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RESEARCH ARTICLE

Development of Salt Tolerant IR64 Near Isogenic Lines Through Marker-Assisted Breeding

Viet The Ho[1,*], Michael J. Thomson[2,3], Abdelbagi M. Ismail[2]

[1]*University of Food Industry, 140 Le Trong Tan, Tan Phu district, Ho Chi Minh city, Vietnam*
[2]*International Rice Research Institute, Los Banõs, Laguna, Philippines*
[3]*Current address: Department of Soil and Crop Sciences, Texas A&M University, Texas, USA*

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Abstract

Salt stress causes considerable damage to rice with a consequent reduction in grain yield, however, conventional breeding for this stress is time-consuming and costly. Recently, marker-assisted breeding has shown enormous potential to accelerate breeding of stress tolerant varieties because of its precision, time saving, and cost effectiveness. The present study was carried out to transfer *Saltol*, a major QTL on chromosome 1 associated with salinity tolerance, from FL478, a tolerant genotype, into IR64, a popular lowland variety through marker-assisted backcrossing (MABC). This technique considerably enhanced the recovery rate of the recurrent parent genome within three backcross generations, which could have saved several backcrosses compared with conventional schemes to achieve the same results. By using this technique, up to 99.7% of the recurrent parent genome was recovered at BC_3F_2 generation, saving at least three backcrosses compared with conventional breeding schemes. Salinity tolerance of IR64-*Saltol* lines was evaluated using saline culture solution adjusted to electrical conductivity of 12 dS m^{-1} using NaCl. Based on selected physiological and growth parameters, the new *Saltol* introgression lines showed a significantly higher tolerance of salinity than their recurrent parent IR64. The results of this study confirm the benefits of using molecular markers in plant breeding to enhance tolerance of abiotic stresses.

Key words : Marker-assisted breeding, Quantitative trait loci (QTL), rice; salinity tolerance

Introduction

Rice (*Oryza sativa* L.) is considered sensitive to salt stress especially during the seedling and reproductive stages (Moradi et al. 2003), and salt stress at either stage causes considerable reduction in growth and grain yield. Conventional breeding has been used for decades to improve tolerance of rice, especially during the seedling stage to ensure good crop establishment when salinity is higher at the beginning of the monsoon season, as in coastal tropical deltas (Babu et al. 2004; Vanaja et al. 2015). However, conventional breeding retains several drawbacks, including the long period of time required for a breeding program to develop varieties with desired yield and quality preferences, the high expenses, the difficult and even sometimes unreliability of phenotyping because of the nature of the target traits or target environment (Babu et al. 2004). With conventional backcrossing, not only are several backcrosses are needed, but also large segments of unwanted chromosomal regions from the donor parent could be retained (Collard and Mackill 2008).

In recent years, DNA markers have shown enormous potential for use to improve the efficiency and precision of conventional plant breeding, ultimately accelerating the development of new more resilient crop varieties. Introgression of desirable traits can be prompted when those traits are mapped with tightly linked DNA-markers and the results of the introgression of desirable genes can be monitored at early generations (Siangliw et al. 2007).

Among several of the mapped quantitative trait loci (QTLs) such as submergence tolerance (Xu and Mackill 1996), chlorophylly content (Bo et al. 2007), salt tolerance (Masood et al. 2004), and yield components (Thomson et al. 2003), the availability of large effect QTLs like *Saltol* for salt tolerance provided opportunities to introduce these

Viet The Ho (✉)
Email: thehv@cntp.edu.vn
Tel: +84.933-025-523

QTLs into mega rice varieties or to combine several QTLs for multiple stress tolerance using marker-assisted backcrossing (MABC) (Thomson et al. 2010a). *Saltol* QTL was initially identified by Gregorio (1997) at the International Rice Research Institute using AFLP markers to genotype a total of 38 tolerant and 42 sensitive recombinant inbred lines (RIL) developed from a cross between IR29, a sensitive variety, and Pokkali, a highly tolerant landrace cultivated along the southeastern coast of India. The significance of this study was the finding of *Saltol* QTL which contributes largely to maintaining low Na^+ concentration in plant tissue and accounts for about 45% of phenotypic variance for Na^+/K^+ ratio and salinity tolerance at the seedling stage in the RIL population. Among 80 RILs of this mapping population, line IR66946-3R-178-1-1 containing the *Saltol* QTL, named FL478 has been identified with higher potential for use as donor in breeding programs in rice because of its numerous superior characteristics in comparison with original Pokkali landrace, such as higher salinity tolerance, photoperiod insensitivity, short stem, and early flowering (Thomson et al. 2010b).

