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Marker-assisted backcrossing (MABC) for improved salinity tolerance in rice (*Oryza sativa* L.) to cope with climate change in Vietnam

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Abstract

Salinity is the most common abiotic stresses leading to the reduction of rice yield in many rice-growing areas of the world. Improvement in salt tolerance of rice for target stress regions is one of the important objectives of many breeding programs. In this study, we are currently applying a MABC (marker-assisted backcrossing) system on foreground selection, recombinant selection followed by background selection for development of Vietnamese rice variety that can tolerate salinity of rised sea water. The highly salt tolerant FL478 was used as a donor to transfer *Saltol* QTL into Bacthom 7 recipient rice cultivar. A total of 368 SSR markers were conducted to identify 8 markers in *Saltol* locus and 81 markers in other loci with total of 89 (24%) polymophic markers between the parents, out of which 88 markers were then applied to analyze genotyping of each backross generation with three steps of selection. The results showed that, the best plants of BC3F1 generation carry segments of the donor (11.16 - 12.6 Mb), which had 96.8% - 100 % of the recipient genome. This study revealed that the introgression lines can be directly developed into the salinity tolerance variety, which suitable for cultivating in coastal areas of Vietnamese Deltas. The MABC aids in the transfer of target chromosome segments and may improve the recovery of the recipient genome if background selection is employed.

Keywords: marker-assisted backcrossing (MABC); rice (*Oryza sativa* L); salt tolerance; *Saltol* QTL; SSR markers.
Abbreviations: AGI- Agricultural Genetics Institute; CLRRI- Cuu Long Rice Research Institute; IRRI- International Rice Research Institute; MABC- marker-assisted backcrossing; QTL- quantitative trait loci; SSR- simple sequence repeats (microsatellites).

Introduction

Vietnam is located in the sub-climatic regions will suffer the negative impacts of climate change to agricultural production. Rice production is affected by global climate change through various pathways. Sea level rise and storm surge, which result from climate change, adversely impact rice production in deltaic regions in Vietnam. Rice (*Oryza sativa* L.) is a key staple food crop and also a valuable cash crop in Vietnam. Although rice is the major provision for half of the world population, it is more sensitive to salt stress than other cereals (Greenland, 1984; Shannon et al., 1998). Rice production is severely affected by the deposition of soluble salts in the soils of arid and semi arid climates of the world (Ashraf et al., 2008). In Vietnam, the Mekong River Delta (MRD) and Red River Delta (RRD) areas where rice is the main cereal crop and soil salinity is a major constraint to the sustainability and expansion of rice cultivation. These regions will be affected by salinity heavily, which is one of most causes of serious reduction to rice production and economy of Vietnam. To ensure food security as well as maintain and develop the rice sector in the context of climate change, sea level rise and increasing populations are big challenges of the whole nation. Therefore, the great urgency task for rice breeders is to research and improve the rice varieties that can resist to adverse harsh conditions such as salt tolerance. Generally, most rice varieties are sensitive to salinity (Grover and Pental, 2003), however, some traditional *indica* rice varieties such as Pokkali, Nona Bokra and Kala-rata are fairly tolerant to salinity (Yeo et al., 1990). Salinity affects rice growth in varying degrees at all stages starting from germination through maturation (Mass and Hoffman, 1977). Rice is comparatively tolerant of salt stress during germination, active tillering, and at maturity, however, becomes salt sensitive during early seedling and reproductive stages (Lafitte et al., 2004). Tolerance for salinity is complex, involving a number of different physiological mechanisms, including sodium exclusion from roots, controlled sodium transport between roots and shoots, and sequestering of sodium in older tissues and in the vacuoles (Thomson et al., 2007). Although many genes likely contribute to salt tolerance, but a major QTL for salinity tolerance named *Saltol*, which responsible for seedling-stage salinity tolerance was identified on the short arm of chromosome 1 and explains more than 40% of the phenotypic variation (Bonilla et al., 2002). Reduced tillering is one of the main phenotypes observed under salinity stress during the vegetative stage that affects final yield (Hoshikawa, 1989). Molecular markers have become important tools for genetic analysis and crop improvement. DNA markers also permits plant breeders to correctly place desirable QTLs/genes into a molecular map. According to Jena and Mackill (2008), a comprehensive molecular genetic map of rice 1488 genes have been identified corresponding to several traits of economic importance. Several genes and quantitative traits loci (QTL) have been identified for abiotic stresses such as drought, salinity, submergence and cold. A major *saltol* QTL provided the opportunity to apply MABC to precisely introduce tolerance into popular salt-sensitive mega varieties. Thus, the improving of modern high yield rice varieties, which better

