chromosome 8 (DT-QTL 8) designated CSSL8-9 4 and CSSL8-116. They investigated for co-expression network and physiological responses to salinity compared to their parents (KDML105; drought and salt sensitive recurrent parent, and DH103; drought tolerant QTL donor).

Tiwari *et al.* (2016) reported a method for rapid identification of QTLs for reproductive stage salt tolerance in rice using bulked segregant analysis (BSA) of bi-parental recombinant inbred lines (RIL). The method was applied to CSR11/MI48 RILs segregating for reproductive stage salt tolerance. Genotyping of the parents and RIL bulks, made on the basis of salt sensitivity index for grain yield, revealed 6,068 polymorphic SNPs and 21 QTL regions showing homogeneity of contrasting alleles in the two bulks. BSA with 50K SNP chip revealed 5,021 polymorphic loci and 34 QTL regions.

In this study, we established QTL maps for traits related to salinity tolerance at different stages in order to select genotypes adapted to both seedling and reproductive stage tolerances that are required for the Mekong Delta region of Vietnam.

MATERIALS AND METHODS

Plant materials

Two indica rice genotypes were used: OM5629, which was considered as the donor of salinity tolerance, and OM7347 as a recurrent parent with good quality traits and drought tolerance. Two hundred fifty three elite lines from the BC_2F_2 population of OM7347/OM5629 were produced and 230 lines from the BC_3F_6 population of OMCS2000/Pokkali were also developed (Lang *et al*., 2015). Of the 769 SSRs screened for polymorphism across parents, 416 were polymorphic and used for QTL mapping.

In addition, 100, 50, and 53 lines from BC_2F_2 of OM6162/Pokkali, BC_3F_2 of OMCS2000/Pokkali, and BC_3F_2 of OM1490/Pokkali, respectively were used to identify promising genotypes tolerant to salinity. Pokkali was considered as the tolerant check.

The following primers were used:
RM 223:
F 5'-GAGTGAGCTTGGGCTGAAAC-3' and R 5'-GAAGGCAAGTCTTGGCACTG-3'.
RM3252:
F 5'-GGTAACTTTGTTCCCATGCC-3' and R 5'-GGTCAATCATGCATGCAAGC-3'.

Creating backcross populations with introgressions for salinity tolerance

The hybridization goal is to introduce salinity tolerance genes/QTLs from Pokkali into high yielding rice genotypes using backcrossing to create recombinants that have desirable agronomic traits, and tolerance to salinity. The OM1490, OMCS2000, and OM6162 varieties are high yielding and have good grain quality, especially OM6162 which is aromate, but they are sensitive to salinity. They were used as recipient female parents in the breeding program. Backcrossing was done to create BC_2 and BC_3 generations.

Screening for salinity tolerance in the screenhouse

Salinity screening experiments were done in the greenhouse (Gregorio, 1997; IRRI, 1996) with Pokkali as a tolerant variety and IR29 as a susceptible variety at the EC of 8 dS/m and 4 dS/m at seedling and reproductive stages, respectively.

The experiment was carried out in three replications, randomized complete block design (RCBD). Germinated seeds were put into the floating styrofoam lids using sterilized water within 3 days. Then, the EC of the Yoshida solution was increased up to 4dS/m and 8 dS/m, at pH = 5.0 – 5.5. After 21 days, phenotypic evaluation was performed on the survival day (SD) of the tolerant and susceptible genotypes. The susceptible variety was almost dead completely.

Genotyping

DNA samples were assessed using agarose gel electrophoresis (0.9%, in TAE 1X buffer). The leaves were collected 2-3 weeks after planting for DNA extraction. Amplification of genes based on PCR using SSR marker: PCR reactions were performed with 769 SSR markers. Amplified PCR products were assessed on agarose gels 3% in TBE buffer (Lang, 2002). Reactions were overlaid with mineral oil and processed in a programmable thermal controller set for 35 cycles of 1 min at 94 ^{0}C, 1 min at 55 ^{0}C, and 2 min at 72 ^{0}C, with a final extension at 75 ^{0}C for 5 min. After amplification, 10 µl of stop solution was added to the PCR product, which was then denatured at 94 ^{0}C for 2 min. Eight microliters of each reaction were run on 5% polyacrylamide gels.

Data analysis

Map construction and QTL analysis: The program MAPMAKER/EXP v.3.0 (Lander *et al.*, 1987; Lincoln *et al.,* 1992) was used to establish a genetic linkage map using the Kosambi mapping function (Kosambi, 1944; Causse *et al.*, 1994; Harushima *et al.*, 1998; Temnykh *et al.*, 2001). MAPMAKER/QTL version 1.1 was used to identify loci affecting quantitative traits on the basis of interval analysis (Paterson *et al.*, 1988; Lincoln *et al.*, 1992). A LOD score of 3.0 was selected as the threshold for the presence of a QTL (Lander and Bostein, 1989). With such a threshold, a false positive QTL would be detected anywhere in the genome with a probability of approximately 0.05 (Paterson *et al.*, 1988). The interaction between all possible loci on the map was performed using QTLMap version 1.0 (Wang *et al.*, 1999). To analyze marker-QTL association for each trait, single-point (single marker) analysis of QGene version 4.0.2 (Nelson, 1997) was performed. Combined data based on QGene, MapMaker, and GGT (Graphical Genotyper) was used to analyze QTL maps.

RESULTS

QTL mapping for salinity tolerance in BC_2F_2 population of OM7347/OM5629 at seedling stage

Two hundred and fifty three individuals of BC_2F_2 population of OM7347/OM5629 were used to identify QTLs as well as to identify regions associated with drought tolerant in OM7347 and salinity

tolerant in OM5629 in previous studies (Lang and Buu, 2011b; Lang and Buu, 2011c). Twelve linkage groups representing all twelve chromosomes constructed were 2,447.5 cM in total length, with average distance of 10.69 cM. To elucidate the genetic basis and physiology of traits related to salinity tolerance in rice, and identify overlapping QTLs.

Table 1. Modified IRRI standard evaluation scoring (SES) system based on the visual symptoms of salinity stress injury of rice.

