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# Microsatellite markers linked to salt tolerance in rice

**Nguyen Thi Lang[1,2], Zhikang Li[2] and Bui Chi Buu[1]**


## ABSTRACT

*An advanced backcross population ($BC_2F_2$) were developed with the parents included OM1706, ChengHui448, FR13A, Type3, Almol3 and Madhadar as the donors of salt tolerance and IR64, IR68552-55-3-2, Tequing as the recurrent parents with good quality traits. One hundred thirty $BC_2F_2$ lines were evaluated at seedling stage in the green house at IRRI. Molecular markers associated with both qualitative and quantitative salt tolerance were identified by using 150 SSR primers.*

*There are common markers from the $BC_2F_2$ populations : IR64/Chenghui 448, IR64/FR13A, IR64/OM1706, NTP/FR13A, NTP/Mahakar, IR68552-55-3-2/FR13A, IR68552-55-3-2/Chenghui 448 in chromosome 1.*

*In chromosome 9, common mapped markers were recognized in the $BC_2F_2$ populations of IR64/Chenghui448, IR68552-55-3-2/FR13A. IR64/type3, NTP/Type3, Teqing/Almol.*

*In chromosome 6, common mapped markers were recognized in the $BC_2F_2$ populations of IR64/OM1706, Tequing/OM1706, IR68552-55-3-2/FR13A, IR68552-55-3-2/FR13A, IR68552-55-3-2/Almol3.*

*In chromosome 8, common mapped markers were recognized in the $BC_2F_2$ populations of IR64/Chenghui448, IR68552-55-3-2/OM1706.*

*In chromosome 11, common mapped markers were recognized in the $BC_2F_2$ populations of IR64/Type3, IR68552-55-3-2/Type3, IR68552-55-3-2/OM1706, and in chromosome 3, IR64/Type 3.*

*This is one of the first reports mentioning salt tolerance gene tagging based on SSR with advanced backcross populations ($BC_2F_2$). IR64/ChengHui 448, IR64/OM1706 and IR64 / FR13A derived alleles nearly located at RM315, associated with stress tolerance to NaCl (EC = 18dS/m) at seedling stage, at a distance of 21.2cM, 1.9 and 0.0cM in chromosome 1. In case of IR68552-55-3-2/Madhukar the alleles are linked with OSR2 of chromosome 1 at a distance of 4.2cM. In the cross of IR68552-55-3-2/OM1706, the alleles are linked with RM223 in chromosome 8 at a distance 7.2cM. This suggests that quantitative trait loci on chromosomes 1 and 8 control salt tolerance at EC = 18dS/m. The identification provides the basis for developing a more efficiently for salt tolerance at 18EC among different crosses. Microsattelite markers on chromosome 1 may be used efficiently in rice breeding marker-assisted selection.*


**Keywords**: Salt tolerance, Microsatellite, SSR (simple sequence repeat), QTL mapping, Mapmarkers, MAS (marker-assisted selection)

## INTRODUCTION

Rice (*Oryza sativa* L.) is sensitive to salinity which can lead to changes in development, growth, and productivity, then severe stress may threaten survival. Many studies have examined the effect of salt stress with the aim of discovering mechanism used by stress-tolerant genotypes that may confer tolerance to sensitive ones. Rice breeding for salt tolerance has been reviewed by several workers (Akbar et al 1986, Maas and Hoffman 1977, Mori et al 1987, Gregorio 1997).

[1] Cuulong Delta Rice Research Institute, Vietnam
[2] Internaltional Rice Research Institute (IRRI), Philippines

Progress in salinity tolerance breeding is slow due to (1) limited knowledge on the genetics of tolerance, (2) complexity of the several tolerance mechanisms involved, (3) inadequate screening techniques, (4) low selection efficiency and (5) poor understanding of salinity and environmental interactions. Through recent developments in molecular marker analysis, it is now feasible to analyze both the simply inherited as well as quantitative traits, and then identify individual genes controlling the traits of interest. Molecular markers could be used to tag quantitative trait loci and to evaluate their contributions to the phenotype by selecting for favorable alleles at these loci in a marker-aided selection scheme aiming to accelerate genetic advance. Advanced backcross QTL analysis can be used to evaluate mapped donor introgression in the genetic background of an elite recurent parent (Tanksley and Nelson 1996). QTLs for salt tolerance in rice via RFLP markers and microsatellites reported by Lang et al (2000), Tuan et al (2000) were based on recombinant inbred population of Tesanai 2/CB, and backcross inbred lines ($BC_1F_8$) of Nipponbare/Kasalath, respectively.

DNA microarrays to monitor transcript abundance and expression patterns in rice (lines Pokkali and IR29) were conducted to determine gene expression profiles during initial phase of salt stress in rice (Kawasaki et al 2001)

QTLs associated with disease resistance, abiotic stress tolerance and yield potential of rice in a range of ecosystems have been identified (McCouch and Doerge 1995). For plant breeding applications, QTL analysis provides a way of selectivity manipulating individual genetic components of a complex trait such as salt tolerance. The progress on rice breeding for salt tolerance at CLRRI has been the identification of a major locus conferring salt tolerance gene in rice at the vegetative and reproductive stage (Buu et al 2000)

### Objectives

- To investigate the genetic basis of salinity tolerance using microsatellites to $BC_2F_2$ populations
- To screen the BC for salinity tolerance under greenhouse
- To tag microsetllite marker linked to salinity tolerance
- To determine whether enhancing QTLs from IR64, NTP Tequing would be detected in 130 BC2F2 families grown under salt tolerance