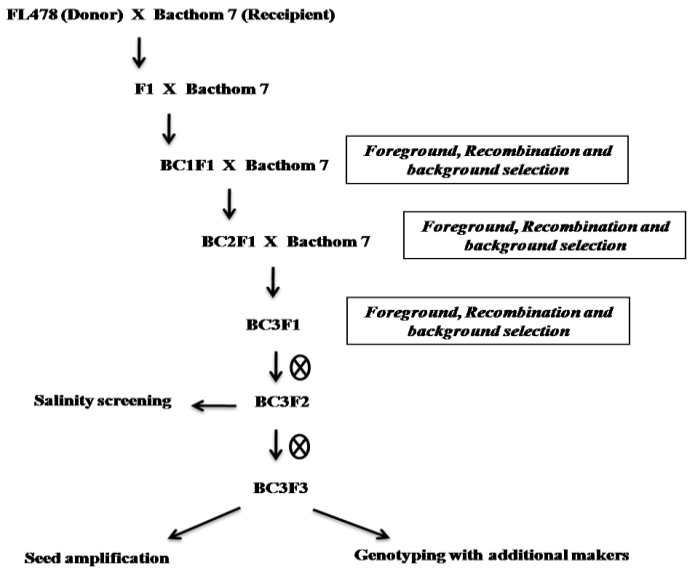

Fig 1. Marker-assisted backcrossing (MABC) scheme.

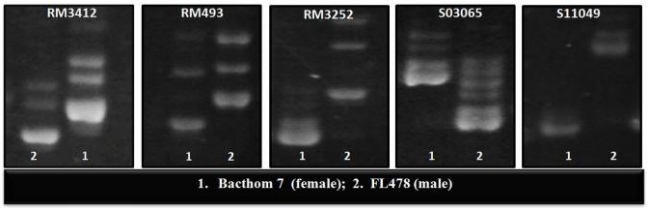

Fig 2. The polymorphic SSR markers between two parents (Bacthom 7 and FL478) were identified for MABC stratergy: PCR products on 6.0 % polyacrylamide gel generated by silver staining.

adaptability to saline areas and thus higher productivity under such ecologies plays an important role in rice breeding program at the present in Vietnam. There have been several solutions to cope with climate change and to reduce salinity damage in rice cultivation. It has been difficult to solve with conventional breeding methods because of the large environmental effects, time consuming and low heritability of salt tolerance (Gregorio et al., 2002). With recent advances in molecular biology, there are opportunities to address these problems using marker-aided selection techniques for salinity tolerant trait in rice. Ismail et al., (2007) conducted mapping study to identify QTLs associated with tolerance to salinity in rice and a major QTL (*Saltol*) was fine mapped and markers linked to the QTL were identified and are being used for their introgression into elite breeding lines and popular rice varieties using MABC. The main objective of this study was to develop a marker-assisted backcrossing (MABC) strategy as valuable tool for rapidly improving the salt tolerance of Vietnamese variety (Bacthom 7) within a two to three year timeframe. The new salt tolerance variety will be disseminated to farmers in coastal provinces of the Vietnamese Mega Deltas for reducing of agricultural losses caused by sea level rise.

