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## Breeding for the Salinity Tolerance Rice Variety in Viet Nam

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This study focus on developing new rice varieties which are salt tolerant combine with high yield potentials, high quality using Molecular Markers and convensional breeding. The Saltol QTL introgression lines were developed and selected through generations from Vietnam popular rice variety AS996 using markers assisted backcrossing (MABC). After three backcross generations, the selfing generations were developed. Conventional breeding were used to select the high yield, high quality lines. Screening for salinity tolerant were carried out to evaluate all the selected lines in the salt affected field and in greenhouse. The important agronomical traits evaluations concerning to high yield and resistances to biotic and abiotic stress (salt tolerance) were applied on the BC<sub>3</sub>F<sub>3</sub> -BC<sub>3</sub>F<sub>6</sub> generations for breeding purpose in several areas. A promising new salt tolerant rice line with high yield potentials, high quality were received. This new variety was sent to the National Variety Trial and Testing Centre” for Value-for-Cultivation-and-Use (VCU) and Distinctness-Uniformity-Stability (DUS) tests and trials at different ecological zones.

**Keywords:** AS996, MABC, rice, salinity tolerant, Saltol.

### Introduction

Rice is the largest foreign exchange earner among Vietnam's agricultural exports and also the main staple food for most of the country's 90 millions people. Developing adaptation rice varieties to cope with climate change and sea level rise is very important to Vietnam economy and food security, it also contributes to the global food security. Recently, many researches in the development of high yielding rice varieties tolerant of abiotic stresses such as submergence and salt stress, have archived good results. These improved varieties were newly developed using both conventional and modern breeding tools. Breakthroughs in salinity tolerance breeding became feasible after the identification of major chromosomal regions (Quantitative trait loci, QTLs) underlining salinity (*Saltol*) stresses, and the development and use of a marker system for their speedy incorporation into modern high yielding and popular

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varieties through marker assisted backcrossing (MABC; Thomson *et al.*, 2010). The basis of MABC strategy is to transfer a gene/QTL from a donor line to a recipient line while selecting against donor introgressions across the rest of the genome (Thomson *et al.*, 2010; Septiningsih *et al.*, 2009; Singh *et al.*, 2010). MABC breeding strategy was applied for increased salinity tolerance in Vietnam's rice lines in previous study.

Objective: Our study focused on breeding for new rice varieties with salt tolerance and high yield potential, high quality using molecular markers and conventional breeding.

## **Materials and methods**

### ***Plant materials***

- FL478 was used as the donor of *Saltol*. The recipient varieties were AS996, which is widely grown cultivars in the South of Vietnam.

- The *Saltol* QTL introgression BC<sub>3</sub>F<sub>3</sub>, BC<sub>3</sub>F<sub>4</sub> lines carrying target gene and almost recipient genome in previous study were used for screening salinity tolerance and conventional breeding.

### ***Methods***

- Target QTL *Saltol* was screened using SSR markers tightly linked to that QTL. Marker genotyping data of those crosses were obtained by running SSR markers using 15 µL PCR reactions on 96-well plates. After initial denaturation for 5 min at 94°C each cycle comprised 30 sec denaturation at 94°C, 30 sec annealing at 55-60°C, and 45 sec extension at 72°C with a final extension for 5 min at 72°C at the end of 35 cycles. The PCR products were mixed with bromophenol blue gel loading dye and were analyzed by electrophoresis on 6% -8% acrylamide gels at 100v (Dual Triple-Wide Mini-Vertical System, C.B.S.Scientific, CA, USA) followed by SYBR-Safe staining (Invitrogen), gel documentation (Alpha Innotech), and manual scoring of the gel pictures. The marker data was analyzed using the software Graphical Genotyper (GGT 2.0) (Van Berloo, 2008). The homozygous recipient allele, homozygous dominant allele and heterozygous allele were scored as 'A', 'B' and 'H'. The percentage of markers homozygous for recipient parent (% A) and the percentage of recipient alleles including heterozygous plants (% R) were calculated.