Using sets of markers selected from the 2240 SSR markers for rice developed by McCouch et al. (2002) available at Gramene (http://www.gramene.org/), *Saltol* QTL has recently been fine-mapped and used to develop several salt tolerant rice varieties (Bimpong et al. 2016; Le et al., 2012; Luu et al. 2012; Thomson et al. 2010a). This study describes successful introgression of *Saltol* QTL from FL478 into IR64, a popular rice variety, widely grown in South and Southeast Asia, using MABC, and partially quantified the role of *Saltol* in conferring salinity tolerance in this variety.

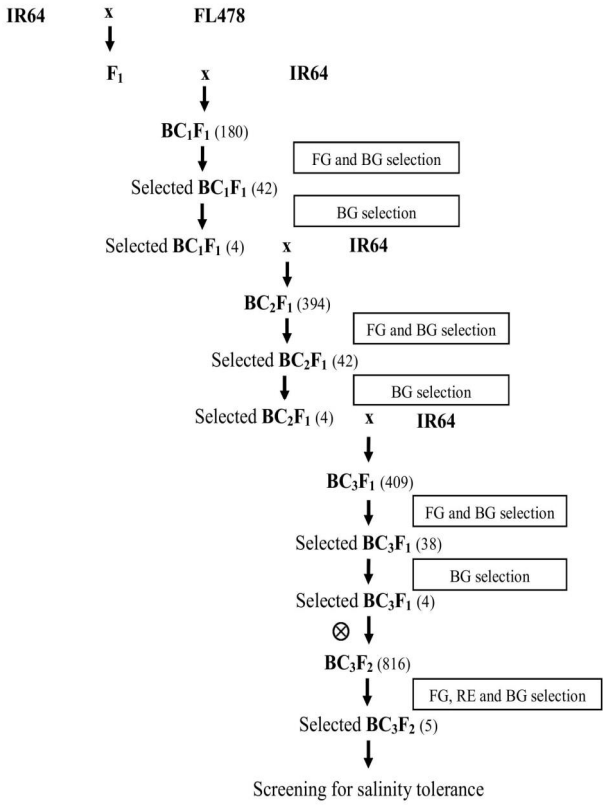

Fig. 1. Marker-assisted backcrossing scheme for the development of IR64-*Saltol* introgression lines with SSR markers used for foreground (FG), recombinant (RE), and background (BG) selection. The "x" symbol represented a cross, whereas ↳ represented a selfing generation during marker-assisted backcross program. The numbers of plants selected in each generation are indicated in parentheses.

Materials And Methods

Plant Material

FL478, a salt-tolerant RIL developed from the cross of Pokkali and IR29 was used as a donor parent because of possessing *Saltol* QTL, which is responsible for high tolerance to salt stress, whereas IR64 was used as the recipient parent because this variety is widely grown in South and Southeast Asian countries as it has several desirable traits such as high yield, relatively high resistance to diseases like blast and bacterial leaf blight, and good grain quality.

Backcrossing Scheme

The marker-assisted backcrossing scheme used in this study followed several cycles of crossing and selfing (Fig. 1). The F_1 plants obtained from the cross of FL478 x IR64 were crossed with the IR64 parent to obtain BC_1F_1 seeds. In the BC_1F_1 generation, individual plants heterozygous at the *Saltol* locus were identified using markers specific for *Saltol* (RM3206f and RM3412b) to reduce the population size for further screening (termed foreground selection; Neeraja et al. 2007). Individuals possessing *Saltol* were then surveyed using two *Saltol* flanking markers, namely RM10694 and RM493 (termed "recombinant selection"; Collard and Mackill 2008). The plants that were homozygous in recurrent parent type at both flanking markers were classified as recombinants, whereas plants homozygous for either of the two flanking markers were grouped as semi-recombinant plants. Recombinant and semi-recombinant plants were then genotyped using markers specific for the recurrent parent background to identify individuals containing the largest number of marker alleles of the recipient parent (termed background or recurrent selection; Thomson et al. 2010a). The individuals with the highest recurrent parent background were crossed with the recurrent parent to obtain BC_2F_1 seeds.