Results and discussion

Parental DNA polymorphic survey

With the recent development in the field of molecular marker analysis, SSR markers have enormous potential to improve efficiency and precision of conventional plant breeding via MAS (Gregorio et al., 2010). In this study, a total of 368 SSR markers covering all 12 rice chromosomes were used to identify DNA polymorphic markers between two parents (Fig 2). The results showed that the 89 polymorphic SSR markers including 8 markers in *Saltol* locus and 81 markers in other loci were detected and the ratio of polymorphic markers on parental survey is approximately 24 %. The positions of polymorphic SSR markers between two parents were identified on each chromosome in rice (Fig 3). The results indicated that the genetic diversity at different locus on chromosome within parental materials. These polymorphic markers could be useful for MABC strategy in each backcross generation.

Marker-Assisted Selection details for BC line development

We have already applied MABC with three main advantages over conventional backcrossing. Firstly, DNA markers can be used for simple and efficient selection of the target locus ('foreground selection'). Secondly, the size of the donor chromosome segment containing the target locus can be minimized ('recombinant selection'). Thirdly, the recovery of the recurrent parent can be accelerated by selecting backcross lines with a higher proportion of recurrent parent genome ('background selection'). MABC is a precise and effective method to introgress a single locus controlling a trait of interest while retaining the essential characteristics of the recurrent parent (Collard and Mackill, 2008). It is known that MABC is effective for genes or QTLs with large variation in phenotype. In this study, we used the *Saltol* QTL to develop a MABC system to transfer the FL478 *Saltol* allele into Bacthom 7 rice cultivar.

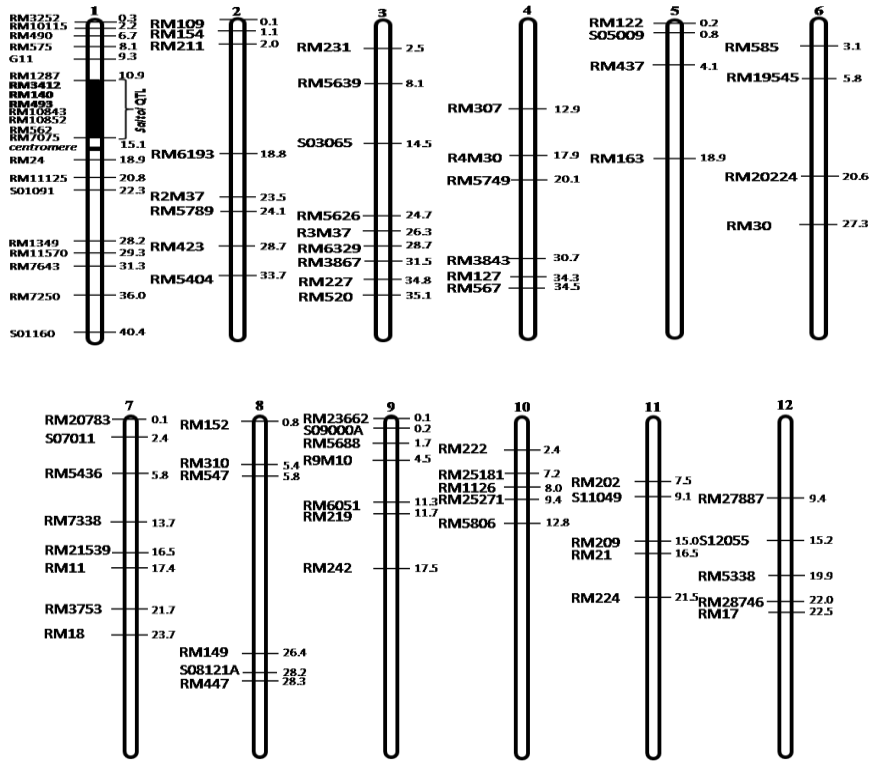

Fig 3. The positions of polymorphic markers on 12 chromosomes (Bacthom 7 and FL478).

