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Research Article

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Improving the Quality of the Salt-Tolerant Rice Lines by Molecular Breeding

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Abstract Salinity is a key abiotic constraint devastating crop production in the world. The developed salt tolerance rice varieties have been recently released and grown in the salt-affected deltas in Vietnam. However, the quality of these varieties is often poor quality due to the adverse influences of soil salinity and alkalinity. The objective of this study was to improve the quality of salt-tolerant rice varieties using the waxy and BAD2 markers. The F2 to F4 populations were examined the target genes of amylose and aromatic contents by marker-assisted selection (MAS). Some potential plants of F4 population with salt tolerance and high quality (waxy, aroma) were successfully improved and developed.

Keywords Waxy, amylose content, aroma, marker-assisted selection, rice

Introduction

Rice (*Oryza sativa* L.) is the most cash crop in Vietnam, providing daily food for over 90 million people in this country. Presently, Vietnam is the second largest rice exporter in the world, which was accounting for 50% of the world rice trade [1]. However, Vietnam is one of the countries most affected by climate change, as a consequence, rice production is particularly vulnerable. Most acute rice growing areas in close vicinity of the sea are vulnerable to salinity. Salt intrusion due to sea level rise has caused adverse effects on 1 million cultivated hectares equally with 3% of total areas of this country, causing the economic loss by salt intrusion in 2005 was up to 45 million USD [2]. To date, approximately 600.000 ha of rice growing areas are severely affected by drought and saline intrusion in the early year of 2016, has caused economic losses up to 15 trillion VND in rice only (≈670 million USD) and reduced rice harvest by up to 50% [1].

Salinity tolerance of rice derives from genes that limit the rate of salt uptake from the soil and the transport of salt across the plant, adjust the ionic and osmotic balance of cells in roots and shoots, and regulate leaf development and the onset of senescence [3]. Salinity is a key abiotic constraint devastating crop production in the world. One-fifth of irrigating arable lands in the world has been reported to be adversely affected by high soil salinity. Saline soils occur naturally in both coastal areas, where groundwateris contaminated by sea water rise, and in areas subjected to irrigation and/or draining. Salt stress is one of the major serious factors limiting the productivity of rice crop in many worldwide areas. According to the report of FAO [4] over 800 million ha of worldwide land are severely salt affected and approximately 20% of irrigated areas. In Asia, 21.5 million hectares of land areas are being influenced by salinity and estimated to cause the loss of up to 50% fertile land by the 21st midcentury.

The flavour or aroma of rice varieties is relatedto the presence of 2-acetyl-1-pyrroline (BAD2). A recessivegene (*fgr*) on chromosome 8 of rice was identified and linked to the aromatic traits [5]. Waxy gene (waxy, Wx) is an important gene coding amylose synthase that was previously cloned in 1990 [6]. This gene is responsible for encoding starch granule-bound starch synthesis to control the synthesis of amylose. Some studies reported that the level of amylose content in the different rice cultivars was recorded by the splicing efficiency of the first intron in Wx gene [6,7].

In some recent years, some salt tolerance rice varieties have been developed by traditional method and molecular breeding in Vietnam. These varieties have been released to the farmers, and being grown in the salt-affected coastal deltas of Vietnam [1, 2, 8]. However, Salt tolerance rice varieties are known as poor quality due to the adverse influences of soil salinity and alkalinity [9]. Therefore, the objective of this study was to improve rice quality of the salt tolerance rice variety.

Materials and Methods

Plant Materials and Cross Scheme

A high-quality rice variety carrying the waxy and BAD2 was used as the donor plant. While the salt tolerance rice variety was used as the recipient material. Both donor and recipient materials were kindly provided by Hung Yen University of Technology and Education in 2016. The scheme of the crosswas summarized in Figure 1. In brief, the donor and recipient plants were crossed to develop F1, then F2 and F3. The F3 population were grown in experimental farm to evaluate some agronomic trait as well as evaluate the quality of the potential lines