## MATERIALS AND METHODS

### Plant materials

Table 1: Response to salt stress (EC = 36dS/m) for $BC_2F_2$ of 12 crosses

| Cross | Response to salt stress |
|---|---|
| IR64/Cheng-Hui 448 | 5 |
| IR64/OM1706 | 5 |
| IR64/FR13A | 5 |
| IR64/Type3 | 3 |
| Teqing/OM1706 | 5 |
| Teqing/Amol3 | 5 |
| IR68552-55-3-2/Cheng-Hui448 | 7 |
| IR68552-55-3-2/OM1706 | 3 |
| IR68552-55-3-2/FR13A | 3 |
| IR68552-55-3-2/Madhuhar | 5 |
| IR68552-55-3-2/Type3 | 3 |
| IR68552-55-3-2/Almol3 | 5 |
| Pokali (check for resistant) | 3 |
| PSBRc88 (check for resistant) | 3 |
| IR29(check for susceptible) | 9 |
| IR64 (check for moderate) | 5 |

Nine cultivars were selected to exhibit various degrees of resistance to salinity. The resistant donors were crossed to the susceptible recurrent cultivars. Subsequently, $F_1$ progenies were selfed and backcrossed to their respective parents to produce the $F_2$ and backcross generations. The parents, $F_1$s , $F_2$s, $BC_1$ (backcross of $F_1$ to resistant parent) and $BC_2$ (backcross of $F_1$ to susceptible) were used.

Three improved rice cultivars were crossed to IR64 and $F_1$ hybrids were backcrossed to the elite cultivars. The promising $BC_1$ plants selected for desirable phenotypic traits were backcrossed to the elite cultivars to generate $BC_2$ plants. 130 $BC_2F_2$ were screen to salt stress. Transgressive segregants were observed under such conditions. The $BC_2F_2$ family were surveyed by microsatellite markers.

### Salt tolerance of $BC_2F_2$ lines

The parents and $BC_2F_2$ were used to determine the optimal eletric conductivity (EC) level for the subsequent experiments. Seeds were placed at 55$^{o}$C for 3 days in order to break dormancy, and rised with 0.1% of HgCl

for 1 minute, then germinated at 37°C for 48 hours. Finally, the most uniform seeds were seeded in cultural solution. Seedling survival day (SD) character was observed at three EC levels: 12, 18, 36 dS/m.

Seedlings were grown in nutrient solution (Yoshida et al 1992) in a randomized complete block (RCB) design with three replications.

In each replication, 50 seeds were maintained in nutrient solution for 4 days before adding NaCl to establish the different EC levels. The pH of the cultural solution was monitored every day to keep pH = 5. The Yoshida solution was renewed every one week to limit the effect of algae. When a plant was completely yellow, no more green tissue was evident, it was considered as dead. Plants survival data was scored. Based on the results from the first experiment, a second one was performed at the optimal EC level using $BC_2F_2$ populations in a RCB design with three replications, then 10 plants/each were harvested as compared to two EC level 12 and 18 dS/m.

**CTAB extraction DNA**

Leaf tissues (4-6 gr) were grinded in liquid nitrogen, and transferred into 50 ml tube (10-15 ml leaf power). The finer the grind gives the higher the yield, but attentions should be paid to prevent the tissues from not thaw.

20 ml 2 X CTAB extraction buffer (preheated to 65°C) were added to the leaf power, they were mixed thoroughly and incubated at 65°C for 30 min (up to 1 hour). Tubles were removed from water bath and let cool briefly. 20 ml 24:1 $CHCl_3$: isoamyl alcohol were added, then incubated at room temperature with moderate shaking for 20min. they were centrifuged at 3000 rpm for 30 min at 4°C. Supernatant (upper phase) was transferred into 50ml tube with filtering through micracloth. One vol (20ml) isopropyl alcohol was added, then mixed by inversion. They were incubated at room temperature or colder (-20 °C) for 1 hour to overnight, then centrifuged at 3000 rpm for 30 min at 4°C. Supernatant was drained away from pellet, then pellet was washed with 70% EtOH. Pellets were in air dry and dissolved nucleic acids in 5 ml TE. Tubles were placed at 65°C to help dissolution. 1/10 vol 3M NaAoc (500 μl) and 2 vol Absolute EtaOH (10-11ml) were added, then they were incubate at –20°C for one hour (or longer) at this step it is possible to leave the samples overnight at –20°C

Centrifuge at 3000rpm for 30 min was done, then wash with 70% EtOH, air dry, dissolve in 500 μl TE, transfer microfuge tube, rinse 50 ml tube with 200 μl TE, collect wash rest of solution.

**Amplification of microsatellites and detection of their polymorphism**

The nucleotide sequences of the primer pairs used for each microsatellite are identified. PCR amplification were performed in 10mM Tris-HCl (pH8.3) 50mMKCl, 1.5mM $MgCl_2$, 0.5 or 1 unit of *Taq* polymerese, 4 nmole of dNTP, 10 pmole of primer with 10ng of genomic DNA per 20μl using a thermal cycler. Thrity five PCR cycles were performed with 30s of denaturation at 94°C, 30s of anaealing at 55°C and 1 min of polymerization at 72°C. Polymorphism in the PCR products was detected by both of ethidium bromide staining after electrophoresis in 3% agarose gel and 5% polyacrylamid gels in which banding patterns were visualized using silver staining.

**Score data**

Markers were scored in 118 individuals of the $BC_2F_2$ for their presence or absence in two different data according to their parental orgin. Only clear cut fragments were recorded.