The same selection strategies were followed in the BC_2F_1 and BC_3F_1 generations to trace the presence of *Saltol* QTL and increase the recovery of the recurrent parent genome. Selected BC_3F_1 plants were self-pollinated to obtain BC_3F_2 seeds. This generation was used to select individuals that are homozygous at the target *Saltol* QTL and with clean background of IR64. BC_3F_3 seeds of selected plants were then used to screen for salinity tolerance at seedling stage to confirm the tolerance level of the newly developed IR64-*Saltol* lines as compared with their parents and a sensitive check, IR29.

DNA Extraction and SSR Marker Analysis

DNA extraction was performed using a slightly modified CTAB method (Thomson et al. 2006). The quality and quantity of the isolated DNA were quantified with A Spectrophotometer (Nanodrop, ND-1000, USA) and diluted to working concentrations of 35 ng μL^{-1} with deionized distilled water. After screening 250 SSR markers distributed in the 12 chromosomes, 82 polymorphic markers were selected for foreground and background selections. SSR markers used for MABC were obtained from Gramene (http://www.gramene.org/). Each PCR reaction was carried out with 15 µL reaction mix containing 1.5 µL 10x buffer, 1 µL of 1 mM dNTPs, 0.5 µL of 5 µM forward primer, 0.5 µL of 5 µM reverse primer, 0.7 µL of 5 U μL^{-1} Taq polymerase, 8.8 µL deionized water, and 2.0 µL of each DNA template at 35 ng mL^{-1} concentration. PCR profiles were programmed as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and elongation at 72°C for 2 min, and a final extension step of 7 min at 72°C. The PCR cocktails were run using a GStorm GS1 thermocycler (Green Technology Limited, UK). Two µL of the PCR products together with 5 µL of 10X loading dye (bromphenol) were loaded into an 8% polyarcylamide gel and run at 100 volts for about 1.5 to 3.5 hours, depending on the size of the PCR products. A 1 kb DNA ladder was used as the size standard. Gels were stained with Syber green safe and visualized by Gel doc system (Bio-Rad, USA). Allelic bands of DNA markers were scored based on the parents' bands and designated as A for IR64, B for FL478, H for heterozygote, and U for unidentifiable band.

Screening for Salt Tolerance at Seedling Stage

Salinity tolerance of selected BC_3F_3 lines was evaluated using the modified IRRI standard evaluation scoring (SES) system based on visual symptoms of salt injury at the seedling stage (IRRI 1996). Visual symptoms were evaluated with scores ranging from 1 to 9, with 1 being the most tolerant and 9 the most sensitive. Seven genotypes were used; the donor parent FL478, the recipient parent IR64, four derived IR64-*Saltol* lines, and IR29 as a sensitive check. Two treatments were used: a control condition using Yoshida nutrient solution (Yoshida et al. 1976) and salt stress treatment using artificially salinized solution. The salinized nutrition solution was prepared by adding analytical grade of NaCl while stirring, until the solution reached the desired electrical conductivity (EC; about 3 and 6 g NaCl L^{-1} nutrient solution for an EC of 6 and 12 dS m^{-1}, respectively). In the salt-stress treatment, pre-germinated seeds were kept growing in nutrient solution for 2 weeks. After this period, seedlings were initially stressed with NaCl at an EC of 6 dS m^{-1} for 1 week, then increased to EC of 12 dS m^{-1} and kept for 3 more weeks when the experiment was terminated.

Graphical Genotyping and Statistical Analysis

Graphical genotyping (GGT 2.0, Wageningen University, the Netherlands) software was used to monitor the recovery rate of the recurrent parent genome and to assess the genome composition of the new genotypes in each backcross generation based on genotyping data. Analyses of variance of the physiological and agronomic data, namely shoot K^+ concentration, shoot Na^+ concentration, shoot length, shoot fresh weight, shoot dry weight, and visual symptoms of injury (SES) were conducted using Statgraphics Centurion XV (Princeton University, USA). Experiments were arranged in a Randomized Complete Block Design with four replications, single factor ANOVAs were used for the analysis of each trait, and standard errors were calculated from pooled error. Mean values of each trait were separated using Least Significant Difference (LSD) test at $P < 0.05$.