The principal idea of MABC is to use widely grown and locally well adapted rice varieties from target countries as recipient parents for the introgression of tolerance QTLs. In each backcross generation, the 2-3 polymorphic markers within the *Saltol* QTL region in the range of 11.16-12.6 Mb on chromosome 1, which were used for foreground selection to determine the heterozygous plants (Xu et al., 2000; Thomson et al., 2010). In the previous study reported that 5 SSR markers RM1287, RM8094, RM3412, RM493 and RM140 were linked to the *Saltol* QTL on chromosome 1 (Niones, 2004). Our results verified that the polymorphic markers between two parents in target region of *Saltol* on chromosome 1 (RM3412, RM140, RM493), which are useful for forground selection of *Saltol* QTL. In addition, the important purpose of recombinant selection is to reduce the size of donor chromosome segment containing the *Saltol* QTL. We therefore used 5 polymorphic markers (RM1287, RM10843, RM10852, RM562 and RM7075) that flank a target *Saltol* QTL were used for recombination selection step (Fig 2). In this step, all heterozygous plants with *Saltol* locus that were obtained after screening of foreground selection, which were examined to identify the individuals with target *Saltol* QTL and recombination events. Furthermore, based on the polymorphism information of two parents, at least 4 polymorphic SSR markers per chromosome were used for screening genotype of background selection step. The full backcrossing scheme for Bacthom 7 + *Saltol* QTL was demonstrated in figure 4.

Genotyping of BC1F1 population (BT7/FL478//BT7)

Firstly, 2 SSR markers (RM3412, RM493) were used for foreground selection to get heterozygous plants from BC1F1 population. Then, a total of 80 SSRs polymorphic markers including the other markers that unlinked to the target QTL on chromosome 1 were used for recombinant (Fig 5a) and background selection to determine genotyping. The results obtained the best 8 BC1F1 plants that heterozygote at target loci and 71% -76% homologous for the recurrent markers at other loci in all 12 chromosomes. These plants were used to backcross with Bacthom 7 in order to develop the BC2F1 population for next step.

Genotyping of BC2F1 population (BT7/FL478//BT7//BT7)

Three polymorphic SSR markers (RM140, RM3412 and RM493) found in the *Saltol* locus in chromosome 1 were used for first step of foreground selection (Fig 5b). The results showed that the 264 heterozygous plants were selected from 852 plants of BC2F1 population. We next used 5 markers that flank to above and below of *Saltol* QTL region on chromosome 1 for recombinant (Fig 5b). According to results, 19 plants were identified for continuing background selection by using 76 polymorphic markers that covering in the remaining 11 chromosomes in rice genome. Finally, four BC2F1 plants were chosen for developing the BC3F1 population. In genome of these selected plants that conferred *Saltol* QTL region with 88.5% - 95.5% homozygous ratio for the recurrent parent.

Genotyping of BC3F1 (BT7/FL478//BT7//BT7//BT7)

Firstly, the 368 plants of BC3F1 population were characterized with two markers that linked very closely with *Saltol* QTL for foreground to get 114 heterozygous plants at *Saltol* loci. Secondly, recombinant selection was done using other 5 polymorphic markers that located on chromosome 1. Then, 19 plants were screened for background selection (Fig 5c) to recover the recipient genome. Finally, the results showed that two BC3F1 plants (#D30 and #D32) were selected after background selection with 1.44 Mb *Saltol* QTL

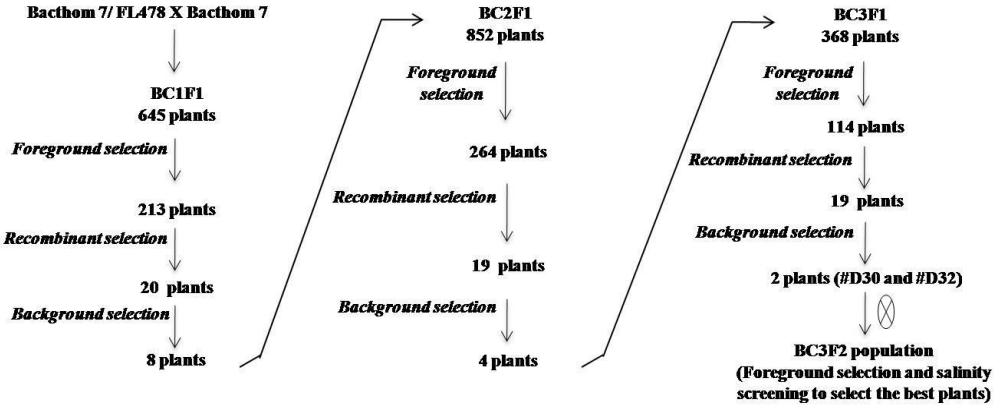

Fig 4. MABC scheme for development of Bacthom 7 + *Saltol* QTL.