SES	Description	Tolerance
1	Normal growth, only the old leaves show white tips while no symptoms on young leaves	Highly tolerant
3	1. Near normal growth, but only leaf tips burn, few older leaves become whitish partially	Tolerant
5	2. Growth severely retarded; most old leaves severely injured, few young leaves elongating	Moderately tolerant
7	3. Complete cessation of growth; most leaves dried; only few young leaves still green	Susceptible
9	4. Almost all plants dead or dying	Highly susceptible

(IRRI, 1996; Gregorio *et al.*, 1997)

Through marker analysis, there were many significant QTLs related to salinity tolerance from BC_2F_2. The QTLs were located on chromosome 1 i.e. RM3532-RM10694; RM3740-RM5336, and RM11125-RM9 explained 13.33%, 30.48%, and 37.14% of phenotypic variance, respectively. QTLs located on chromosome 1 had large effects and explained more than 50% of the phenotypic variance. In the backcross population of OM7347/OM5629, the QTLs related to salinity tolerance (EC=8 dS/m) at seedling stage were located on chromosomes 1, 6, 8, and 9 (Lang *et al*., 2000; Lang *et al.*, 2001a; 2001b).

LOD peaks for QTLs associated with salinity tolerance at seedling stage on chromosome 1 (RM3532-S-RM10694; RM3740-RM5336, and RM11125-RM9), on chromosome 3 (RM3867-RM6959 and RM6876-RM4425), and on chromosome 12 (RM453-RM511) were detected (Figure 1) at EC = 8 dS/m at seedling stage and 4 dS/m at reproductive stage.

At seedling stage, the QTLs related to survival day and SES score were detected on chromosomes 1 and 3. Three QTLs were identified within the intervals of RM3532-RM10694, RM3740-RM5336, and RM11125-RM9 with genetic distance of 4.4, 4.5, and 18.0 cM on chromosome 1, respectively. Two QTLs within the intervals of RM3867-RM6959 and RM6876-RM4425 with genetic distance of 4.5 and 18.0 cM on chromosome 3, respectively. They explained 11.41 % to 37.14 % of the phenotypic variation (Table 1). Results of plant height of BC2F4 lines in OM1490/IR64-Sub1 in conditions before and after complete submergence are shown in Table 2.

At reproductive stage, 253 individuals of the BC_2F_2 population from the OM7347/OM5629 population were screened under salt stress of EC

= 4 dS/m. Yield components and grain yield were evaluated. The results showed that QTLs associated with salinity tolerance were mainly located on chromosomes 1 and 12. Three QTLs located within the intervals of RM1324-RM2412, RM1185-RM24, and RM1282-RM2560 on chromosome 1 corresponded to grain yield. One QTL within the region of RM453-RM511 on chromosome 12 was detected using the SES (standard evaluation system developed by IRRI) (Table 3).

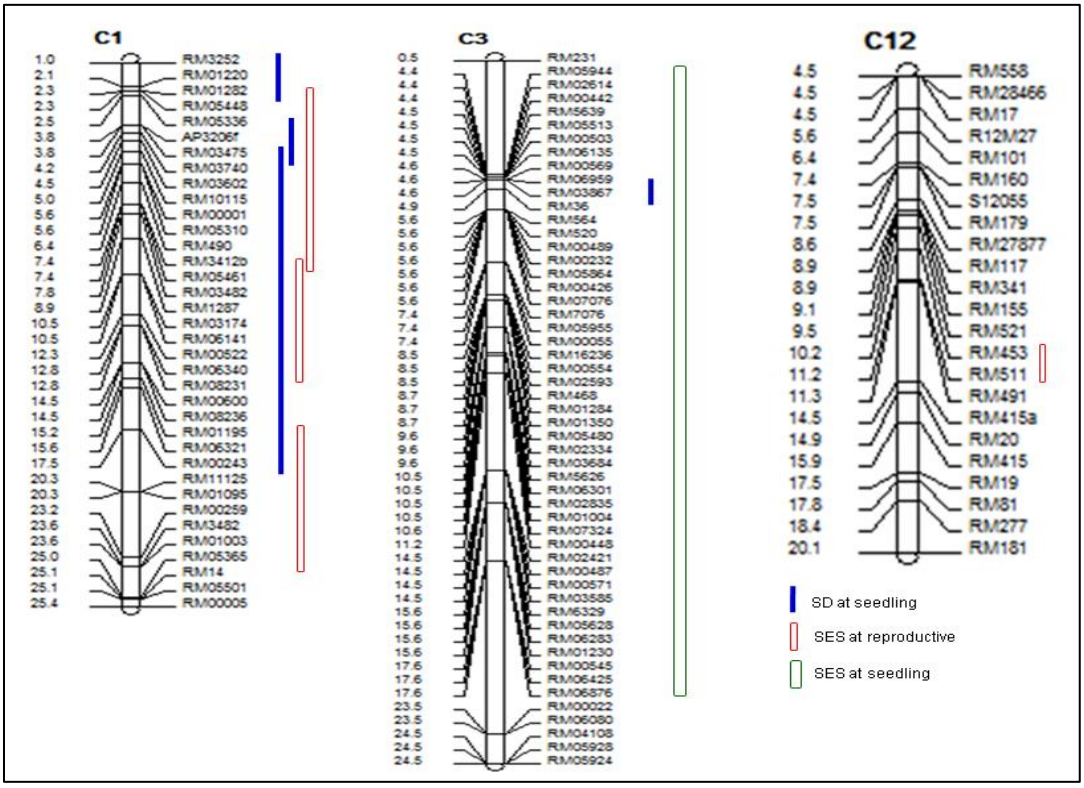

Figure 1. QTL map for the traits related to salinity tolerance on chromosomes 1, 3 and 12 in the BC_2F_2 population of OM7347/OM5629 (blue bar: survival day at seedling, red bar: SES score at reproductive, green bar: SES score at seedling).

In addition, Lee *et al.* (2007) reported that salinity tolerance QTLs located on chromosomes 1 and chromosome 3 were QST01 and QST03, respectively. Recently, Thomson *et al.* (2010) reported that four QTLs related to salt tolerance were located on chromosomes 1, 2, 3, and 12. Results from the QTL mapping analysis showed that marker RM3532 was tightly linked to *Saltol* locus (4.6 cM) on chromosome 1.