- Evaluation for agronomic characteristics:

The BC<sub>3</sub>F<sub>3</sub>, BC<sub>3</sub>F<sub>4</sub> lines with the parents AS996 and FL478 were grown at Can Tho and Bac Lieu province. The plants were laid in a 15x15 cm distance

and evaluated for 8 traits: (1) days to maturing (dtm) were evaluated as the number of days from sowing until the seed matured; (2) plant height (ph) was measured in centimeters from soil surface to the tip of the tallest panicle (awns excluded); (3) panicle numbers (pn) was calculated as the number of panicles per plant; (4) seed per panicle (sp) was calculated as the number fully filled seeds per panicle; (5) 1,000 seed weight (tsw) was measured in grams as the weight of 1,000 fully filled seeds per plant; (6) spikelets per panicle (spp) was calculated as the number of spikelets per panicle; (7) real yield (ry) was measured in ton as the weight of seeds per hecta and (8) salinity tolerance ability on the field.

- Evaluation of salinity tolerance :

Pre-germinated BC<sub>3</sub>F<sub>3</sub> and BC<sub>3</sub>F<sub>4</sub> seeds were sown in holes on styrofoam floats with a net bottom suspended on trays filled with Yoshida nutrient solution (Yoshida *et al.* 1976). Three replications were used for each experiment, with nine individual plants per line evaluated for each replication. Salt stress was imposed 14 days after germination by adding NaCl to an EC of 12 dS m<sup>-1</sup> in Yoshida nutrient solution until final scoring. IR29 (sensitive) and FL478 (highly tolerant) were used as checks. The pH of the nutrient solution was adjusted daily to 5.0, and the culture solutions were replaced every 5 days. Entries were scored based on visual symptoms using IRRI's Standard Evaluating Score (SES) for rice, with ratings from 1 (highly tolerant) to 9 (highly sensitive; IRRI, 2014).

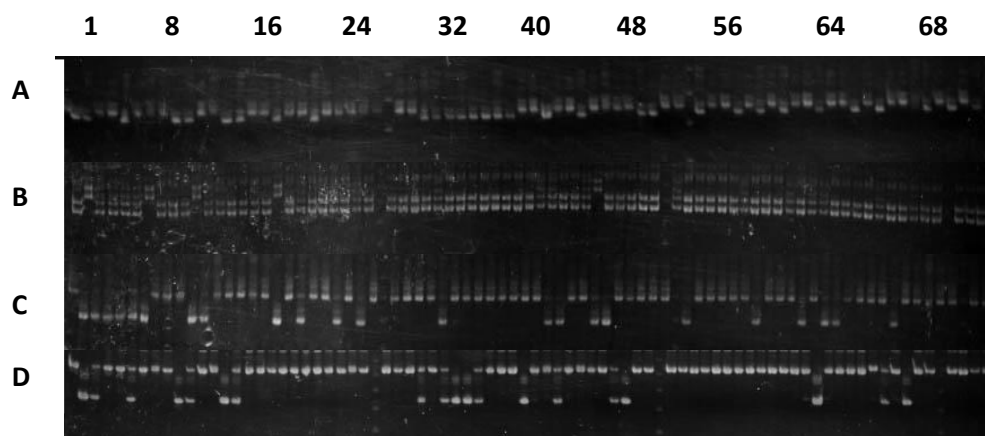
## Results

### *Molecular markers analysis*

Genotyping was carried out on 3 backcross generations, plant P284-112-209 was the best BC<sub>3</sub>F<sub>1</sub> individual with all the recipient alleles screened based on total of 63 markers. The four plants P307-305- 21, P284-112-195, P284-112-198, P284-112-213 were the second ranking with only one loci heterozygous. All those 5 plants were chosen as the breeding lines for result of *Saltol*-AS996 introgression in this study.

Total of 512 individuals developed from above results were selected for screening to find out the target region *Saltol* QTL. The 4 primers RM3412, RM10694, RM10711 and RM10793 were used for screening.

After this step, only the lines having the DNA band from the tolerance variety FL478 were chosen to develop in the field for breeding purpose. Combine with molecular breeding, conventional breeding were applied on the BC<sub>3</sub>F<sub>3</sub>, BC<sub>3</sub>F<sub>4</sub>, BC<sub>3</sub>F<sub>5</sub> for selection of the new salt tolerance rice lines with good agronomic traits and high yield.



