1. APPLICATION OF MARKER ASSISTED BACKROSSING IN RICE BREEDING TO COPE WITH CLIMATE CHANGE IN VIETNAM

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ABSTRACT

Our findings contribute to enhancing and sustaining future livelihoods and food security of smallholder farmers in Vietnam vis-a-vis climate change. FL478 was used as the donor of Saltol.

IR64*Sub1* was used as the donor of *Sub1*. The recipient varieties were AS996 and Khang dan mutant, which are widely grown cultivars in the South and North of Vietnam. The best BC3F1 plant was P284-112-209 with all the recipient alleles and *Saltol* region. Four plants P307-305-21, P284-112-195, P284-112-198, P284-112-213 were ranked second with only one loci heterozygous (63 markers on 12 chromosomes). These five plants were chosen as the breeding lines for *Saltol*-AS996 introgression. After three generations of backcrossing, application of marker assisted backcrossing (MABC) resulted in the best BC3F1 individual P422-14-177 with 100% of recipient alleles; based on 52 markers the introgression size of *Sub1* was 0.3Mb between ART5 and SC3. Phenotyping was carried out on BC3F1 and BC2F2 of the selected lines. The survival ratios of these selected lines compare to FL478 and IR64*Sub1* were the same. We were able tosuccessfully introgress *Saltol* and *Sub1* into the AS996 variety. The breeding lines BC4F1, which having a 100% genetic background of the donor variety, are ready for the development of new salinity tolerance and submergence tolerance varieties to assist rice farmers to adapt to climate change. These findings support the use of MABC as an effective method in rice breeding.

Keywords: Climate change, MABC, rice breeding, salinity tolerance, submergence tolerance.

I. INTRODUCTION

Vietnam is among the five countries hardest hit by climate change; the frequency of natural disasters including typhoons and flooding are increasing annually. Submergence caused by flash-flooding is one of the most serious impacts to the agricultural productions of the country. In Vietnam, rice is the number one agricultural product. Globally, Vietnam is the second largest exporter of rice. Nationally, rice accounts for 90% of national food production. Of the 4.5 million ha of rice grown annually, more than one million ha are affected by flooding for 1-2 weeks. Depending on the timing of a flood with respect to the growth stage of rice, shallow flash floods can result in less than 10% production loss, whereas deeper floods and stagnant water for two weeks at >100cm depth, can cause damage ranging from 40% to 77% (Manzanilla et al., 2011). Developing new rice varieties to assist farmers to cope with climate change and sea level rise for the Red River Delta and Mekong River Delta is crucial to Vietnam's economy and food security. Increased rice production in Vietnam also contributes to global food security.

Breakthroughs in salinity and submergence tolerance breeding became feasible after the identification of major chromosomal regions (Quantitative trait loci, QTLs) associated with salinity (*Saltol*) and submergence (*Sub1*) stresses, and the development and use of a marker system for

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their speedy incorporation into modern high yielding and popular varieties through marker assisted backcrossing (MABC) (Thomson et al., 2010). The basis of the 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. The MABC breeding strategy in this study was targeted at introgressing the *Saltol* and *Sub1* QTLs into Vietnamese varieties while still maintaining the background of the varieties. These varieties will be grown in the coastal area of Vietnam to reduce the affects of climate change.

II. MATERIALS AND METHODS

The rice variety FL478 was used as the donor of *Saltol*. IR64Sub1 was the donor of *Sub1*. The recipient varieties were AS996 and the Khang dan mutant, which are widely grown cultivars in the South and North of Vietnam.

Genotype data were obtained by analysing DNA with SSR markers. The PCR products were analyzed by electrophoresis on 4.5% acrylamide gel followed by silver staining steps and scoring; or electrophoresis on 6% -8% acrylamide gels followed by SYBR-Safe staining (Invitrogen).

2.1. Data analysis

The marker data were 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 percent markers homozygous for recipient parent (%A) and the percent recipient alleles including heterozygous plants (%R) were calculated.

2.2. Evaluation of salinity and submergence tolerance

Entries were scored based on visual markers using IRRI's Standard Evaluating Score (SES) for rice, with ratings from 1 (highly tolerant) to 9 (highly sensitive). Submergence screening was performed in the greenhouse following standard protocols of Pamplona et al. (2007).

III. RESULTS AND DISCUSSION

Saltol, a major QTL associated with the Na-K ratio and seedling-stage salinity tolerance, was identified on chromosome 1. In a hydroponic screen test at the seedling stage this QTL explained 43% of the variation for the seedling shoot Na-K ratio in the population of rice plants.