**Data analysis**

ANOVA was performed for each single marker and each combination of two markers to be identified as putatively associated with salt tolerance. This was done to confirm the association between the markers and salt tolerance loci. All markers were tested for the expected 1:1 segregation by chi-square test at P=0.05 level. MAPMARKER/QTL was used to identify putative quantitative trait loci (QTLs) coding for quantitative salinity tolerance in the rice genome. Results from the two different analysis approaches were compared.

**Construction of SSR map and assignment of linked groups to chromosomes**

A set of SSR markers which were present in the test population were identified to construct the SSR map and to assign the linked groups in chromosomes. Linkage groups were ordered by using MAPMARKER (Lander et al 1987). Linkage groups were

reconfirmed by using the "GROUP". Map units (cM) were derived by using the Kosambi function (Kosambi 1944).

**QTL map for salt tolerance**

Two different analyses were performed to identy the major genes and QTLs for salinity tolerance. First, the visual rating at seedling stage was noticed. The data were analyzed by using MAPMARKER to locate genes for salinity tolerance. For QTLs, interval analysis was conducted with MAPMARKER/QTL. The threshold for declaring a QTL for salinity tolerance was at LOD> 3.0. Interval mapping developed by Lander and Botstein ( 1989) is able to define the most likely position of a QTL and precisely estimaste the phenotypic effect of the QTL if it does not lie exactly at the marker locus. Log of odds peaks for each significant QTL was used to position the QTL on the SSR map. The proportion of the total phenotypic variation explained by each QTL was calculated as $R^2$ value ($R^2$ = ratio of the sum of square explained by the QTL to the total sum of square)

**RESULTS**

**Developing population**

Mapping population is an important factor for gene tagging and QTL analysis.

Backcrossing populations were created for this research. From the original these cross, 50 $BC_1F_1$ were produced that exhibited sufficient fertility for further backcrossing or selfing. Of these 30 $BC_1F_1$ eventually produced viable backcross to $BC_2F_2$.

**Phenotypic variation in salinity tolerance**

***Performance of $BC_2F_2$ for salt tolerance****:* The first experiment was designed to find out the optimal EC level for evaluation of salt tolerance in the $BC_2F_2$ populations.

***For the cross IR64/Chenghui 448*****:** The basic principle in screening salinity tolerance at seedling stage is the ability of the seedlings to grow in salinized culture solution. Distribution of salt tolerance (36EC) at seedling stage of $BC_2$ $F_2$ bulk population at score 5 was noticed. The distribution frequency of salinity reaction of the cross was continuous and nearly normal.

*For the cross IR64/OM1706*: There was a wide variation observed for yield. Distribution of salt tolerance (36EC) at seedling stage of $BC_2F_2$ bulk population at score 5 was noticed (Table 1). This reaction was obtained as well as IR64/FR13A, Teqing/OM1706, Teqing/Amol3, IR68552-55-3-2/Madhuhar, IR68552-55-3-2/Almol3. However, distribution of salt tolerance (36EC) for $BC_2F_2$ bulk population at score 3 was noticed in the crosses as IR64/Type3, IR68552-55-3-2/OM1706, IR68552-55-3-2/FR13A, IR68552-55-3-2/Type3.

$BC_2F_2$ populations are ideally considered for QTL mapping. Recombinant inbred (RI) lines were produced by continuously selfing the progeny of individual members of the $F_2$ population until homozygosity is obtained. $BC_2F_2$ populations have several advantages over than $F_2$. The major advantages are homozygosity which allows easy propagation and experimental replication and greater of recombination which breaks up linkage blocks and increases there solution of analysis (Burr and Burr 1991).

$BC_2F_2$ are very useful for tagging several traits in single population because the same set of RFLP data can be used to analyze different sets of phenotypic data. $BC_2F_2$ populations are also very useful for tagging resistance genes for complex pathogens e.g. blast which has many races in different geographical regions. It is worthwhile to mention that the population also segregated for other traits such as plant height, photoperiod sensitivity and disease resistance

Table 2: The performance of $BC_2F_2$ under 18 EC level and he segregation of molecular markers

| $BC_2F_2$ from the cross | Code | Plant number | Response to salt tolerance at 18EC | Confirmed marker |
|---|---|---|---|---|
| IR64/Cheng-Hui448 | 32005 | 11 | R | RM292,RM242 |
| IR64/OM1706 | 32058 | 11 | R | RM276,RM315 |
| IR64/FR13A | 32035 | 11 | R | RM315 |
| Teqing/OM1706 | 32134 | 8 | R | |
| IR68552-55-3-2/Chenghui448 | 32146 | 11 | R | |
| IR68552-55-3-2/OM1706 | 32197 | 11 | R | RM223 |
| IR68552-55-3-2/FR13A | 32175 | 5 | R | |
| IR68552-55-3-2/Madhukar | 32177 | 24 | R | OSR2 |
| IR64/Type3 | 32044 | 6 | R | |
| NTP/Type3 | 32184 | 13 | R | |
| Tequing/Almol3 | 32088 | 10 | R | |
| IR68552-55-3-2/Almol3 | 32160 | 11 | R | |