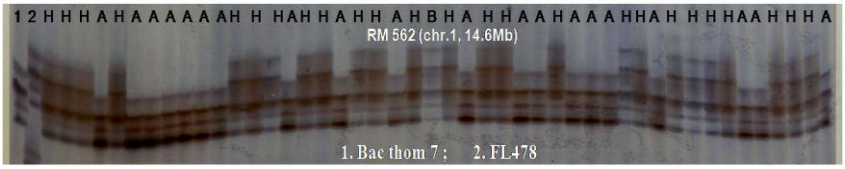

Fig 5a. Genotype scoring for markers linked to *Saltol* QTL on chromosome 1 for recombinant selection in BC1F1 population. P1: Bacthom 7; P2: FL478, A: Homozygous sensitive (Recombinant); B: Homozygous tolerant (Donor); H: Heterozygous.

region transferring successful in Bacthom 7. For plant #D30, chromosomes from number 2 to 12 were completely homologous for recurrent parent alleles. Overall, 100% of the genome of this plant was homozygous for the recurrent parent (Bacthom 7 variety). For the selected plant #D32, all the non-target *Saltol* QTL chromosomes were completely homozygous also. The overall, recurrent parent genome percentage of this BC3F1 plant was 96.8% based on the tested markers. These homozygous plants were allowed to self again to generate BC3F2 and BC3F3 for developing the salinity tolerant varieties. Salinity affects almost all plant processes because of effects on soil properties, the osmotic effects of high ionic concentrations and toxic effects within the plant tissue (Yeo and Flowers 1989). Rice plants are highly sensitive to salinity during the early seedling stage (Pearson and Bernstein, 1959; Flowers and Yeo, 1981). Two donors FL478 at IRRI and FL378 at Dhaka University for the MABC transfer of different *Saltol* alleles into popular varieties to test the efficacy of these alleles to provide seedling stage tolerance. Since MABC can transfer a QTL with minimal linkage drag, it is best to choose recurrent parents that have already been accepted by farmers, such as the mega-varieties that are cultivated on over 1 million hectares (Thomson et al., 2010). The application of advanced molecular technique to be used in this study is MABC. 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 (Septiningsih et al., 2009, Thomson et al., 2010; Singh et al., 2010). The results showed that the recover of the recipient parent genome was greatly increased of using markers to assist selection in each backcross generation. This study has indicated that *Saltol* QTL was introgressed in genetic background of Bacthom 7 cultivar. The background analysis in the introgression line revealed the recovery up to 96.8% - 100 % of recurrent parent alleles based on the screened markers after 3 generations. Salinity is particularly a major problem in coastal regions in the tropics. IRRI has long been developing and using modern biotechnology tools in breeding modern varieties. The *Saltol* QTL was identified at IRRI and a MABC system was developed and successfully used for their introgression into numerous modern rice varieties, some of them have already been released in Philippines, India, Bangladesh, Nepal an Indonesia through networks involving farmers. This technology is now transferred and institutionalized in Vietnam. The development of the Bacthom 7-*Saltol* variety is still a work in progress. The tolerant level of promising introgresion lines needs to be confirmed under greenhouse and field conditions. This is in part of the first project in Vietnam on rice breeding specifically designed to cope with climate change effects. Additionally, the recent success in the development of salt-tolerant rice variety has demonstrated the MABC on food security for poor farmers. Thus, the improved introgression lines by using MABC aproach can be directly developed into salinity varieties, which will have an impact on the yield stability in Bacthom 7-producing target areas in Vietnam.