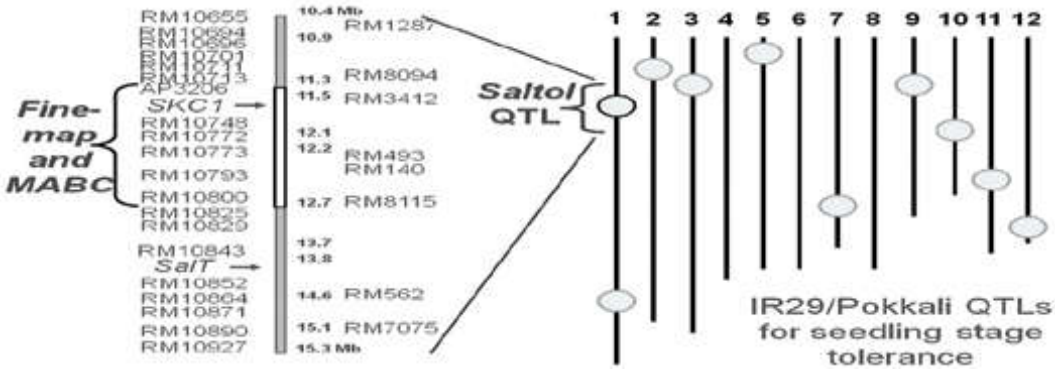

Figure 1: Fine-map and MABC strategy of Saltol QTL

An analysis of single feature polymorphisms 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 (Thomson et al., 2010). 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.

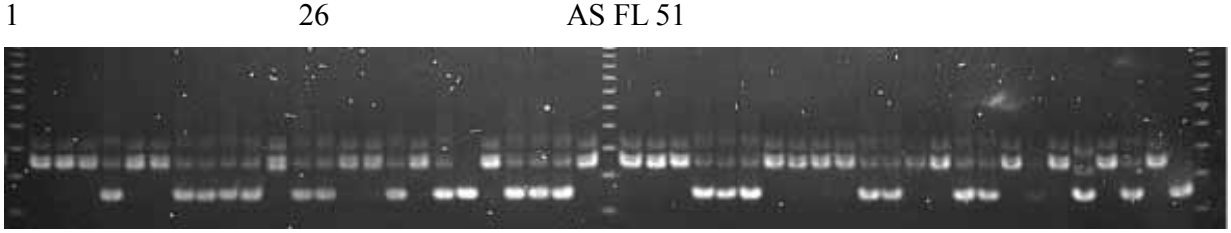

Figure 2: Screening individuals on crossed BC1F1 (AS996/FL478) using primer RM310.
Wells 1, 26, 51: 25bp marker, 2-25 and 27-48: BC1F1 individuals, Well 49:AS996, Well 50: FL478

Genotyping was carried out on 3 backcross generations. Plant P284-112-209 was the best BC3F1 individual with all the recipient alleles screened based on a total of 63 markers. The four plants P307-305- 21, P284-112-195, P284-112-198, P284-112-213 ranked second with only one loci heterozygous. All of these 5 plants were chosen as the breeding lines for *Saltol*-AS996 introgression.

For submergence tolerance, *Sub1* is a major QTL on chromosome 9 explaining almost 70% of the phenotypic variance (Xu et al. 2006).

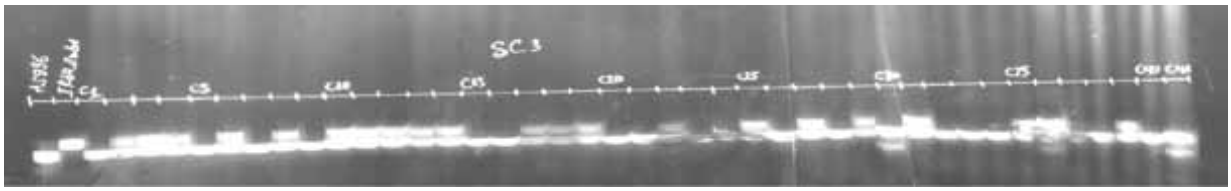

Figure 3: BC2F1 (AS996xIR64Sub1) individuals screening using primer SC3
1. AS996, 2. IR64Sub1, C1-C41: BC2F1 individuals