The lines from population BC_3F_4 of OM1490/Pokkali were screened and evaluated under EC = 8 dS/m based on survival day and SES score. In addition, genotyping using 342 polymorphic SSRs (Figure 2) facilitated more precise selection of progenies at the region around 200 cM on chromosome 1.

Table 2. QTLs associated with salinity tolerance in the BC_2F_2 population of OM7347/OM5629 at seedling stage (EC=8 dS/m).

Chr.	QTL	Location (cM)	CIM (Interval cM)	LOD	A	D	R^2
1	SD QTL-1	4.4	RM3252--RM10694	4.3	0.29	15.18	13.33
1	SESQTL-1	18.0	RM11125-RM9	3.0	11.43	81.08	37.14
3	SD QTL-3	4.5	RM3867-RM6959	4.6	12.56	23.67	11.41
3	SESQTL-3	18.0	RM6876-RM445	17.1	4.85	24.50	17.40

P < 0.05; A: Additive, D: Dominant, R^2: phenotypic variance explained, Chr: chromosome; SD: survival day

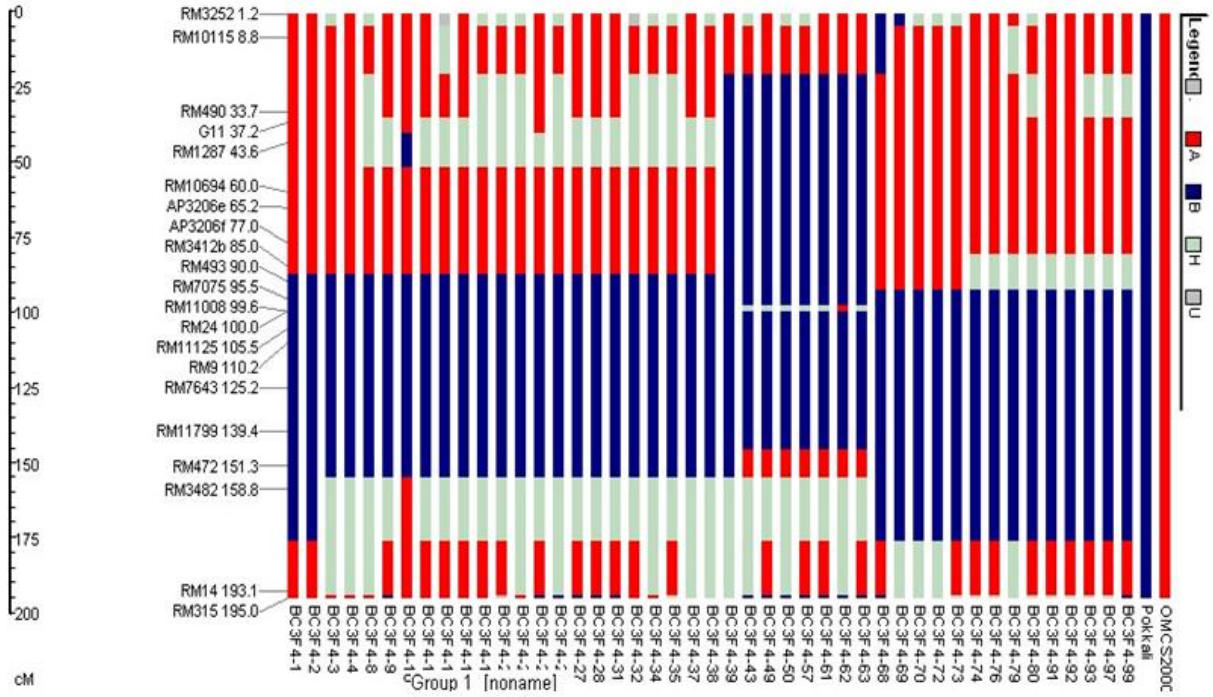

Figure 2. Graphical genotypes of BC_3F_4 backcross population of OMCS2000/Pokkali on chromosome 1.

Table 3. Data based on combined analysis of phenotype and genotype on BC2F2 populations of OM6162//OM6162/Pokkali.

Lines	Lines	Phenotype EC=8Ds/m	Score
OM6162	OM6162	S	S
Pokkali	Pokkali	3	T
1	BC2F2-1	3	T
2	BC2F2-2	3	T
3	BC2F2-3	9	S
4	BC2F2-4	9	S
5	BC2F2-5	9	S
6	BC2F2-6	9	S
7	BC2F2-7	9	S
8	BC2F2-8	9	S
9	BC2F2-9	9	S
10	BC2F2-10	9	S
11	BC2F2-11	7	S
12	BC2F2-12	5	S
13	BC2F2-13	5	S
14	BC2F2-14	7	S
15	BC2F2-15	7	S
16	BC2F2-16	7	S
17	BC2F2-17	9	S
18	BC2F2-18	5	S
19	BC2F2-19	7	S
20	BC2F2-20	7	S
21	BC2F2-21	7	S
22	BC2F2-22	7	S
23	BC2F2-23	9	S
24	BC2F2-24	9	S
25	BC2F2-25	9	S
26	BC2F2-26	9	S
27	BC2F2-27	9	S
28	BC2F2-28	9	S
29	BC2F2-29	9	S
30	BC2F2-30	7	S
31	BC2F2-31	9	S
32	BC2F2-32	7	S
33	BC2F2-33	5	S
34	BC2F2-34	5	S
35	BC2F2-35	5	S
36	BC2F2-36	5	S
37	BC2F2-37	5	S
38	BC2F2-38	5	S
39	BC2F2-39	7	S
40	BC2F2-40	7	S
41	BC2F2-41	7	S
42	BC2F2-42	7	S
43	BC2F2-43	7	S
44	BC2F2-44	7	S
45	BC2F2-45	7	S
46	BC2F2-46	7	S
47	BC2F2-47	7	S
48	BC2F2-48	3	T
49	BC2F2-49	7	S
50	BC2F2-50	5	S
51	BC2F2-50	7	S
52	BC2F2-52	3	T
53	BC2F2-53	5	S
54	BC2F2-54	9	S
55	BC2F2-55	9	S
56	BC2F2-56	9	S
57	BC2F2-57	9	S
58	BC2F2-58	9	S
59	BC2F2-59	9	S
60	BC2F2-60	9	S
61	BC2F2-61	3	T
62	BC2F2-62	3	T
63	BC2F2-63	7	S
64	BC2F2-64	3	T
65	BC2F2-65	3	T
66	BC2F2-66	9	S
67	BC2F2-67	3	T
68	BC2F2-68	3	T
69	BC2F2-69	7	S
70	BC2F2-70	7	S
71	BC2F2-71	3	T
72	BC2F2-72	7	S
73	BC2F2-73	9	S
74	BC2F2-74	9	S
75	BC2F2-75	9	S
76	BC2F2-76	5	S
77	BC2F2-77	5	S
78	BC2F2-78	9	S
79	BC2F2-79	5	S
80	BC2F2-80	5	S
81	BC2F2-81	7	S
82	BC2F2-82	9	S
83	BC2F2-83	9	S
84	BC2F2-84	9	S
85	BC2F2-85	5	S
86	BC2F2-86	9	S
87	BC2F2-87	9	S
88	BC2F2-88	9	S
89	BC2F2-89	9	S
90	BC2F2-90	7	S
91	BC2F2-91	9	S
92	BC2F2-92	5	S
93	BC2F2-93	9	S
94	BC2F2-94	7	S
95	BC2F2-95	7	S
96	BC2F2-96	7	S
97	BC2F2-97	9	S
98	BC2F2-98	9	S
99	BC2F2-99	9	S
100	BC2F2-100	9	S