Results and Discussion

Foreground and Recombinant Selection

The foreground selection was performed on 180, 394, and 337 plants of BC_1F_1, BC_2F_1, and BC_3F_1 generations, respectively. The banding patterns of R3206f and RM3412b markers used in foreground selection showed differences in three genotypes, namely, the homozygous recurrent parent IR64 (A), the homozygous donor parent FL478 (B), and the heterozygous genotype (H) (Fig. 2).

The foreground markers effectively detected the presence of *Saltol* QTL located in the short arm of chromosome 1; however only two recombinants for *Saltol* locus were identified in the BC_1F_1 generation. This result was expected since according to Semagn et al. (2006), the probability of obtaining double homozygous individuals for two flanking markers in a single backcross generation is relatively rare. Even in BC_2F_1 generation, Zhou et al. (2003) did not detect any recombinants between the waxy marker and C688 in 49 plants containing the *wx*-MH gene. The frequency of the

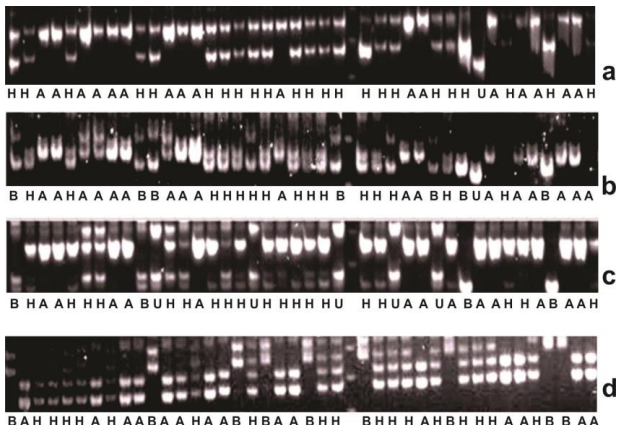

Fig. 2. Examples of the genetic profiles of two foreground markers (RM3206f, RM3412b) and two flanking markers (RM10694, RM493) in the cross of IR64 and FL478. A: homozygous to IR64 parent; B: homozygous to FL478; H: heterozygous; U: unidentified genotypes. a: RM493; b: RM3412b; c: RM3206f; d: RM10694.

recombinants was higher in subsequent backcross generations with 11 and 37 plants identified in BC_2F_1 and BC_3F_1, respectively (Fig. 1).

Background Selection

A total of 32 markers were used for background selection of 42 BC_1F_1 plants identified through foreground and recombinant selection, and the proportion of the recipient parent genome in these plants ranged from 64.7 to 85.9%. Four plants, BC_1-144, BC_1-161, BC_1-110, and BC_1-63 with the recovery rate of the recurrent parent genome at 85.9, 82.0, 80.5, and 78.2%, respectively, were selected for backcrossing. In BC_2F_1, 16 new SSR markers were added to fill the chromosomal gaps identified in BC_1F_1 background selection. A total of 48 markers were then used to screen 42 BC_2F_1 plants, which revealed the recurrent parent contributed from 79.3 to 94.9% of the genomes of these selected plants. Four plants, BC_2-114-266, BC_2-63-119, BC_2-63-128, and BC_2-144-218, with the recovery of the recurrent parent genome at 94.9, 88.2, 88.2, and 87.9%, respectively, were selected for backcrossing. In the last backcross, BC_3F_1, a total of 82 markers were used to assess the recovery rate of the recurrent parent genome in 94 BC_3F_1 plants. The results showed that the contribution of the recurrent genome in these plants varied from 85.5 to 97.7% and four plants, BC_3-63-119-53, BC_3-63-119-73, BC_3-63-119-289, and BC_3-63-119-330 with recovery rates at 95.7, 95.8, 95.2, and 95.1%, respectively, were selected and used for selfing.

The proportion of the recurrent parent genome among individual plants varied largely in its distribution throughout the genome, and consequently background selection processes were performed to identify individuals with the lower proportion of the undesirable genome from the donor parent. This enabled the selection of plants that are closer to their IR64 recurrent parent except for the presence of the introgressed QTL. Theoretically, the proportion of recurrent parent genome in BC_1F_1 generation would be 75%, but some individuals are expected to possess more recurrent parent genome than others, and these could be exploited through MABC, to select for a higher proportion of the recurrent parent genome (Collard et al. 2005). Plants with recovery rates higher than the theoretical estimates of 87.50 and 93.75% of the recurrent parent genome were identified and selected in BC_2F_1 and BC_3F_1 generations, respectively. Using the same approach, Chen et al. (2001) achieved relatively higher recovery rate of the recurrent parent genome of over 90% in BC_1F_1 when transferring genes for bacterial blight resistance in rice. Additional selection criteria were followed in this study to select plants for further genotyping on the rest of the 11 chromosomes, including individuals with the highest percentage of recurrent parent genome, and phenotypic resemblance of IR64.