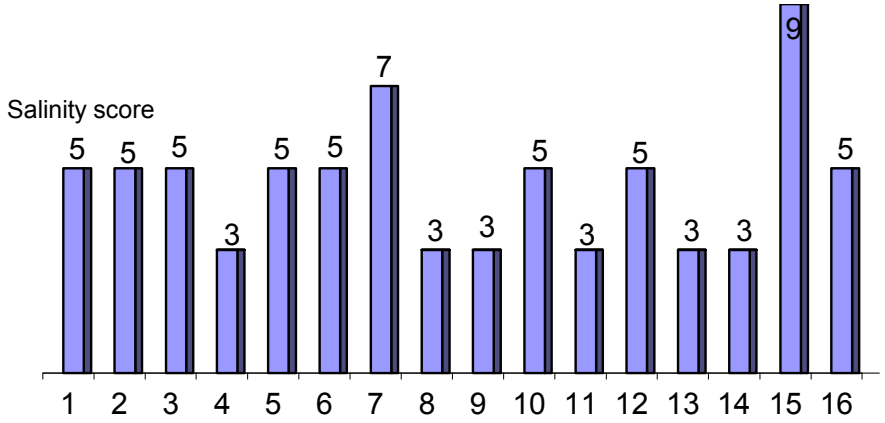



Figure 1: Distribution of $BC_2F_2$ from 12 crosses under salt stress (36EC) at seedling stage in nutrient solution

1-12: $BC_2F_2$ populations
13: Pokkali (tolerance check)
14: PSBRc88
15: IR29 (susceptible check) and
16: IR64

**Microsatellite marker survey among parents**

Out of 150 primers pairs tested in these crosses, some markers amplified specific DNA fragment related to resistance based on polymorphism between the DNA of resistant and susceptible plants (Table 2).

Of the 150 primers markers tested, thirty eight markers were polymorphic between IR64/ Chenghui 448; 29 markers between IR64/OM1706, 44 markers between IR64/FR13A; 28 markers between Teqing/OM1706; 40 markers between IR68552-55-3-2/Chenghui 448; 39 markers between IR68552-55-3-2/OM1706; 47 markers between IR68552-55-3-2/FR13A; 38 markers between IR68552-55-3-2/Mahukar; 35 markers between IR64/Type3; 28 markers between IR68552-55-3-2/Type 3; 39 markers between IR68552-55-3-2/Type3; 14 markers between Teqing/Almol3; 9 markers between IR68552-55-3-2/Almol3. Three markers were polymorphic among parents: OSR1, RM234 and RM 204 (figure 2)

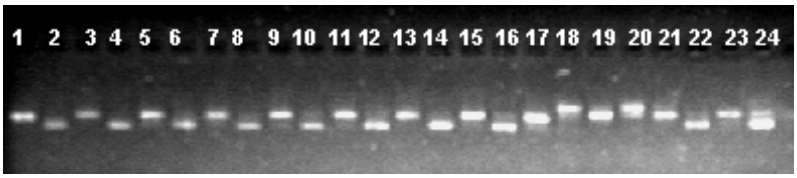



Figure 2: Polymorphism exhibition of OSR1 among parents.

Lane 1: IR64, lane 2: Chenghui 448
Lane 3: IR64, lane 4: OM1706
Lane 5: IR64, lane 6: FR13A
Lane 7: Teqing, lane 8: OM1706
Lane 9: IR68552-55-3-2, lane 10: Chenghui 448
Lane 11: IR68552-55-3-2, lane 12: OM1706
Lane 13: IR68552-55-3-2, lane 14: FR13A
Lane 15: IR68552-55-3-2, lane 16: Mahakar
Lane 17: IR64, lane 18: Type 3
Lane 19: IR68552-55-3-2, lane 20: Type 3
Lane 21: Teqing, lane 22: Almol3
Lane 23: IR68552-55-3-2, lane 24: Almol3

**Polymorphism of marker and marker segregation:**

**For IR64/Chenghui 448**

Polymorphism obtained 95% of the SSR in case of IR64 and Chenghui 448. The $BC_2$ $F_2$ population was evaluated by using 34 SSRs. The expected segregation ratio would be 98% homozygotes, 2% heterozygotes. Resulting in an allele frequencing of 47.6% in IR64, and 51.58% in Chenghui 448 was due to skewed allele frequencies at 34 out of 150 (22.6%) of marker loci. Skewing toward the adapted elite parent can be explained by the selection improved in the $BC_1$ and $BC_2$ generations during population development but skewing toward Chenghui 448 was not expected. This may gain a recombination due to the genetic distance between the parental lines as suggested by Grandillo and Tanksley (1996). Some markers did not introgress into the population such as RM283, RM286, RM339.

**For IR64/OM1706**

Polymorphism obtained 97% of the SSR in case of IR64 and OM1706. The $BC_2F_2$ population was evaluated by using 30 SSRs. The expected segregation ratio would be 97.5% homozygotes, 2.5% heterozygotes. Resulting in an allele frequencing of 43.2% in IR64, and 53.5% in OM1706 was due to skewed allele frequencies at 47 out of 150 (23.3%) of marker loci.

**For IR64/FR13A**

Polymorphism obtained 92% of the SSR in case of IR64 and FR13A. The $BC_2F_2$ population was evaluated by using 44 SSRs. The expected segregation ratio would be 97.5% homozygotes, 2.5% heterozygotes. Resulting in an allele frequencing of 55.5% in IR64, and 37.5% in FR13A was due to skewed allele frequencies at 44 out of 150 (31.3%) of marker loci. Some markers did not introgress into the population such as RM218, RM333.