S: susceptible; T: Tolerant.

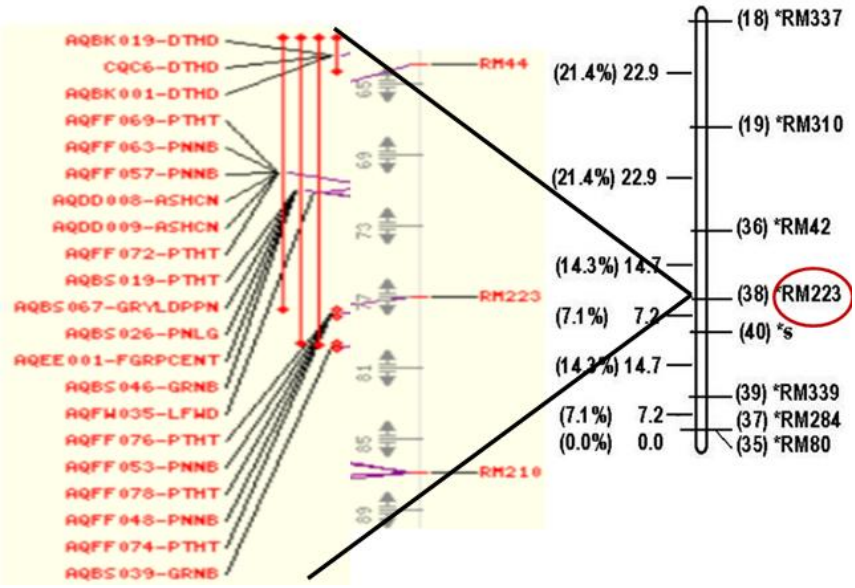

Figure 3. Genetic map of QTL linked to salt tolerance on chromosome 8 at locus RM223 (Lang *et al.* 2000, 2001c). New markers for fine mapping were identified by comparative mapping using Gramene.

Previously, a QTL linked to salinity tolerance was detected on chromosome 8 near the locus RM223 (Lang *et al.*, 2000, 2001c). The map was constructed using a RIL population of IR28/Doc Phung. The QTL which is linked to RM223 on chromosome 8, explained 33% of phenotypic variation of survival day at seedling stage under EC = 6 dS/m (Figure 3). Following information in the Gramene database, six new markers were tested in our segregating populations at Cuu Long Delta Rice Research Institute (CLRRI): RM23550, RM23554, RM23562, RM23571, RM23582, and RM23584 on chromosome 8 at the locus RM223.

Conventional breeding and marker-assisted selection

Salinity tolerance of OM6162/Pokkali population

The BC_2F_2 population of OM6162/Pokkali was screened and evaluated under EC = 8 dS/m and 15 dS/m at seedling stage. At EC = 8dS/m, most of lines at seedling stage exhibited their survival within the range of 20-25 days. Out of 100 individuals, eighteen segregants survived after 27-30 days, which indicates good tolerance to salinity.

At EC= 15 dS/m, most of lines did not survive. Of 100 individuals, six segregants had the same survival rate as tolerant check i.e. BC_2F_2-1, BC_2F_2-60, BC_2F_2-61, BC_2F_2-63, BC_2F_2-64, and BC_2F_2-66.

Salinity tolerance of OMCS2000/Pokkali population

At EC = 8dS/m, four lines at seedling stage had a SES score of 1. Out of 50 lines, only three lines as BC_3F_2-4, BC_3F_2-9 and BC_2F_3-12 exhibited SD of 27 days. At EC = 15 dS/m, there was

only one lines i.e. BC_3F_2-4, which had the same SD as the tolerant check.

Salinity tolerance of OM1490/Pokkali

At EC = 8dS/m, of 53 lines, three progenies exhibited high tolerance to salt stress with their SES score of 1 and SD of 27 days. They were selected as promising genotypes i.e. BC_3F_2-10, BC_3F_2-37, and BC_3F_2-40.

At EC= 15dS/m, two lines exhibited high SD namely BC_3F_2-10 and BC_3F_2-40 with the survival percentage of 65.0% and 41.9%, respectively, after 20 days.

Marker-assisted selection

Major QTLs for salinity tolerance (including *Saltol*) was mapped on chromosome 1 and chromosome 8 (Lang 1999, Lang 2001). RM223 was linked to salt tolerance at the position 6.3 cM on chromosome 8 at vegetative stage under EC = 10 $dS.m^{-1}$ from F_3 population of IR28/Doc Phung (Lang *et al.*, 1999). Table 1 and 2 indicated that RM3252-S1-1 on chromosome 1; RM3867, RM6959 on chromosome 3 also to be used for marker-assisted selection.