Selection in BC_3F_2 generation

From four selfed BC_3F_1 plants, 816 BC_3F_2 seedlings were produced and screened to select plants with IR64 cleaner background and homozygous for *Saltol* QTL. Foreground and background selection were combined in one step using 16 SSR markers including two markers for foreground selection, two markers for recombinant selection and the remaining 12 markers were located on the heterozygous chromosome fragments identified in the previous generation. Four plants introgressed with *Saltol* with clean IR64 genomic composition (99.7%) were identified, namely: IR64-119-73-168; IR64-119-73-181; IR64-119-73-284; and IR64-119-73-557 (hereafter, IR64-*Saltol* 168, IR-*Saltol* 181, IR64-*Saltol* 284, and IR64-*Saltol* 557, respectively).

This study showed the efficiency of the *Saltol*-specific markers (RM3206f, RM3412b) and the flanking markers (RM10694, RM493) in selecting individuals carrying this QTL. Previous studies also showed similar results using different QTLs (Hospital and Charcosset; 1997; Neeraja et al. 2007). These co-dominant SSR markers also showed their advantages over dominant markers. Liu et al. (2000) used dominant markers to trace the introgression of genes responsible for powdery mildew resistance in wheat, but they had to develop one more selfing generation to determine the genotype of their plants after each backcross. Flanking markers play an important function in recombinant selection to minimize linkage drag while recovering the desirable trait in the recipient parent, and they also facilitate the identification of double recombinants with relatively smaller donor fragment being introgressed (Neeraja et al. 2007).

The recovery of the recurrent parent genome was greatly accelerated using background markers after each backcross. It would have taken from two to three additional generations of backcrossing to achieve similar percent recovery of the recurrent parent genome using conventional backcrossing (Frisch et al. 1999; Thomson et al. 2010a). Moreover, using molecular markers, the inheritance of the target QTL and the remaining recurrent parent genome can be effectively assessed. *Saltol* QTL from FL478 was successfully introduced into IR64 in three generations of backcrossing followed by one generation of selfing. Derived IR64-*Saltol* lines contained a fragment from the donor parent with relatively small size of less than 1.2 Mb, that contain the *Saltol* region, and with the rest of the genome of the recipient parent (Fig. 3). The introgression of this QTL enhanced the vigor of the new lines under salt stress, and reduced the concentration of Na^+ in the shoot, both of which were recognized as important mechanisms that contribute to salinity tolerance in rice (Ismail et al. 2007).

Phenotypic Evaluation of IR64-Saltol for Tolerance of Salt Stress at Seedling Stage

Seven genotypes, including the donor parent FL478, the recurrent parent IR64, the four IR64-*Saltol* lines, and the sensitive control IR29, were grown under controlled condition and salt stress of 12 dS m^{-1} at the seedling stage using Yoshida nutrient solution. The four lines had uniform growth and their agronomic features such as shoot length and shoot fresh and dry weights, were similar to that of IR64 under control