**For Teqing/OM1706**

Polymorphism obtained 90% of the SSR in case of IR64 and Type3. The $BC_2F_2$ population was evaluated by using 28 SSRs. The expected segregation ratio would be 98.5% homozygotes, 1.5% heterozygotes. Resulting in an allele frequencing of 46.0% in IR64, and 52.2% in Type3 was due to skewed allele frequencies at 28 out of 150 (18.60%) of marker loci. Except marker RM333 did not introgress into the population

**For IR68552-55-3-2/Chenghui 448**

Polymorphism obtained 98% of the SSR in case of IR64 and Chenghui448. The $BC_2$ $F_2$ population was evaluated by using 40 SSRs. The expected segregation ratio would be

97.5% homozygotes, 2.5% heterozygotes. Resulting in an allele frequencing of 57.0% in IR64, and 42.0% in Chenghui 448 was due to skewed allele frequencies at 40 out of 150 ( 26.6%) of marker loci. Except marker RM218 did not introgress into the population

**For IR68552-55-3-2/OM1706**

Polymorphism obtained 97% of the SSR in case of IR68552-55-3-2 and OM1706. The $BC_2F_2$ population was evaluated by using 37 SSRs. The expected segregation ratio would be 93.7% homozygotes, 7.25% heterozygotes. Resulting in an allele frequencing of 41.9% in IR68552-55-3-2 56.47% OM1706 was due to skewed allele frequencies at 37 out of 150 (24.6%) of marker loci. Except RM39 did not introgress into the population

**For IR68552-55-3-2/FR13A**

Polymorphism obtained 90% of the SSR in case of IR68552-55-3-2 and FR13A. The $BC_2F_2$ population was evaluated by using 47 SSRs. The expected segregation ratio would be 96.5% homozygotes, 2.8% heterozygotes. Resulting in an allele frequencing of 58 % in IR68552-55-3-2, and 40.0% in FR13A was due to skewed allele frequencies at 47 out of 150 (31.3) of marker loci. Some markers did not introgress into the population such as RM225, RM339, RM337, RM302

**For IR68552-55-3-2/Madhukar**

Polymorphism obtained 90% of the SSR in case of IR68552-55-3-2/Madhukar. The $BC_2$ $F_2$ population was evaluated by using 37 SSRs. The expected segregation ratio would be 75% homozygotes, 25% heterozygotes. Resulting in an allele frequencing of 44.46% in IR68552-55-3-2 and 52.8% in Madhakar was due to skewed allele frequencies at 37 out of 150 (24.6%) of marker loci.

**For IR64/Type3**

Polymorphism obtained 94% of the SSR in case of IR64/Type3. The $BC_2F_2$ population was evaluated by using 30 SSRs. The expected segregation ratio would be 75% homozygotes, 25% heterozygotes. Resulting in an allele frequencing of 44.46% in IR64 and 52.8% in Type3 was due to skewed allele frequencies at 30 out of 150 (20%) of marker loci.

**For IR68552-55-3-2/Type3**

Polymorphism obtained 90% of the SSR in case of IR68552-55-3-2 / Type3. The $BC_2$ $F_2$ population was evaluated by using 28 SSRs. The expected segregation ratio would be 97.5% homozygotes, 2.5% heterozygotes. Resulting in an allele frequencing of 55.5% in IR68552-55-3-2 and 48.2% in Type3 was due to skewed allele frequencies at 28 out of 150 (18.6%) of marker loci.

**For Teqing/Almol**

Polymorphism obtained 95% of the SSR in case of Teqing and Almol. The $BC_2F_2$ population was evaluated by using 14 SSRs. The expected segregation ratio would be 97.5% homozygotes, 2.5% heterozygotes. Resulting in an allele frequencing of 54% in Teqing and 45.2% in Almol was due to skewed allele frequencies at 14out of 150 (9%) of marker loci.

**For IR68552-55-3-2/Almol**

Polymorphism obtained 90% in case of Teqing and Almol. The $BC_2F_2$ population was evaluated by using 28 SSRs. The expected segregation ratio would be 9.75% homozygotes, 2.5% heterozygotes. Resulting in an allele frequencing of 89% in IR68552-55-3-2 and 11% in Almol was due to skewed allele frequencies at 28 out of 150 (18.6%) of marker loci.

**SSR analysis of $BC_2F_2$**

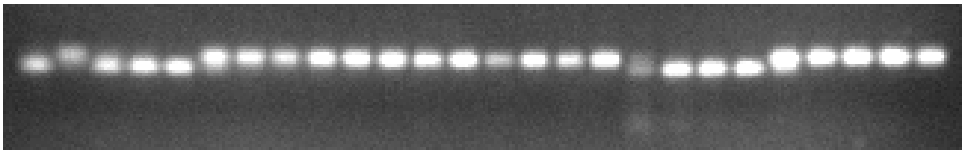


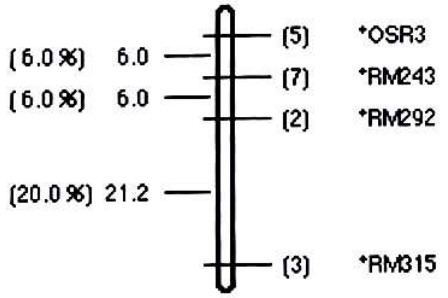
Figure 3 shows the introgression into IR64/FR13A population with RM315

For the population of IR64/ChengHui 488, the allele segregation ratios on chromosomes 1, 8, 9 are deviated from the expected 1:1 ratio for the parents (figure2). This population was created by crossing two indica varieties. IR64 is an improved variety, and Chenghui 448 from coastal area of China. The segregation distribution favored Chenghui 448. Of the seven markers evaluated from chromosome 1, there were three markers OSR2, RM302, RM283 which showed no linkage with four markers OSR3, RM243, RM292, and RM315. This is the same with the $BC_2$ $F_2$ of IR64/FR13A. Out of seven markers evaluated in chromosome 1, there was no linkage between OSR3 and six other markers. While in the cross IR64/OM1706, of the 8 markers evaluated from chromosome 1, all these markers very closely linked together.

In case of IR68552-55-3-2/Madhukar, of the five markers evaluated on chromosome 1, linkage was detected among data of them using this study .