Materials and methods

Plant materials and marker suveys used for Saltol genotyping

The experiments were done at Agricultural Genetics Institute (AGI) and Cuu Long Rice Research Institute (CLRRI) in the early year 2010 up to 2012. FL478 and Bacthom 7 rice cultivars were used as the parents in this study. FL478 is one of the cultivars identify from the recombinant inbred line (RIL) population carrying major *Saltol* QTL was used as the donor, whereas Bacthom 7, a popular aromatic cultivar with good cooking and eating quality in Vietnam was used as the recipient. Introgression lines were developed through backcross breeding using MABC strategy (Fig 1). Marker surveys were first conducted to identify polymorphic markers between the parents and 368 SSR markers that covered all 12 rice chromosomes from the available rice SSR markers (IRGSP 2005) were screened. These polymorphic markers were used for genotyping with three general levels of MABC approach. In the first level, the markers which are tightly linked to *Saltol* QTL were used for foreground selection. The second level, the polymorphic markers that flank a *Saltol* QTL (less than 5 cM on either side) were used for

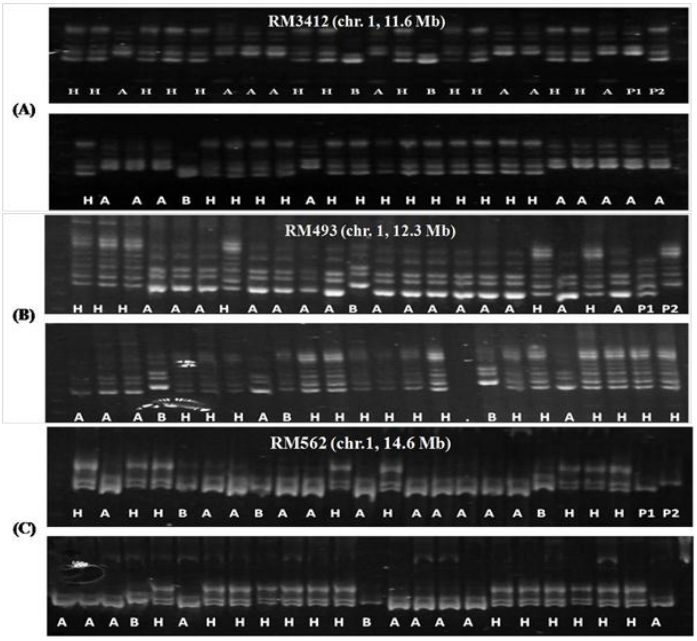

Fig 5b. Genotype scoring for markers linked to *Saltol* QTL on chromosome 1 for foreground and recombinant selection in BC2F1 population (BT7/FL478//BT7): (A) RM3412 and (B) RM493 for forground selection; (C) RM562 for recombinant selection. P1: Bacthom 7; P2: FL478; A: Homozygous sensitive (Recombinant); B: Homozygous tolerant (Donor); H: Heterozygous.

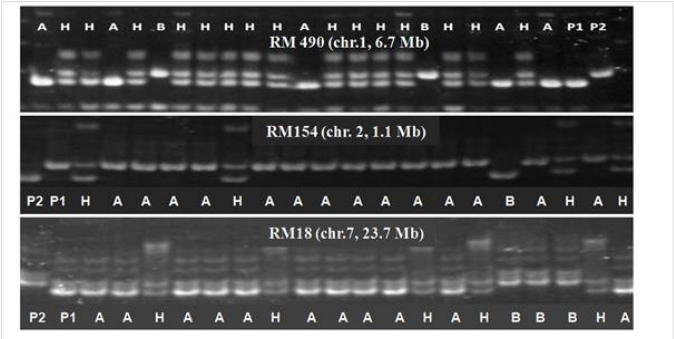

Fig 5c. Genotype scoring for markers unlinked to *Saltol* QTL for background selection in BC3F1 population. P1: Bacthom 7; P2: FL478; A: Homozygous sensitive (Recombinant); B: Homozygous tolerant (Donor); H: Heterozygous.