**Figure 1.** Screening individuals  $BC_3F_4(AS996/FL478)$  using marker RM3412 (A), RM10694 (B), RM10711 (C), RM10793 (D).  
Lane 1: AS996, Lane 2: FL478. Lanes 3-68: individuals of  $BC_3F_4$

In the Summer- Autumn crop season in 2013, the yield of the selection lines were shown from 5.0 to 6.5 ton per hecta. We only selected the lines with high yield and useful agronomic characteristics for the next crop season. On table 2 show the agronomic characteristics related to yield of promising lines  $BC_3F_5$  in winter – Spring crop season 2013.

**Table 1:** Evaluating the agronomic traits related to yield of promising lines BC3F3 of AS996/FL78 in winter- spring season 2012 in BacLieu

No	Lines	Days to maturing (days)	Plant height (cm)	Panicle /m <sup>2</sup>	Full Filled seed/ Panicle	P1000 (g) seed weight	Real yield (t/ha)
1	B111	95	95.0	424	121.5	27.6	7.80
2	B 112	101	98.0	342	132	27.3	7.55
3	B 75	100	100.0	344	131	27.3	7.54
4	B 299	100	102.0	334	136	27.2	7.52
5	B 291	98	101.5	345	130	27.0	7.44
6	B 221	102	96.5	339	132	27.0	7.42
7	B 63	100	95.0	340	128	27.0	7.40
8	B 190	100	100.0	338	134	27.5	7.35
9	B 195	100	97.5	335	132	27.2	7.34
10	B 214	99	102.8	338	135	27.0	7.33
11	B 198	100	98.0	342	127	27.0	7.25
12	B 316	101	100.0	325	128	27.3	7.25
13	B 296	99	100.0	328	132	27.0	7.16
14	B 97	100	95.8	328	132	27.0	7.16
15	B 190	100	100.0	323	131	27.0	7.15
16	B 293	100	95.2	333	133	27.2	6.88
17	B 344	100	93.8	315	127	27.3	6.86
18	B 349	100	95.0	312	122	27.3	6.85
19	B 115	98	95.	317	132	27.2	6.78
20	B 21	101	102.2	314	125	27.0	6.75
21	B 213	100	102.5	313	120	27.0	6.74
22	B 112	100	93.0	325	134	27.0	6.72
23	B 322	100	99.2	320	135	27.0	6.70
24	B 11	100	96.5	318	124	27.0	6.58
25	B 305	97	103.5	315	120	27.3	6.50
	AS996	100	95.0	335	135	27.5	6.94
	FL478	100	110	335	120	25.2	5.87
	CV %						6.8
	LSD						0.68

In table 1, there were 15 lines having the yield higher than the the parent AS996 and FL478. Those lines also selected for the folowing evaluation.

**Table 2:** Evaluating the agronomic characteristics related to yield of promising lines BC3F5 in winter- spring season 2013 in BacLieu

No	Lines	Days to maturing (days)	Plant height (cm)	Panicle /m2	Full Filled seed/ Panicle	P1000 (g) seed weight	Real yield (t/ha)
<b>1</b>	<b>B111-5</b>	<b>95</b>	<b>95,0</b>	<b>424</b>	<b>121.5</b>	<b>27,6</b>	<b>7.90</b>
2	B112-1	95	92,4	406	134.2	27,7	7.60
3	B291-1-2	95	93,4	385	126.9	27,8	7.50
4	B198-9	95	95,0	387	96.4	27,5	7.10
5	B291-2	95	92,5	405.	117.7	27,5	7.00
6	B190-9	92	90,1	341	107.7	27,6	7.00
7	B291-2-3	95	92,0	362	117.7	27,6	7.00
8	B190-2	94	95,6	340	125.0	27,5	7.00
9	OMCS2009	92	92,4	355	115.3	27,5	7.00
10	B198-13	97	96,0	361	123.7	27,0	6.90
11	B190-1	95	95,0	328	120.5	27,3	6.60
12	B190-4	95	95,5	354	110.3	27,0	6.50
13	B190-6	97	90,2	305	104.7	27,4	6.25
14	AS996	95	94,6	340	126	27,4	6.91
15	FL478	98	100	300	102	26,5	5,50
16	IR29	92	90,2	302	90.1	26,0	5,40
	CV%						5.6
	LSD <sub>50</sub>						0.62

In table 2, among 12 tested breeding lines, B111-5 was found the short days to maturing (95days), the useful agronomic characteristics, the highest grain yield 7.9 ton/ha.