Progeny selection from OM6162/Pokkali population

PCR products at the locus RM3252-1-1 on chromosome 1 among 100 lines of BC_2F_2 derived from OM6162/Pokkali in agarose gel (3%) indicated that two bands of alleles were addressed at 220 bp and 230 bp corresponding to Pokkali and OM6162, respectively (Figure 3). Of 100 lines, eight segregants in BC_2F_2 population exhibited *Saltol* QTLs (Lines: 1, 47, 51, 60, 61, 63, 64, and 66). The six phenotypes under Yoshida solution test as BC_2F_2-1, BC_2F_2-60, BC_2F_2-61, BC_2F_2-63, BC_2F_2-64, and BC_2F_2-66 were noticed among the eight lines.

Figures 4 and 5 showed the amplification of RM223 on chromosome 8, with two allele sizes of 200 bp and 220 bp corresponding to Pokkali and OM6162, respectively. Once again, the six lines BC_2F_2-1, BC_2F_2-60, BC_2F_2-61, BC_2F_2-63, BC_2F_2-64, and BC_2F_2-66 inherited their salinity tolerance QTLs from Pokkali. There were 57% heterozygous segregants.

PCR products at the locus RM3252-1-1 on chromosome 1 among 50 lines of BC_3F_2 derived from OMCS2000/Pokkali revealed that two alleles (200 bp and 230 bp) corresponding to Pokkali and OMCS2000, respectively. Of 50 lines, five segregants in the BC_3F_2 population exhibited salinity tolerance (lines 4, 5, 9, 20, and 46). The heterozygous alleles were observed for lines 19, 29, and 43, which meant that most of them were homozygous and were selected (Figure 6).

PCR products at the locus RM223 on chromosome 8 among 50 lines of BC_3F_2 derived from OMCS2000/Pokkali revealed that two marker alleles (200 bp and 220 bp) corresponded to OMCS2000 and Pokkali, respectively. Four segregants in the BC_3F_2 population exhibited *Saltol* QTLs as 4, 7, 9, and 12. Two lines i.e. 4 and 9 based on both two markers were considered for further breeding. They are corresponded to BC_3F_2-4 and BC_3F_2-9.

PCR products at the locus RM3252-1-1 on chromosome 1 among 53 lines of BC_3F_2 derived from OM1490/Pokkali revealed that two bands of alleles (220 bp and 230 bp) corresponded to Pokkali and OM1490, respectively (Figure 7). On chromosome 3, two bands of alleles

separated 200 bp (Pokkali) from 220 bp (OM1490) at the RM223 locus. Of 53 lines, three genotypes had the same allele as Pokkali at both loci RM3252-1-1 and RM223, i.e. BC_3F_2-10, BC_3F_2-37, and BC_3F_2-40 (Figure 8).

DISCUSSION

Development of rice varieties tolerant to salinity, high-yielding, and good quality are essential for poor farmers in saline conditions in the Mekong Delta, Vietnam. The purpose of this experiment was to develop rice varieties tolerant to salinity on the basis of a combination of two breeding methods by molecular markers and backcrossing. Our results confirmed the major QTL *Saltol* associated with salt tolerance at the seedling stage, which was mapped on chromosome 1 in rice on OM7347/OM5629 (at EC= 8dSm). Genotyping was performed using three advanced backcross populations were developed as BC_2F_2 of OM6162/Pokkali (100 lines), BC_3F_2 of OMCS2000/Pokkali (50 lines), and BC_3F_2 population of OM1490/Pokkali (53 lines) to select for salinity tolerance. Their phenotypes were evaluated at seedling and reproductive stages. Marker-assisted selection was applied to identify promising lines using the SSR markers RM3252-S1-1 and RM223.

Using co-dominant DNA markers, it is possible to fix specific alleles in their homozygous state in the BC2F2 or BC_3F_2 generation. For salinity tolerance in the OM6162/Pokkali population at EC = 8dS/m, most of lines at seedling stage exhibited their survival within the range of 20-25 days.

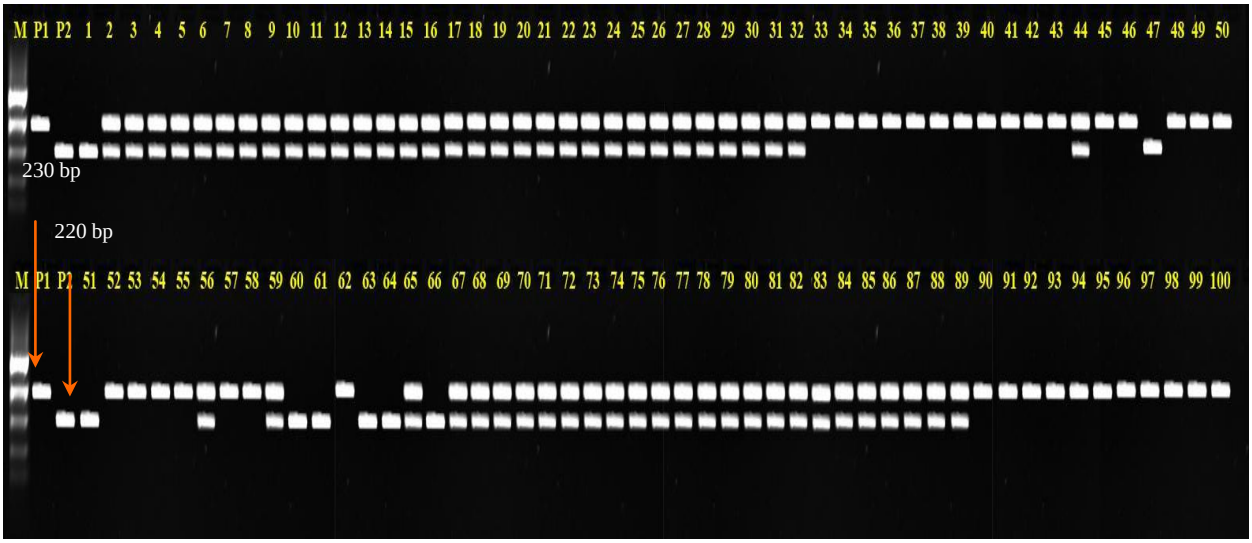

Figure 4. PCR products at the locus RM3252-on chromosome 1 among 100 lines of BC_2F_2 derived from OM6162/Pokkali in agarose gel 3% P1: OM6162; P2: Pokkali; 1-100: BC_2F_2 of OM6162/Pokkali.