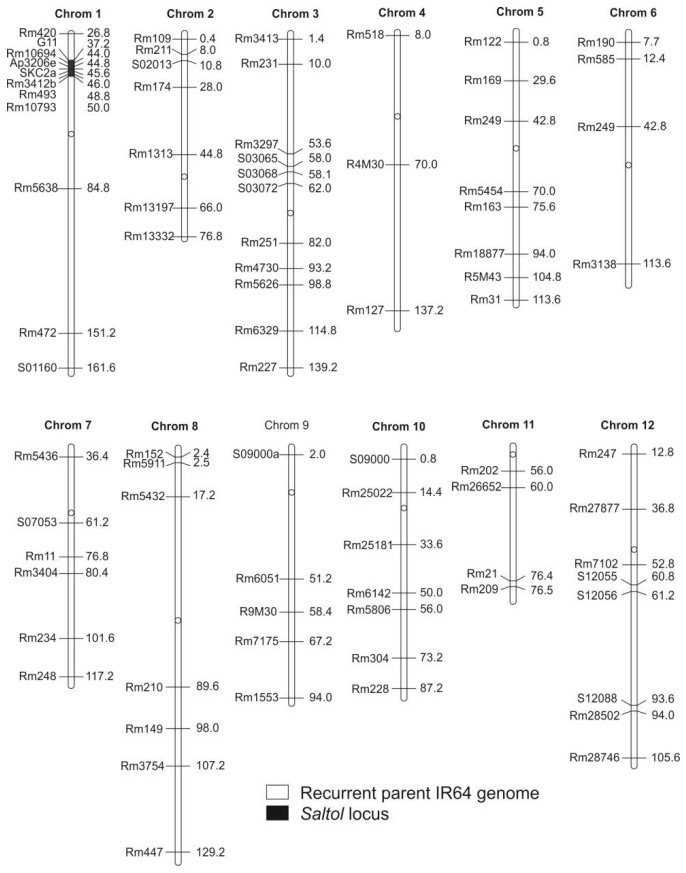

Fig. 3. Graphical representation of IR64-*Saltol*-119-73-284. Chromosome numbers are located at the top of the bar. The black portion of chromosome 1 is the *Saltol* region derived from FL478 genotype. Names and corresponding positions of markers are labeled on the left and right sides of the chromosomes, respectively. The circles indicate positions of chromosome centromeres.

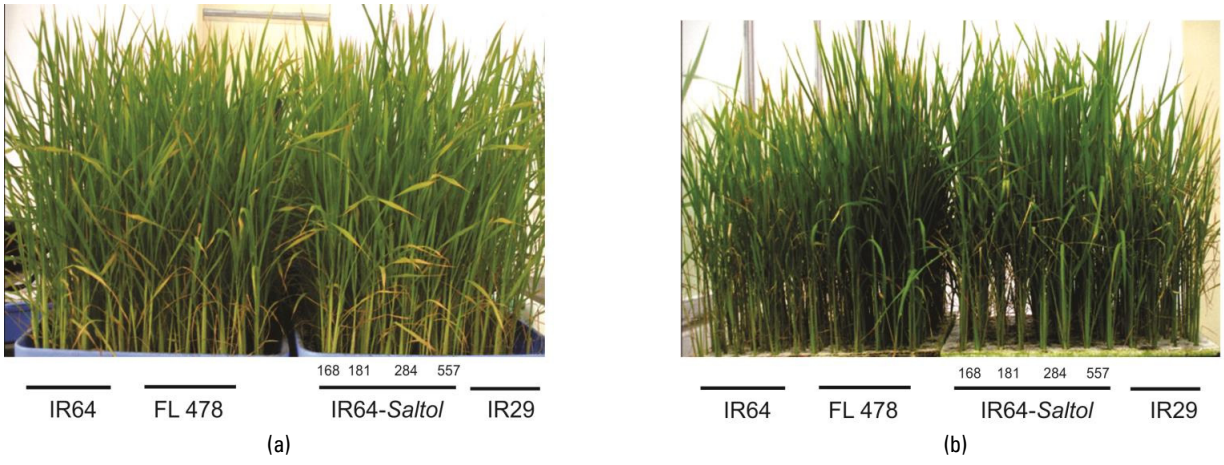

Fig. 4. Evaluation of different rice genotypes using Yoshida hydroponic culture solution under (a) control and (b) salt stress of 12 dS m^{-1}. The numbers above "IR64-*Saltol*" indicate different IR64-*Saltol* derived lines at BC_3F_3 generation.

condition (Fig. 4a and Table 1). This suggests that in the absence of salt stress, the presence of *Saltol* QTL do not have any apparent effects on morphological features of the recipient parent.

Under salt stress, different genotypes showed different levels of salt injury. Symptoms such as leaf rolling, brownish and whitish leaf tips were more evident on the first and second leaves. Stunted growth, cessation of elongation and dying of seedlings in the sensitive lines were also observed (Fig. 4b). The sensitive check, IR29, showed the poorest performance with an average SES score of 8, while the donor parent, FL478, showed the highest tolerance with

Table 1. Variations in shoot length and shoot fresh and dry biomass of four rice *Saltol* introgression genotypes, parents (IR64; FL478) and one sensitive check (IR29) under control and salt stress conditions (saline) of 12 dS m^{-1}.