Integrating MAB and conventional breeding, we focused on combining the useful agronomic traits of AS996 with *Saltol* QTL, which attached salt tolerance in backcross breeding lines by conversion to the recurrent parent genotype using molecular genotyping with SSR markers. We selected successfully the promising line B111-5 with high real yield 7.9 ton per hecta with salt tolerance and disease resistance on the field trial.

The winter- spring season 2014 in Bac Lieu, the new rice resilience line was grown for agronomic and disease resistance evaluations. The promising rice line was resulted real grain yield 8.17 ton/ha. The rice line was sent to the National Plant testing centre under way to release the National new rice variety for Cuu Long Rice Delta and the coastal areas in the South of Viet Nam.

***Evaluation of salinity tolerance***

As described in the part of method, BC<sub>3</sub>F<sub>5</sub> seeds were screening to evaluate the tolerance level of the AS996-Saltol promising lines. Salt stress was imposed 14 days after germination by adding NaCl to an EC of 12 dS m<sup>-1</sup> (6<sup>0</sup>/<sub>00</sub>) in Yoshida nutrient solution until final scoring. Based on visual symptoms using IRRI's SES for rice, when the susceptible variety IR29 (sensitive) scored 9 and variety FL478 were used as highly tolerant checks scored 3, almost the promising lines having the same score as the tolerant checks. It means that the Saltol fragment working well in BC<sub>3</sub>F<sub>5</sub> generation.

**Table 3:** Tolerance level of BC<sub>3</sub>F<sub>3</sub> rice lines by SES (IRRI, 2014)

<i>No</i>	<i>Genotypes</i>	<i>Score</i>	<i>Tolerance level</i>
1	B111-5	2	R
2	B112-1	2	R
3	B291-1-2	3	R
4	B198-9	2	R
5	B291-2	5	M
6	B190-9	2	R
7	B291-2-3	5	M
8	B190-2	2	R
9	<b>OMCS2009</b>	9	S
10	B198-13		R
11	B190-1	2	R
12	B190-4	5	MR
13	B190-6	5	MR
14	AS996	7	S
15	FL478	1	HR
16	IR29	9	S



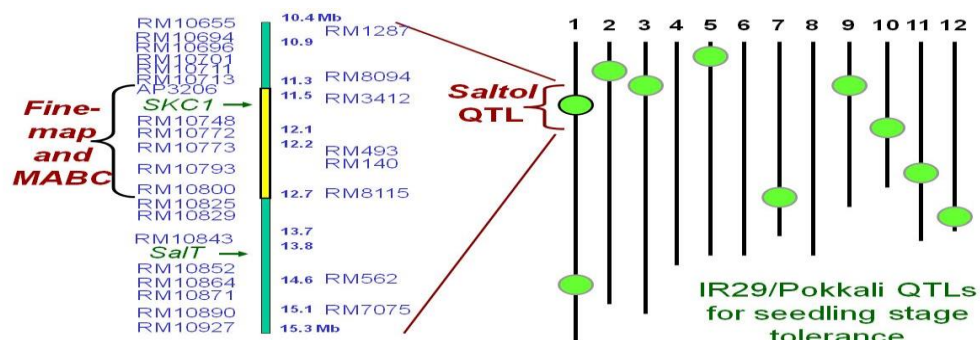
**Figure 2:** Evaluating the selected lines for salinity tolerance

*Lane 1: IR29; lane 2-8: AS996-Saltol lines; lane 9: FL478; lane 10: AS996* Integrating MAB and conventional breeding, the resilience new rice line B111-5 were identified. This line was name OM352 to send to the National Variety Trial and Testing Centre” for Value-for-Cultivation-and-Use (VCU) and Distinctness-Uniformity-Stability (DUS) tests and trials at different ecological zones.