recombinant selection. The third level of MABC, the SSR markers that are unlinked to the *Saltol* QTL on all 12 rice chromosomes were used to hasten the background selection to recover the recipient genome. The F1 plants were obtained from cross between two parents and identified by using some polymorphic markers that in the target *Saltol* QTL. The F1 hybrid was then backcrossed with recurrent parent to produce a first backcross generation (BC1). The second and third rounds of backcrossing (BC2 and BC3) were derived from the cross of selected tolerance BC1 and BC2 plants based on linked markers. A large amount of backcross plants in each generation was produced for MABC breeding strategy to obtain the individuals that conferring *Saltol* QTL with a highest percent of Bacthom 7 genome.

Methods

Seedling tissues were collected and frozen with liquid nitrogen. Genomic DNA samples were extracted following CTAB method as described by Zheng et al., (1995). Agarose gel electrophoresis was used to estimate DNA concentration, and each sample was then dilluted to approximately 5-10 ng/ µl. Polymerase chain reaction (PCR) was carried out in a final volume of 15 µl per reaction which consist of 2 µl DNA template, 1.5 µl PCR buffer, 1 µl 2 mM of dNTPs, 0.5 µl of each the forward and reverse primers, 8.5 µl Q-water and 1 µl Tag polymerase (Fermentas, California, USA). A PCR mixture was run using the Eppendoft thermocycler (Mastercycler Pro S, Germany) with 96-well plates. The PCR profile starts with 94°C for 5 min, followed by 35 cycles each of denaturation at 94^{o}C for 1 min, annealing at 55^{o}C for 1 min, extention at 72^{o}C for 2 min, and then a final extention step at 72^{o}C for 5 min. The amplified PCR products were mixed with bromophenol blue dye and were then resolved by using the 6% polyacrylamide gels at 100 volts (Dual Trip-Wide Mini-Vertical System, C.B.S. Scientific, CA, USA), followed by SYBR-Safe staining (Invitrogen), gel document (Alpha Innotech). Marker genotype data were obtained by running SSR markers and the individual bands were scored for further analysis based on the parent's band.

Statistical analysis

The molecular weights of the different alleles were measured using Alpha Ease Fc 5.0 software. All the genotypes were scored for the presence and absence of the SSR bands. For each marker, allelic bands were scored based on the tolerant and sensitive parental bands of the amplified products and were designated as 'A', 'B', 'H' for homozygous sensitive, homozygous tolerant and heterozygote, respectively. The excel file containing these data was imported into excel data exchange of GGT 2.0 software program for further analysis. The marker data were analyzed using graphical genotype (GGT 2.0) software package (Van Berloo, 2008). The percentage of homozygous markers for recipient parent (A%) and the percentage of recipient alleles conferring heterozygous at target loci (R%) were calculated.

Conclusions

Rice variety with tolerance to salinity stress can adapt better to areas which are prone to abiotic stresses, especially during the wet season in the coastal saline areas. Marker-assisted backcrossing (MABC) developed in this study was useful for improvement of salinity tolerance in rice. It is also suggested that using MABC should be done not only for the target segment but also for the background selection. The recovery of the recipient Bacthom 7 genome was greatly accelerated emphasizing the increased efficiency of using markers to assisted selection of backcross lines. The generated backcross introgression lines of Bacthom 7 (#D30 and #D32 plants) will be assessed for phenotypic performance under salinity stress to fully evaluate the success in transferring *Saltol* QTL using MABC. Salt stress tolerant variety will deliver to farmers for enhancing the resilience of agriculture even with the anticipated salt stress problems as the results of climate change. The application of molecular genetics in breeding programs is bounded by precision of the effects of associated markers as well as by the cost effectiveness of marker-assisted selection.

Acknowledgements

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References

Ashraf M, Athar RH, Harris CJP, Kwon RT (2008) Some prospective strategies for improving crop salt tolerance. Adv Agron 97: 45-110.

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 Agric Sci 85: 68-76.

Collard BC, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos Trans R Soc Lond B Biol Sci 363: 557-572.