Genotype	Shoot length (cm)		Shoot fresh weight (g)		Shoot dry weight (g)		SES
	Control	Saline	Control	Saline	Control	Saline	
IR29	54.4	33.0^{c}	1.9	0.5^{c}	0.51	0.17^{bc}	7.8^{a}
168	59.0	44.1^{a}	2.1	1,0^{a}	0.50	0.24^{ab}	4.23^{b}
181	58.1	48.5^{a}	2.0	1.6^{a}	0.48	0.40^{a}	4.50^{b}
284	60.4	46.9^{a}	2.3	1.2^{a}	0.52	0.28^{a}	4.25^{b}
557	55.6	45.5^{a}	1.7	1.1^{a}	0.41	0.26^{ab}	4.25^{b}
IR64	54.8	38.9^{b}	1.8	0.7^{b}	0.44	0.17^{b}	5.75^{ab}
FL478	60.8	48.9^{a}	3.4	1.6^{a}	0.88	0.44^{a}	3.25^{b}

Values with different letters within a column are significantly different at $P < 0.05$
SES: Standard evaluating score of visual salt injury at seedling stage

Table 2. Na^+ and K^+ concentrations and Na^+-K^+ ratios of four IR64-*Saltol* introgression lines, parents (IR64; FL478) and one sensitive check (IR29) under salt stress of 12 dS m^{-1} using hydroponic culture solution.

LINES	K^+ CONCENTRATION (mM mg^{-1})	Na^+ CONCENTRATION (mM mg^{-1})	Na^+/K^+ ratio
IR 29	15.25^{b}	45.98^{a}	3.06^{a}
168	18.58^{a}	26.00^{b}	1.48^{b}
181	18.00^{a}	22.15^{b}	1.23^{b}
284	18.78^{a}	26.80^{b}	1.44^{b}
557	18.18^{a}	34.00^{ab}	1.88^{ab}
IR 64	17.18^{a}	35.68^{ab}	2.37^{ab}
FL 478	19.05^{a}	21.13^{b}	1.11^{b}

Values with different letters within a column are significantly different at $P < 0.01$.

an average SES score of 3. The four IR64-*Saltol* lines were significantly better than IR64 ($P < 0.01$; Table 1). Salt stress also significantly reduced shoot length and shoot fresh and dry weights of all genotypes; however all *Saltol* introgression lines were significantly taller than IR64 under salt stress.

Rice growth is considerably affected by salt stress, especially at the seedling stage and this have enormous impact during crop establishment in salt affected areas. High-yielding varieties with higher salt tolerance during the seedling stage are needed to ensure good crop stand and high grain yield. The reduction in survival and growth related traits during crop establishment, contributes directly to losses in stand and productivity of rice in salt-affected areas. Seedling survival, shoot length, and shoot fresh and dry weights are usually seriously affected under salt stress. Studies conducted in the past showed that shoot growth in rice is controlled by multiple genes scattered on different chromosomes (Haq et al. 2008; Koyama et al. 2001). Vigorous vegetative growth at seedling stage contributes to salt stress tolerance through diluting Na^+ concentration in shoot tissue, leading to better survival and growth in saline soil (Yeo et al. 1990).

The derived IR64-*Saltol* lines had significantly higher shoot biomass than that of the recurrent parent IR64 under salt stress. Some of these lines also showed significantly higher shoot fresh and dry weights than those of IR64. The tolerant parent FL478 seems to be more tolerant than the *Saltol* introgression lines, possibly because FL478 contains additional QTLs for salinity tolerance from the original donor Pokkali (Ismail et al. 2007; Thomson et al. 2010b). This suggests that transferring *Saltol* QTL alone may not result in salinity tolerance similar to that of FL478, and that transferring additional QTLs may be necessary to achieve higher tolerance (Singh et al. 2007).

Variation in physiological traits associated with salinity tolerance among the IR64-*Saltol* introgression lines and checks

Concentrations of K^+ in seedling shoots were similar among IR64-*Saltol* lines, IR64, and FL478 but significantly lower in the shoots of the sensitive check, IR29. On the other hand, Na^+ concentration in shoots was considerably higher in the sensitive check, IR29. Three of the four IR64-*Saltol* (168, 181, 284) and FL478 similarly had significantly lower Na^+ concentration as well as Na^+/K^+ ratios in their shoots compared with IR64 (Table 2) suggesting that *Saltol* plays an important role in salt exclusion during ion uptake. Similar observations were made before (Haq et al. 2008; Moradi et al. 2003; Moradi and Ismail 2007; Rahman et al. 2016)

where high concentration of Na^+ in plant tissue commonly observed under salt stress conditions, was the main reason for the reduction in seedling growth and survival (Platten and Ismail 2013).