In case of Teqing/Almol, polymorphism frequency between two parents was very low. Of the 5 markers evaluated from chromosome 9, there was very close linkage between RM219 and RM242 (0.0cM).

In case of IR68552-55-3-2/FR13A, polymorphism frequency between two parents was very high. However, out of 5 markers evaluated from the chromosome 6, no linkage between RM204 and the four others was recognized. This may be due to multiple band patterns of RM204 in the two bulks, the polymorphism band showed in the bulk DNA survey may reside in another chromosome. The seven generations of selfing tended to increase recombination distances between flanking markers. If the recombination frequency is particularly high, the genetic distance in $BC_2F_2$ may be extended so that no linkage was detectable.




These may affect the results of QTL analysis. The approach used in this study provided an efficient way to identify a few genes with large effects at seedling stage under salt stress condition.

**Construction of genetic linkage map**

Grouping of markers was carried out by a two point linkage analysis with a LOD score of 4 and recombination fraction D of 0.3.

For the $BC_2F_2$ of IR64/Chenghui 448, thirty four markers were used to construct a frame work map. A total length of 148.6 cM was covered in three chromosome 1, 8, and 9 (Figure 4). The makers were mapped based on indica x indica cross (IR64/Chenghui 448) and associated with salt tolerance genes conferring RM315 in chromosome1 at a distance of 21.2 cM.

For the $BC_2F_2$ of IR64/OM1706, thirty five markers were used to construct a frame work map. A total length of 50.5cM was covered in chromosome 1 with 8 markers. Markers RM315 closely linked to salt tolerance gene (1.9cM) under 18EC salt stress (Fig.5)

For the $BC_2F_2$ of IR64/FR13A, fourty seven markers were used to construct a frame work map. A total length of 191.6 cM and 109.3 cM was covered in chromosome 1 and chromosome 11, respectively. Using this population, salt tolerance gene was remarkably linked with RM315 of chromosome 1 at a distance of 0.0cM (Fig.6)

For the $BC_2F_2$ of Teqing/OM1706, twenty eight markers were used to construct a frame work map. A total length of 95.4 cM was covered in two chromosomes (Fig. 6)

For the $BC_2F_2$ of IR68552-55-3-2/Chenghui 448, fourty markers were used to construct a frame work map. A total length of 253.1 cM was covered in chromosome 1 and 9. In chromosome 6, 8 and 11, polymorphism of the marker was obtained. Linkage group size furtherly ranged.

Figure 4: SSR linked map of rice Chr.1 and showing the position of RM315. The marker was mapped in the indica / indica cross with $BC_2F_2$ from IR64 / ChengHui 448

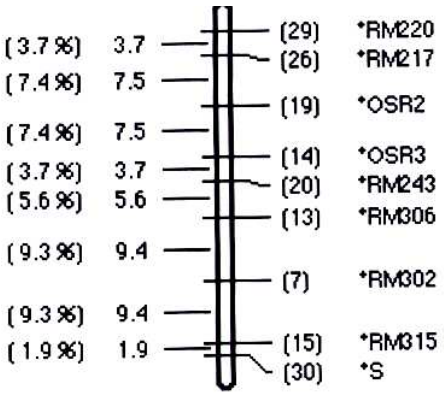



Figure 5: SSR linked map of rice Chr.1 and showing the position of RM315. The marker was mapped in the indica x indica cross with $BC_2F_2$ of IR64 / OM1706

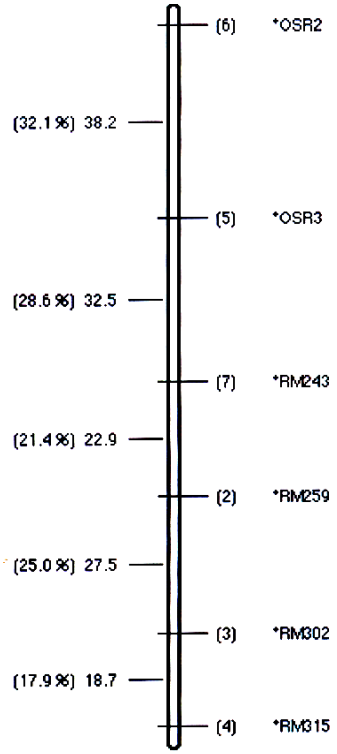



Figure 6: SSR linked map of rice Chr.1 and showing the position RM315. The marker was mapped in the Indica/Indica cross with BC2F2 from IR64/FR13A

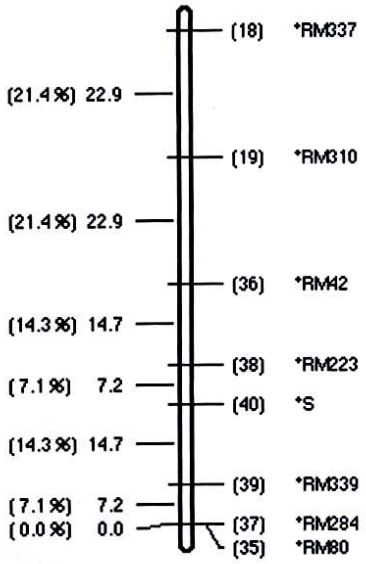



Figure7: SSR linked map of rice Chr.8 and showing the position RM223. The marker was mapped in the Indica/Indica cross with BC2F2 from NTP/OM1706

For the $BC_2F_2$ of IR68552-55-3-2 / OM1706, thirty seven markers were used to construct a frame work map. A total length of 311.5 cM was covered in two chromosomes: 139.5cM in chromosome 8 and 172 cM in chromosome 11. Remain chromosomes were not represented (Fig 8). Microsatellite linkage map of chr.8 showed the position RM223 linked with salt tolerance gene at a distance of

7.2 cM. This is in the same position reported by Lang et al. (2000).