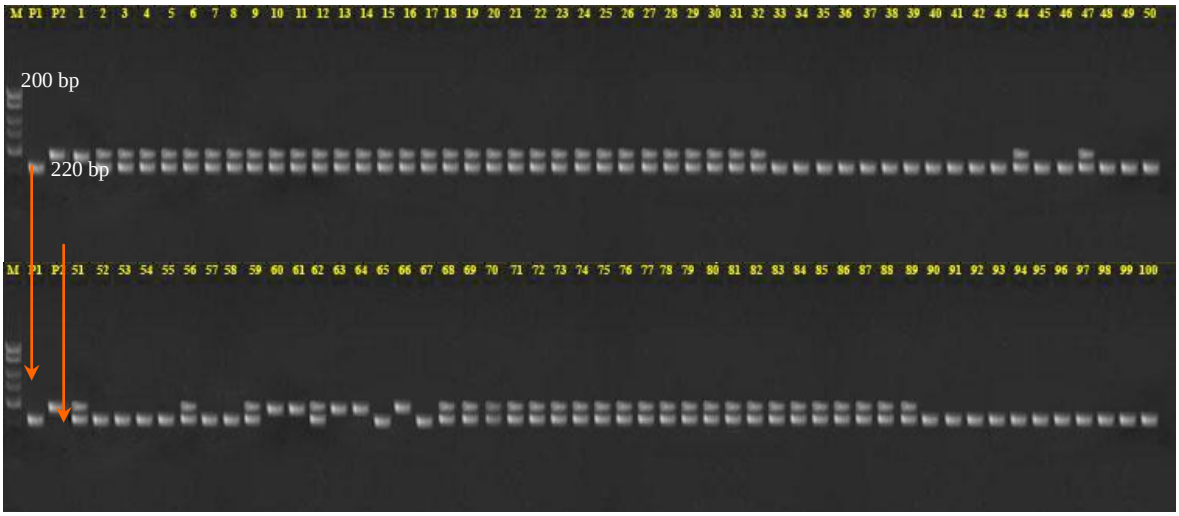

Figure 5. PCR products at the locus RM223 on chromosome 8 among 100 lines of BC_2F_2 derived from OM6162/Pokkali in agarose gel 3% P1: OM6162; P2: Pokkali; 1-100: BC_2F_2 of OM6162/Pokkali.

Progeny selection of OMCS2000/Pokkali

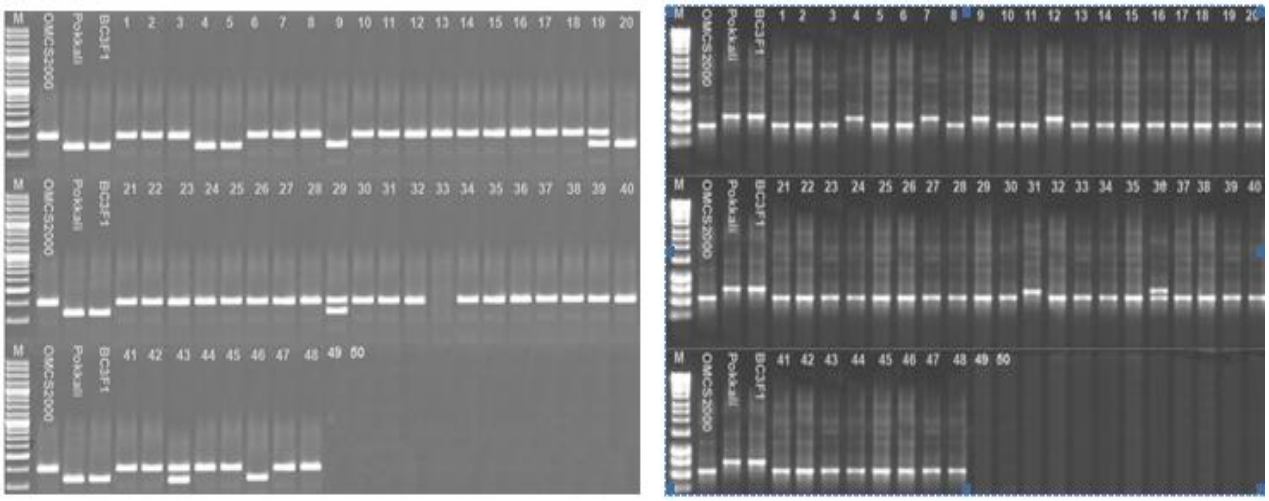

Figure 6. PCR products at the locus RM3252-1-1 on chromosome 1 (left) and RM223 on chromosome 8 (right) among 50 lines of BC_3F_2 derived from OMCS2000/Pokkali in agarose gel 3%. [M: ladder; 1: OMCS2000; 2: Pokkali; 1-50: individuals of BC_3F_2 population].