Flowers TJ, Yeo AR (1981) Variability in the resistance of sodium chloride salinity within rice (*Oryza sativa* L.) varieties. New Phytol 88: 363-373.

Greenland DJ (1984) Exploited plants: rice. Biologist 31(4): 219-225.

Gregorio GB, Senadhira D, Mendoza RD, Manigbas NL, Roxas JP, Guerta CQ (2002) Progress in breeding for salinity tolerance and associated abiotic stresses in rice. Field Crops Res 76: 91-101.

Gregorio GB, Aliyu R, Adamu AK, Muazu S, Alonge SO (2010) Tagging and validation of SSR markers to salinity tolerance QTLs in rice (*Oryza spp*). International conference on biology, environment and chemistry IPCBEE vol.1, IACSIT Press, Singapore, pp: 328-332.

Grover A, Pental D (2003) Breeding objectives and requirements for producing transgenic for the major field crops of India. Curr Sci 84: 310-320.

Hoshikawa K (1989) The Growing Rice Plant: An Anatomical Monograph, edn. l, Nosan Gyoson Bunka, Kyokai (Nobunkyo), Tokyo, Japan.

International Rice Genome Sequencing Project (IRGSP) (2005) The map-based sequence of the rice genome. Nature. 436: 793-800.

Ismail AM, Heuer S, Thomson MJ, Wissuwa M (2007) Genetic and genomic approaches to develop rice germplasm for problem soils. Plant Mol Biol 65: 547-570.

Jena KK, Mackill DJ (2008) Molecular markers and their use in marker-assisted selection in rice. Crop Sci 48: 1266-1276.

Niones JM (2004) Fine mapping of the salinity tolerance gene on chromosome 1 of rice (*Oryza sativa* L.) using near-isogenic lines. MS dissertation. Laguna: University of the Philippines Los Baños.

Lafitte HR, Ismail AM, Bennett J (2004) Abiotic stress tolerance in rice for Asia: progress and the future. "New directions for a diverse planet" Proceeding of 4th International crop Science Congress, 26 Sep. - 1 Oct., 2004, Brisbane, Australia. Published on CDROM. Website www. Cropscience.org.au.

Maas EV, Hoffman GJ (1977) Crop salt tolerance-current assessment. J Irrig Drain Div ASCE 103: 115-134.

Pearson GA, Bernstein L (1959) Salinity effects at several growth stages of rice. Agron J 51: 654-657.

Shannon MC, Rhoades JD, Draper JH, Scardaci SC, Spyres MD (1998) Assessment of salt tolerance in rice cultivars in response to salinity problems in California. Crop Sci 38: 394-398.

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, pp: 387-415.

Thomson MJ, De Ocampo M, Egdane J, Katimbang M, Rahman MA, Singh RK, Gregorio GB, Ismail AM (2007) QTL mapping and marker-assisted backcrossing for improved salinity tolerance in rice. In: Proceedings of BioAsia 2007: 6th Asian Crop Science Association Conference and 2nd International conference on rice for the future, Bangkok, Thailand, 5-9 November 2007, pp: 6-12.

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

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, pp: 451-469.

Van Berloo R (2008) GGT 2.0: versatile software for visualization and analysis of genetic data. J Hered 99: 232-236.

Xu K, Xu X, Ronald PC, Mackill DJ (2000) A high-resolution linkage map of the vicinity of the rice submergence tolerance locus *Sub1*. Mol Gen Genet 263: 681-689.

Zheng K, Subudhi PK, Domingo J, Magpantay G, Huang N (1995) Rapid DNA isolation for marker assisted selection in rice breeding. Rice Genetics Newsletter 12: 255-258.

Yeo AR, Flowers TJ (1989) Selection for physiological characters – examples from breeding for salt tolerance. In: Jones HG, Flowers TJ, Jones MB (Eds.), plants under stress biochemistry, physiology and ecology and their application to plant improvement. Cambridge University Press, Cambridge, pp: 217-234.

Yeo AR, Yeo ME, Flowers SA, Flowers TJ (1990) Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. Theor Appl Genet 79 (3): 377-384.