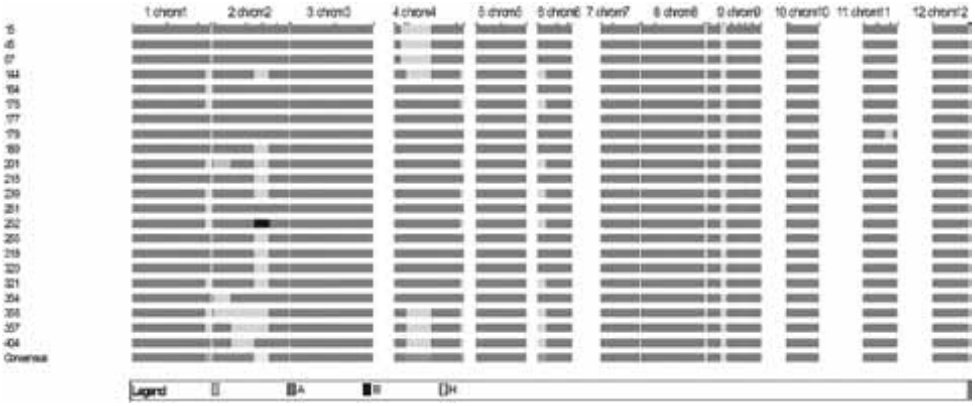

Figure 4: Data on 22 recombinant individuals of BC3F1 (AS996xIR64SUB1) were analysed using GGT3.2 . Individuals BC3F1 number 177 was the best candidate.

After three generations of backcrossing, application of MABC resulted in 93.75% recipient alleles in both plant numbers P422-11 and P422-14 in BC2F1. The best BC3F1 individual was P422-14-177 with 100% of recipient alleles based on 52 markers. The introgression size of *Sub1* was 0.3Mb between ART5 and SC3 position. The breeding line BC4F1 had 100% genetic background of the donor variety. This variety is ready for developing the new submergence tolerant variety ASS996-*Sub1* to assist farmers to cope with climate change.

The BC2F2 and BC3F1 seeds were screening to evaluate the introgression of *Saltol* fragment into AS996. Salt stress was imposed 14 days after germination by adding NaCl to an EC of 12 dS m−1 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, all the BC2F2 of the selected plants, P284-112, P307-305 and P307-322, had the same score as the tolerant checks. Therefore he homozygous *Saltol* fragment performed well in the BC2F2 generation.

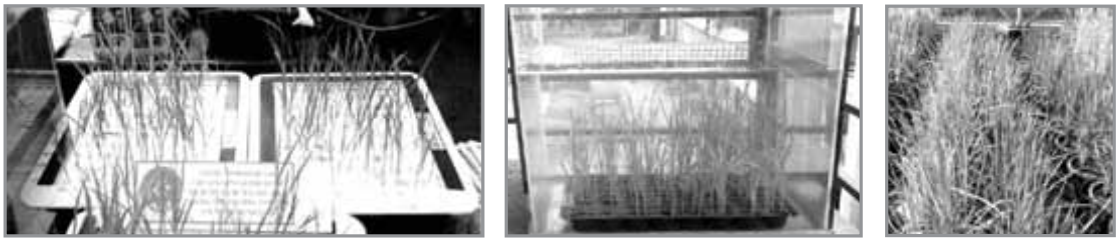

Photo 1 Photo 2 Photo 3

Figure 5. Evaluating the salinity and submergence tolerance of BC2F2 and BC3F1 lines (Photo 1, 2) and their recovering ability after stressed (Photo 3)

The selected BC2F2 and BC3F1 lines showed submergence tolerance just after de-submergence and for 21 days after recovery against the submergence stress. The submergence tolerance scores of these BC2F2 lines were similar to those of the tolerant donor IR64*Sub1*. This confirmed the successful introgression of *Sub1* QTL in these lines.

III. CONCLUSION

The MABC approach described in the present study was successfully adopted to introgress *Saltol* and *Sub1* into AS996 and the Khang dan mutant, and could be successfully used to introgress other important varieties with a minimum introgression segment and within a short timeframe. 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 and provide flexibility to smallholder farmers to adapt to the consequences of climate change.

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2. APPLICATION OF MOLECULAR MARKER IN AROMATIC RICE BREEDING

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ABSTRACT

Application of DNA molecular marker in aromatic rice breeding has been carried out since 2007 at Field Crop Research Institute (FCRI). The results showed that the ASA (Allen Specific Amplification) marker BADH2 including four primers EAP; ESP; IFAP and INSP can be selected for identifying *fgr* gene controlling fragrant flavour of rice in rice materials with high confidence. Application of this marker in aromatic rice selection, in the total of 800 rice individuals of F2-F3 generation was examined for *fgr* gene, 250 of them carried *fgr* gene and 109 of which carried homogeneities of this gene. The lines holding homogenous *fgr* gene have been developed and selected for phenotype evaluation of aroma and further characteristics such as the quality, yield, and biotic, also abiotic stress tolerances. One of them, named HDT8, containing aroma and other good characteristics has been selected to be put in the system of National trials, also in ecological trails in Northern region of Vietnam since 2010. The HDT8 rice variety, highly appreciated for the quality and yield, has been released into production in the Northern region of Vietnam since the summer season of 2011.

Keywords: DNA molecular marker, frg gene, marker assisted selection, fragrance, aroma.

I. INTRODUCTION

In Vietnam, the demand for high quality rice is now growing. Because the domestic production does not meet the demand, so the food companies still have to import high quality rice from

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