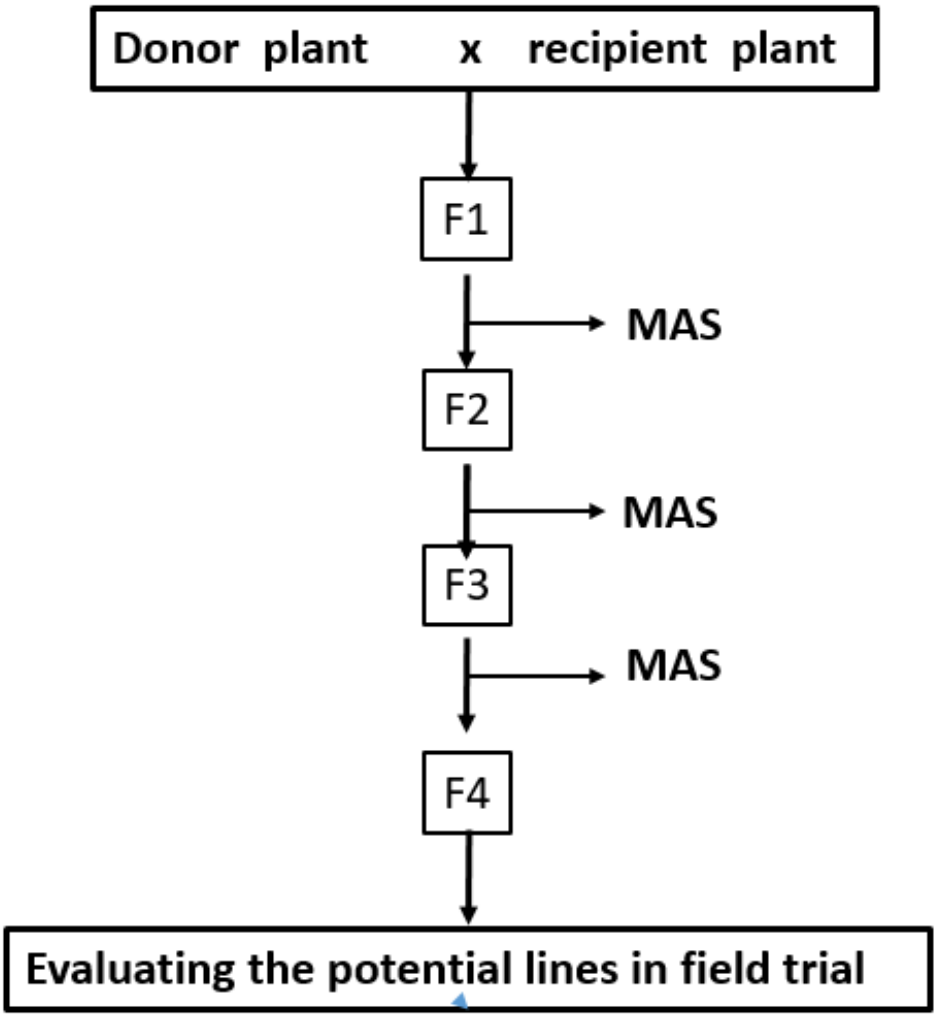

Figure 1: The scheme of applying MAS to improve the quality of salt tolerant rice variety

Molecular Makers and Analyses

In this study, two markers were used the Waxy and BAD2. The information in details as shown in Table 1.

Table 1: The information of primers used in this study

Trait linkage	Primer name	Primer sequences
Amylose content	Waxy	WxF: AGAGGGGGAGAGAGAGAaCG
		WxT: CAGGAAGAACATCTGCgAGT
		WxR: CCTAACCAAACATAACGAACG
Aroma	BAD2	IFAP: ATAGGAGCAGCTGAAATATATACC
		INSP: TGGTAAAAAGATTATGGCTTCA
		EAP: AGTGCTTTACAAAGTCCCGC

DNA was extracted from juvenile leaves of 2-week-old plants using a modified protocol as described by Zheng et al [9]. PCR was performed in 10µL reactions containing 5–25ng of DNA template, 1µL 10X TB buffer (containing 200mM Tris-HCl pH 8.3, 500mM KCl, 15mM $MgCl_2$), 1µLof1mM dNTP, 0.50µL each of 5µM forward and reverse primers, and 0.25µL of Taq DNA polymerase (4U/µL) using an MJ Research single or dual 96-well thermal cycler. After initial denaturation for 5 min at 94 ◦C, each cycle comprised 1 min denaturation at 94◦ C, 1min annealing at 55◦C, and 2min extension at 72◦C with a final extension for 5min at 72◦C at the end of 35 cycles.

The PCR products were mixed with bromophenol blue gel loading dye and were analyzedby electrophoresis on 8% polyacrylamide gel using mini vertical polyacrylamide gels for high throughput manual genotyping (CBS Scientific Co. Inc., CA, USA). The gels were stained in 0.5mg/mL ethidium bromide and were documented using Alpha Imager 1220 (Alpha Innotech, CA, USA). Microsatellite or simple sequence repeat (SSR) markers was used for selection.