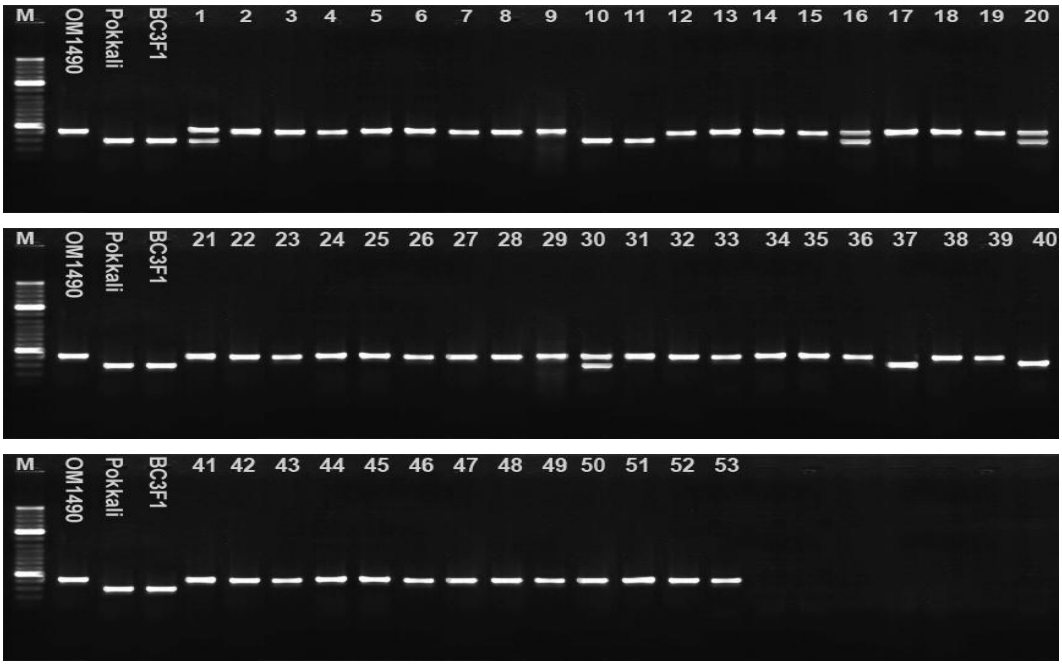

Figure 7. PCR products at the locus RM3252 on chromosome 1 among 53 lines of BC_3F_2 derived from OMCS1490/Pokkali in agarose gel 3%. [M: ladder; 1: OM1490; 2: Pokkali; 1-53: individuals of BC_3F_2 population].

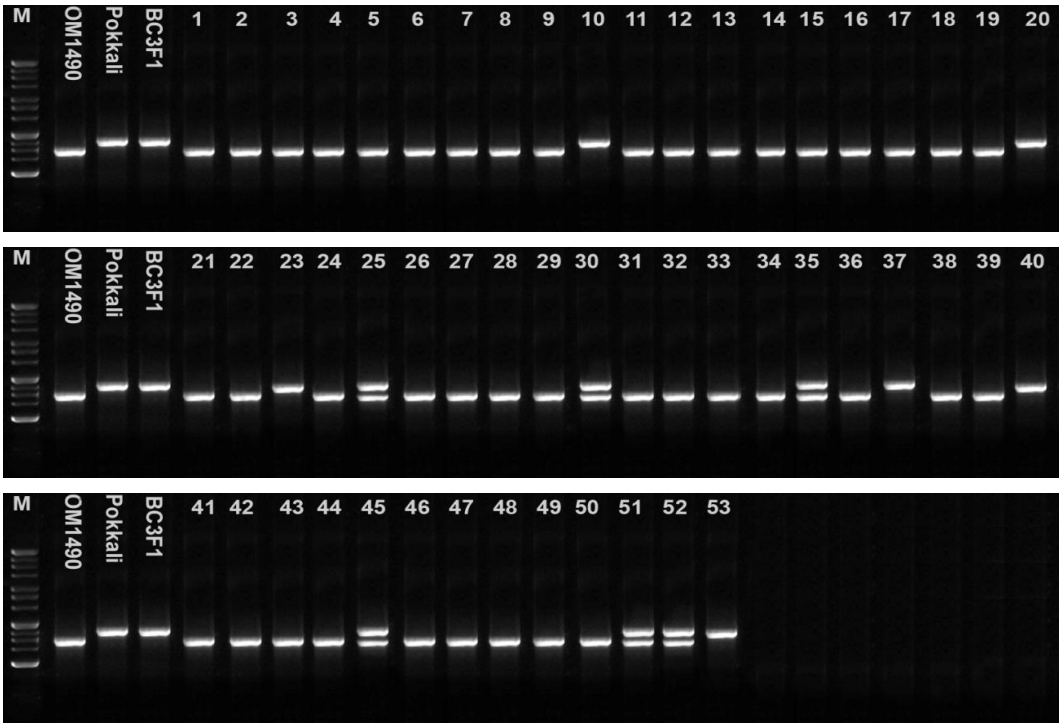

Figure 8. PCR products at the locus RM223 on chromosome 8 among 53 lines of BC_3F_2 derived from OMCS1490/Pokkali in agarose gel 3%. [M: ladder; 1: OM1490; 2: Pokkali; 1-53: individuals of BC_3F_2 population].

Of 100 individuals, 18 segregants offered their SD of 27-30 days, which means good tolerance to salinity. At EC= 15 dS/m, out of 100 individuals, six segregants had the same SD as tolerant check i.e. BC_2F_2-1, BC_2F_2-60, BC_2F_2-61, BC_2F_2-63, BC_2F_2-64, and BC_2F_2-66 lines. Regarding salinity tolerance for the OMCS2000/Pokkali population, only three lines as BC_3F_2-4, BC_3F_2-9, and BC_2F_3-12 exhibited SD of 27 days at EC=8dS/m and only one line i.e. BC_3F_2-4, which had the same level of tolerance as the tolerant check at 15 EC=15dS/m.

Regarding salinity tolerance of the OM1490/Pokkali population there were 3 lines which were selected at EC = 8dS/m. Out of 53 lines and at EC= 15dS/m, two lines exhibited their high survival days namely BC_3F_2-10 and BC_3F_2-40 with survivals of 65.0% and 41.9%, respectively, after 20 days.

All of these lines have introgressions from Pokkali conferring salinity tolerance. This is an opportunity to breed for new rice varieties with salinity tolerance in Vietnam. A total of 11 new breeding lines showed similar salinity tolerance as Pokkali, but with desirable agronomic characters. Our study demonstrates the usefulness of SSR markers for marker-assisted selection.

ACKNOWLEDGEMENTS

We are grateful to the project "Research and application of advanced technologies to breed drought and salinity tolerance rice genotypes adapted to climate change in Mekong Delta" supported by MOST and MARD in Vietnam.

REFERENCES

Ammar MHM, Pandit A, Singh RK (2009). Mapping of QTLs controlling Na^+, K^+ and Cl^- ion concentrations in salt tolerant indica rice variety CSR27, *J. Plant Biochem. Biotech*. 18: 139–50.

Bonilla P, Dvorak J, Mackill D, Deal K, Gregorio G (2002). RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines, *Philipp. J. Agric. Sci.* 85: 68–76.

Bui CB, Nguyen VT, Nguyen TL (1993). Preserving crop genetic of Vietnam. Vietnam Agriculture Publisher, Vietnam. pp. 9-15.