The accumulation of sodium into the cytoplasm could lead to the inhibition of numerous enzymes, but the extent of inhibition is dependent on the concentration of potassium in plant tissue, suggesting the importance of maintaining a balanced concentration of these cations (Zhu 2007). Apparently all the genotypes used in this study maintained higher concentration of K^+ in their shoots under salt stress, except the most sensitive, IR29, which is also reflected in the Na^+/K^+ ratios. The lines that showed lower Na^+/K^+ ratio in shoots also had better shoot growth and less symptoms of salt injury as reflected by SES scores. Similar observations were previously made by Flowers and Yeo (1981) where they found that Na^+/K^+ ratio correlates with growth of rice seedlings and grain yield under saline condition. Previous studies also associated *Saltol* locus with lower Na^+ uptake and Na^+/K^+ ratio in rice seedling shoots (Elahi et al. 2004; Gregorio et al. 2002). The importance of *Saltol* locus for salinity tolerance has been reported in numerous studies (Bohra and Dorffling 1993; Lee et al. 2003; Ren et al. 2005). Thomson et al. (2007) reported the presence of a cation-chloride co-transporter, a stress-inducible membrane pore protein, a universal stress protein ER6, a Myb-like transcription factor, and a methionine synthetase in this region, beside the *SKC1* reported before by Ren et al. (2005). Most or all of these genes are strong candidates for the underlying tolerance attributed to this region.

The *SKC1* gene identified in the *Saltol* locus is probably one of the important genes responsible for K^+ homeostasis (Ren et al. 2005). The relatively smaller differences detected between IR64 and IR64-*Saltol* introgression lines in the present study is probably because IR64 has intermediate tolerance of salt stress (Castillo et al. 2007), mainly through its ability to maintain higher uptake of K^+ and lower uptake of Na^+ under salt stress, compared with IR29. IR64 has high K^+ uptake close to that of FL478, and this inherent tolerance might have partially masked the effects of *Saltol* locus in IR64 background. Monna et al. (2002) and Eshed and Zamir (1995) proposed that the interaction between chromosomal regions with QTLs containing several genes could modify the effect of the introgressed gene(s) or QTL. Whereas, Semagn et al. (2006) suggested the main reason is epistases, either between the introgressed QTLs or between the QTLs and the genetic background.

The differences among IR64-*Saltol* lines in terms of agronomic and physiological traits observed in this study were not expected, assuming that they all have similarly clean IR64 background. However, this variation might be explained by that the presence of few genes that were introgressed from the donor parent into chromosomal regions of the recurrent parent genome where the marker density was not saturated enough to detect these introgressions. FL478 is an F_8 recombinant inbred line from the cross between Pokkali; a salt tolerant landrace and IR29, a salt-sensitive variety, and theoretically should contain about 50% of the genome of IR29. Given that, in rice, there is an average of 200 genes per 2 Mb chromosomal fragment (Neeraja et al. 2007) even a short chromosomal fragment originating from IR29 could be transferred to the recurrent parent which might have resulted in the small variation between these IR64-*Saltol* lines in response to salt stress. This problem was also reported by Neeraja et al. (2007) when they transferred *SUB1* QTL for submergence tolerance into the lowland rice variety Swarna. The high throughput Single Nucleotide Polymorphism (SNP) genotyping platform developed at IRRI with extremely dense marker system will be useful to evaluate the background of these introgression lines for any alien introgressions.

Conclusions

This study demonstrated the effectiveness of using molecular markers in speeding the recovery rate of a recurrent genome in backcross breeding programs and also the precision in tracing target loci. Through this approach, breeding cycles could be shortened by two to three generations of backcrossing compared with conventional backcross methods. Besides, MABC could have significant economic impact on breeding once QTLs of large effects were identified. IR64-*Saltol* lines developed in this study are apparently similar to the recurrent parent, IR64 with the exception that these lines acquired higher tolerance of salt stress and can grow better on saline soils than the recurrent parent. The derived genotypes could be useful for farmers in areas where irrigation water or soil is saline, as in coastal areas affected by tidal inundation or salt intrusion from saline underground water.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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