For the $BC_2F_2$ of IR68552-55-3-2 / FR13A, fourty seven markers were used to construct a frame work map. A total length of 296.3 cM was covered in two chromosomes: 133,7 cM in chromosome 1 with 4 markers, one marker no linked, then 56.2cM in chromosome 9 with 5 markers. Beside that, length of 106.1 cM was covered in chromosome 6 with five markers including only four markers linked with salt tolerance gene. The number of markers per linkage group greatly varied. Some indicated very close link such as RM253 and RM111 (0.0 cM) in chromosome 6. Chromosome 2, 3, 4, 5, 7, 8, 10, 11 and 12 were not represented in the genetic map due to the lack of detectable genetic polymorphism between the parents of a given mapping population.

For the $BC_2F_2$ of IR68552-55-3-2 / Madhakar, thirty seven markers were used to construct a frame work. A total length of 1152 cM was covered in target chromosomes including 129.2.5cM in chromosome 1. It is showing the position of OSR2 linked with salt tolerance at a distance of 4.2cM.

For the $BC_2F_2$ of IR64 / Type3, thirty markers were used to construct a frame work map. A total length of 51.1cM was covered in chromosome 6, 129.2.5cM in chromosome 2, then 129.2.5cM in chromosome 11. Remaining chromosomes were not represented.

For the $BC_2F_2$ of IR68552-55-3-2 / Type3, twenty eight markers were used to construct a frame work map. A total length of 101.11 cM was covered in chromosome 9, and 11 including 64.3 cM with 4 markers in chromosome 9, then 36.8cM with four markers in chromosome 11. Remaining chromosomes were not represented.

For the $BC_2F_2$ of Teqing / Almol3, fourteen markers were used to construct a frame work map. A total length of 40.4 cM was covered in chromosome 9.

For the $BC_2F_2$ of IR68552-55-3-2 / Almol, twenty eight markers were used to construct a frame work map. A total length of 50.1 cM was covered in chromosome 11. Remaining chromosomes were not represented

**Correspondence between maps**

Almost makers were found to be homology in both parents and segregate in a 1:1 ratio in the progeny. Two-point linkage analysis between these markers and markers was carried out in order to locate them and identify homologous map regions between two varieties. Six of these markers were tightly linked with placed marker segregating 1:1. Four other markers segerating 3:1 were linked with markers of one single marker. Some makers were found to be heterozygous in both parents and segregated in a 3:1 ratio in the progeny. These maps revealed a direct homology respectably between linkage groups and linkage group which additionally are the longest ones in both maps and show approximally the same length between species.

All 38 of the probes which showed polymorphism between the two parents were surveyed in the bulked $BC_2F_2$ of IR64 / Chenghui 448. Of the 29 markers showed polymorphism among the tolerant and sensitive bulks. RM292 linked with salt tolerance at 18 EC in chromosome 1. However, the number of markers used in parent survey in this study is not enough to identify QTLs. Almost crosses were found with marker polymorphism which covered in 12 chromosomes, except the cross IR64 / Chenghui 448

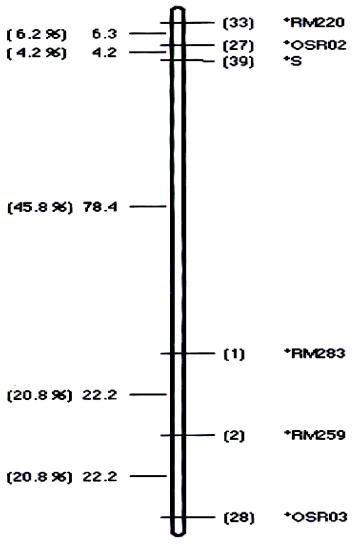



Figure 8: SSR linked map of rice Chr.1 and showing the position OSR2. The marker was mapped in the Indica/Indica cross with BC2F2 from NTP/Mahdukar

Ten chromosomes, except chromosome 7 and 12 were noticed as target chromosomes. The lack of target markers in chromosome 12 was recognized in case of IR64 / Type 3. Fourteen of the probes which showed polymorphism between the two parents were surveyed in the bulked $BC_2F_2$ of

Teqing / Amol. Of the 14 markers showed polymorphism and covered in chromosomes 5, 6, 7, 9 and 11.

There are common for markers from the population $BC_2F_2$ of IR64 / Chenghui 448, IR64 / FR13A, IR64 / OM1706, NTP / FR13A, NTP / Mahakar, IR68552-55-3-2 / FR13A, IR68552-55-3-2 / Chenghui 448 located in chromosome 1.

Similarly, it obtained (1) from the population $BC_2F_2$ of IR64 / Chenghui 448, IR68552-55-3-2 / FR13A, IR64 / Type3, NTP / Type3, Teqing / Almol in chromosome 9,
(2) from the population $BC_2F_2$ of IR64 / OM1706, Teqing / OM1706, IR68552-55-3-2 / FR13A, IR68552-55-3-2 / FR13A, IR68552-55-3-2 / Almol3 in chromosome 6,
(3) from the population $BC_2F_2$ of IR64 / Chenghui 448, IR68552-55-3-2 / OM1706 in chromosome 8.
(4) from the population $BC_2F_2$ of IR64 / Type3, IR68552-55-3-2 / Type3, IR68552-55-3-2 / OM1706 in chromosome 11
(5) from population $BC_2F_2$ of IR64 /Type 3 in chromosome 2

**QTL mapping**

The number of markers used for QTL tagging by bulked segregant analysis should be greater that by SSR analysis. The number of markers used in parent is not enough to identify QTLs. In addition, the spacing of polymorphism markers in chromosomes and all maps was not uniform. Therefore, the interesting of informative markers was lacking in some regions. However, it depends on the cross so selected markers linked with salt tolerance region were identified in a given chromosome under 18EC salt stress.