Buu BC (2017). Vietnam agriculture in 2016: Passing a tough examination**.** The Institute of Agricultural sciences for Southern Vietnam (http://iasvn.org/en/homepage/)

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Xu K, Xian J, Yu Z, Ronald Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Xu K, Xian J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994). Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics*. 138: 1251–1274.

Cheng L, Wang Y, Meng L (2012). Identification of salt-tolerant QTLs with strong genetic background effect using two sets of reciprocal introgression lines in rice. *Genome*. 55: 45–55.

Gregorio GB, Senadhira D, Mendoza RD (1997). Screening rice for salinity tolerance, *IRRI Discussion paperSeries* No.22. International Rice Research Institute, Los Baños. Laguna, Philippines.

Harushima Y, Yano M, Shormura A, Sato M, Shimano T, Kuboi Y, Yamamoto T, Lin SY, Antinio BA, Parco A, Kajiya H, Huang N, Yamamoto K,

Nagamura N, Kurata N, Khush GS, Sasaki T (1998). A high-density rice genetic linkage map with 2,275 markers using a single F_2 population. *Genetics*. 148: 479–494.

IRRI (1996). Standard Evaluation System. Rice Note Book, Philippines.

Kosambi DD (1944). The estimation of map distance from recombination values. *Ann. Eugen.* 12: 172–175.

Kumar V, Singh A, Amitha Mithra SV, Krishnamurthy SL, Parida SK, Jain S, Tiwari KK, Kumar P, Rao AR, Sharma SK, Khurana JP, Singh NK, Mohapatra T (2015). Genome-wide association mapping of salinity tolerance in rice (*Oryza sativa*). *DNA Res*. 22(2): 133–145.

Lander ES, Botstein D (1987). Mapping mendelian factors underlying quantitative traits using rflp linkage map. *Genetics*. 121: 185-199.

Lander ES, Green P, Abrahamson J, Barlow A, Daly DJ, Lincoln SE, Newburg L (1987). MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics*. 1: 174–181.

Lang NT, Loc LQ (2015). Screening salinity tolerance in the backcross populations. In book: Breeding salinity and submergence to supply for Mekong delta. Vietnamese. Vietnam Agriculture Publisher, Vietnam. Page: 190-229.

Lang NT, Yanagihara S, Buu BC. (2000). Quantitative trait loci for salt tolerance in rice (*Oryza sativa* L.) via molecular markers. *OmonRice* 8: 37-48.

Lang NT, Yanagihara S, Buu BC (2001a). A microsatellite marker for a gene conferring salt tolerance on rice at the vegetative and reproductive stages. *SABRAO J. Breed. Genet.* 33(1): 1-10.

Lang NT, Yanagihara S, Buu BC. (2001b). QTL analysis of salt tolerance in rice (*Oryza sativa* L.). *SABRAO J. Breed Genet*. 33(1): 11-20.

Lang NT, Li Z, Buu BC (2001c). Microsatellite markers linked to salt tolerance in rice. *OMONRICE*. 9: 9-21.

Lang NT, Zhang G, Magpantay G, Virmani SS, Huang N, Brar DS, Bennet J, Khush GS, Li Z (2000). PCR-base DNA markers for fertility restoration gene Rf-3 and thermosensitive male sterility genes. *Nethern*. 22-36.

Lang NT (1999). QTL mapping for salt tolerance in rice. Final report. Japan fellowship.

Lang NT (2002). Manual for Biotechnology Lab. Vietnamese. Vietnam Agriculture Publisher, Ho Chi Minh City, Vietnam.

Lee SY, Ahn JH, Cha YS (2007). Mapping QTLS related to salinity tolerance of rice at the young seedling stage. *Plant Breed*. 126: 43-46.

Lincoln S, Daly M, Lander E (1992). Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. Whitehead Institute Technical Report 2nd edn, Whitehead Institute, Cambridge, Massachusetts

Nelson JC (1997). QGene: Software for marker-based genomic analysis and breeding. *Mol. Breed.* 3: 239-245.

Nounjan N, Siangliw JL, Toojinda T, Chadchawan S, Theerakulpisut P (2016). Salt-responsive mechanisms in chromosome segment substitution lines of rice (*Oryza sativa* L. cv. KDML105). *Plant Physiology and Biochemistry*. 103: 96 -105.

Pandit A, Rai V, Bal S (2010). Combining QTL mapping and transcriptome profiling of bulked RILs for identification of functional polymorphism for salt tolerance genes in rice (*Oryza sativa* L.). *Mol. Genet. Genomics.* 284: 121–36.

Paterson AH, Lander ES, Hewitt JD, Paterson S, Lincoln S, Tankley SD (1988). Resolution of quantitative traits into Mendelian factors by using a complete linkage map of

restriction fragment length polymorphisms. *Nature*. 335: 721–726.

Salvi S, Tuberosa R (2005). To clone or not to clone plant QTLs: present and future challenges. *Trends in Plant Science*. 10: 297–304.

Tanksley SD, Young ND, Patterson AH, Bonierbale MW (1989). RFLP mapping in plant breeding: New tools for an old science. *Bio/Technology*. 7: 257.

Tanksley SD (1993). Mapping polygenes. *Annu. Rev. Genet.* 27: 205-233.

Temnykh S, DeClark G, Lukashova A, Lipovich L, Cartinhour S, McCouch SR (2001). Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon association, and genetic marker potential. *Genome Res*. 11: 144–1452.

Thomson MJ, Ocampo MD, Egdane J, Rahman MA, Sajise AG, Adorada DL, Tumimbang-Raiz E, Blumwald E, Seraj Z, Singh RK, Gregorio GB, Ismail AM (2010). Characterizing the saltol quantitative trait locus for salinity tolerance inrice. *Rice*. 3: 148-160.

Tiwari S, Krishnamurthy SL, Kumar V, Singh B, Rao AR, Amitha Mithra SV, Rai V, Singh AK, Singh NK (2016). Mapping QTLs for Salt Tolerance in Rice (*Oryza sativa* L.) by Bulked Segregant Analysis of Recombinant Inbred Lines Using 50K SNP Chip. *PLoS One*. 2016, 11(4): e0153610.

Wang D, Zhu J, Li Z, Paterson AH (1999). Manual for QTLMapper. Version 1.0. A computer software for mapping QTLs.