## Discussions

*Saltol* is a major QTL associated with the Na–K ratio and seedling-stage salinity tolerance, was identified on chromosome 1. This QTL was tested in a hydroponic screen at the seedling stage revealed that this QTL explained 43% of the variation for seedling shoot Na–K ratio in the population (Thompson *et al.* 2010).





**Figure 3:** Fine-map and MABC strategy of Saltol QTL (IRRI, 2010)

An analysis of single feature polymorphism in the *Saltol* region suggested that FL478 contained a DNA fragment smaller than 1 Mb from Pokkali at 10.6–11.5 Mb on chromosome 1, flanked by IR29 alleles (Kim *et al.* 2009). Based on the map of *Saltol* QTL region, the best markers within the *Saltol* QTL region were AP3206 and RM3412, the most useful markers flanking the *Saltol* region were RM10694 (telomeric to *Saltol*) and RM493 and RM10793 (centromeric to *Saltol*), while nearby markers that can be used for negative selection are RM490 above *Saltol* and RM7075 below. Microsatellite markers unlinked to *Saltol* covering all the chromosomes, that were polymorphic between the two parents, were used for recombinant and background selection to recover the recipient genome. The MABC approach was applied successfully to introgress *Saltol* QTL into AS996 and could be successfully utilized to introgress other important varieties with a minimum introgression segment and within a short time frame. By limiting the size of the introgression, the chance of introducing donor genes that might change the essential characteristics of this popular variety was reduced. It is expected that the newly developed lines will be able to increase rice production in the salinity and submergence coastal areas of Vietnam. This study has succeeded in breeding for new salinity tolerance variety from the local variety and the imported variety FL478 from IRRI, combined molecular and conventional breeding together.

## Conclusions

Integrating MAB and Conventional breeding was applied on the introgression Saltol QTL with all recipient genome of AS996 in BC<sub>3</sub>F<sub>3</sub> to BC<sub>3</sub>F<sub>6</sub> generations.

The salinity tolerance of the selection lines were evaluated in green house.

The promising rice line B111-5 possessing Saltol QTL with Salt tolerance and high yield potential was obtained. The new resilience rice line is under way to release as a new rice variety for the coastal areas in Cuu Long rice Delta.

### **Acknowledgement**

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### **References**

- IRRI, “Standard Evaluation System for Rice” (2014), 5th Edition, International Rice Research Institute, Manila, p. 52
- Kim, S.H., Bhat, P. R.; Cui, X.; Walia, H. ; Xu, J.; Wanamaker, S. (2009), “Detection and Validation of Single Feature Polymorphisms Using RNA Expression Data from a Rice Genome Array,” *BMC Plant Biology*, Vol. 9, p. 65. [doi:10.1186/1471-2229-9-65](https://doi.org/10.1186/1471-2229-9-65)
- Septiningsih EM, Pamplona AM, Sanchez DL, Neeraja CN, Vergara GV, Heuer S, Ismail AM, Mackill DJ (2009). Development of submergence tolerant rice cultivars: the Sub1 locus and beyond. *Ann. Bot.* 103:151–160.
- Singh RK, Redoña ED, Refuerzo L. (2010). Varietal improvement for abiotic stress tolerance in crop plants: special reference to salinity in rice. In: Pareek A, Sopory SK, Bohnert HJ, Govindjee, editors. *Abiotic stress adaptation in plants: physiological, molecular and genomic foundation*. New York: Springer p. 387–415.
- Thomson MJ, Ismail AM, McCouch SR, Mackill MJ. (2010). Marker assisted breeding. In: Pareek A, Sopory SK, Bohnert HJ, Govindjee, editors. *Abiotic stress adaptation in plants: physiological, molecular and genomic foundation*. New York: Springer; p. 451–69.
- Van Berloo R (2008) GGT 2.0: versatile software for visualization and analysis of genetic data. *J Hered* 99:232–236.
- Yoshida S, Forno DA, Cock JK, Gomez KA. (1976). *Laboratory manual for physiological studies of rice*. Manila: International Rice Research Institute; p. 38.