**DISCUSSION**

Potential application of a $BC_2F_2$ have been discussed. $BC_2F_2$ can be use a mapping source to rapidly map new cloned genes and are especially useful for the study of quantitative traits. $BC_2F_2$ genotypes can be replicatied in several environments to study specific QTL x environment interactions. They can be crossed with different testers to study QTL x genetic background interaction or other $BC_2F_2$ to study specific QTL x QTL interaction. $BC_2F_2$ can also be a useful tool for gene discovery. The current $BC_2F_2$ dramatically differs for many traits.

Chromosomes 3, 5, 7, and 12 were not represented in our genetic map due to the lack of detectable genetic polymorphism between the parents of the mapping population. This is most likely due to the fact that these parents are indica genotypes. This does not mean that putative QTLs for traits relating to salt tolerance located in these chromosomes are not available, but merely that they cannot be detected. Because there are no discernible allelic differences. Or rice breeding programs have mainly used the same group for crosses e.g. japonica / japonica or indica / indica. The QTL identified among closely related varieties are more interesting and useful to rice breeders. To date, only one QTL analysis of japonica / japonica cross has been reported (Mackill et al. 1996).

The first experiment was designed to find out the optimal EC level for evaluation of salt tolerance in the population. The results summarized in table demonstrated that the optimal EC level was 18 EC for both populations. At 36 EC, different reactions among parents were noticed. The result also supplies useful data of 130 evaluated $BC_2F_2$ lines for salt tolerance in nutrient solution for further analyses. For the $BC_2F_2$ population of NTP / OM1706, there are the same result with $F_2$ population from DocPhung / IR28 ( Lang et al. 2000), RM223 closely linked to salt tolerance gene in chromosome 8. In this study, we compared the finding of this set of experiments with those previously reported by other researchers who are evaluating similar characters in different environments, with different crosses.The use of a common set of molecular markers made it possible to determine whether QTLs from all the different studies were in similar regions of the rice genome.

The duration of survival days of seedling in culture solution at EC=18dS/m was considered as a critical index for phenotyping. The 12 populations of 130 $BC_2F_2$ lines were evaluated by using this system. This may be a first report on salt tolerance genes tagged by SSR with different $BC_2F_2$ populations. Based on this work, it will be possible to test the hypothesis that loci within population. IR64 / ChengHui 448, IR64 / OM1706 and IR64 / FR13A derived alleles linked to markers RM315 (in chromosome 1) which was associated with tolerance to NaCl stress at seedling stage under 18EC. Similarly, IR68552-55-3-2 / OM1706 derived alleles linked to RM223 in chromosome 8, IR68552-55-3-2 / Madahar linked to OSR2 in chromosome1. However, different loci were separately noticed among three populations e.g. IR64 / ChengHui 448, IR64 / OM1706 and IR64 / FR13A. This information could be

used to construct $BC_2F_2$ in several genetic backgrounds with different parents. IR64 was used as a recurrent parent with three donors: ChengHui 448, OM1706 and FR13A, their crosses have a common locus RM315 in chromosome 1 under salt stress phenotyping at 18EC. In case of IR68552-55-3-2 used as a recurrent with Madhukar as a donor, new locus OSR2 was also located in chromosome 1, but different from RM315. In case of IR68552-55-3-2 / OM1706, salt tolerance gene located in chromosome 8.

In summary, salt tolerance genes under salt stress phenotyping at 18EC located at loci in chromosomes 1, and 8. Developing a more efficient protocol for salt tolerance at 18EC under nutrient solution is recommended. Microsatellite markers on chromosome 1 may be efficiently used in rice breeding marker-assisted selection.

A complete molecular marker data set including markers in all 12 chromosomes is needed for further studies to determine if any other genes are segregating for salt tolerance among populations.

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*SUMMARY IN VIETNAMESE*

## Nghiên cứu bản đồ liên kết giữa microsatellites với gen chống chịu mặn

**Nguyễn Thị Lang Zhikang and Bui Chi Buu**

*TÓM TẮT*

*Quần thể hồi giao cải tiến $BC_2F_2$ bao gồm 12 tổ hợp lai, với các vật liệu cho gen chống chịu mặn dùng làm bố là OM1706, FR13A, Cheng Hui 448, Almol 3, Type 3, và Madhadar, các vật liệu được dùng làm mẹ (tái tục) là IR64, IR68552-55-3-2, Teqing.*

*Số cặp primer tương ứng với 150 microsatellite markers đã được sử dụng trong phân tích*

*Sự thể hiện đa hình giữa bố mẹ, sự truyền vào của gen mục tiêu (introgression) thông qua biểu hiện của con lai $BC_2F_2$ được ghi nhận trên nhiễm thể số 1, 6, 8 và 9.*

*Đánh giá kiểu hình giai đoạn mạ, trong điều kiện thanh lọc mặn ở môi trường dinh dưỡng được khuyến cáo ở EC = 18dS/m. Chiến lược chọn giống chống chịu nhờ marker phân tử (MAS) nên tập trung trên nhiễm thể số 1 và số 8, đặc biệt là nhiễm thể số 1, với marker RM315*

*Marker OSR1 rất hữu ích được khuyến cáo cho điều tra sơ khởi tính đa hình của bố mẹ*