Results and Discussion

Evaluation of amylose content of F2 plants

In this study, the waxy marker was used to detect the individual plants of the F2 population. A total of fifty individual plants F2 were screened. The results showed that 12/50 were carrying the waxy gene including the number 1, 11, 23, 25, 29, 30, 32, 48, 49, 50, 51, and 52, which indicated two bands at the position of 425bp and 228bp as these results were agreed with the previous report of Gao et al [7]. Similarly, the F3 populations, 11 individual plants were identified amylose content ≤15.6% based on the electrophoresis results obtaining two bands at the position 425bp and 228bp (Figure 2)

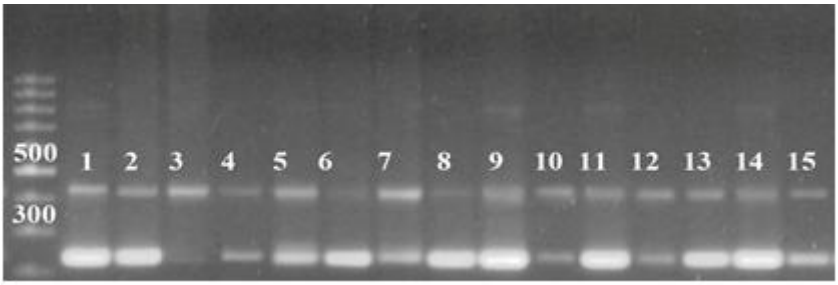

Figure 2: Electrophoresis of the F3 individual plants by use waxy primers
1-15: individual plants of F3, L: Marker 100 bp

In the selfing F4 population, we have detected almost samples carrying two bands at 425 and 228 bp except for band number 2 as presented in Figure 3These results were similar with the previous report of Gao et al [7].

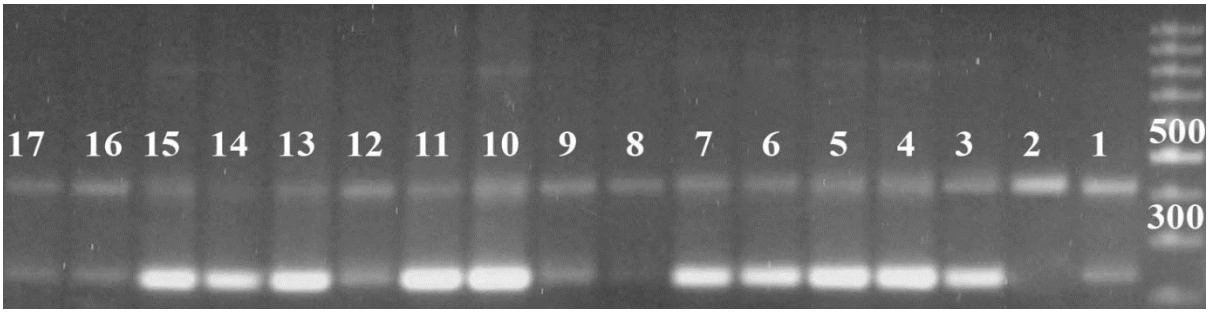

Figure 3: Electrophoresis of the F4 individual plants by use waxy primers
1-17: individual plants of F4 population; last band on the right: marker (100bp)

Evaluation of the aromatic content of F2, F3 and F4 populations

We have also evaluated the aromatic content of the F2 plants by use BAD2 primer. As the previous report of Brabury et al [5] the rice plant is non-aromatic if it is obtained the DNA bands at the position of 585bp, 385bp and 257 bp or two bands at 585bp and 385, respectively. However, the aromatic contents are identified at the position of DNA bands 585bp and 257bp.

In this study, we have found that the individual plants number: 5, 6, 7, 8, 9, 10, 12, 17, 20, 21, 22, 23, 24, 29, 34, 35, 40, 41, 42, 43, 44, 45, 46, 47, 51, 52, which carried the aromatic gene as shown in Figure 4. In fact, the BAD2 appears not to be responsible for aroma in all aromatic rice varieties, since this gene is not controlled for a dominant aroma traits of aroma mutant [11], indicating that in the most rice aroma varieties, is controlled by a single recessive gene mapped to the chromosome 8 of rice [12].

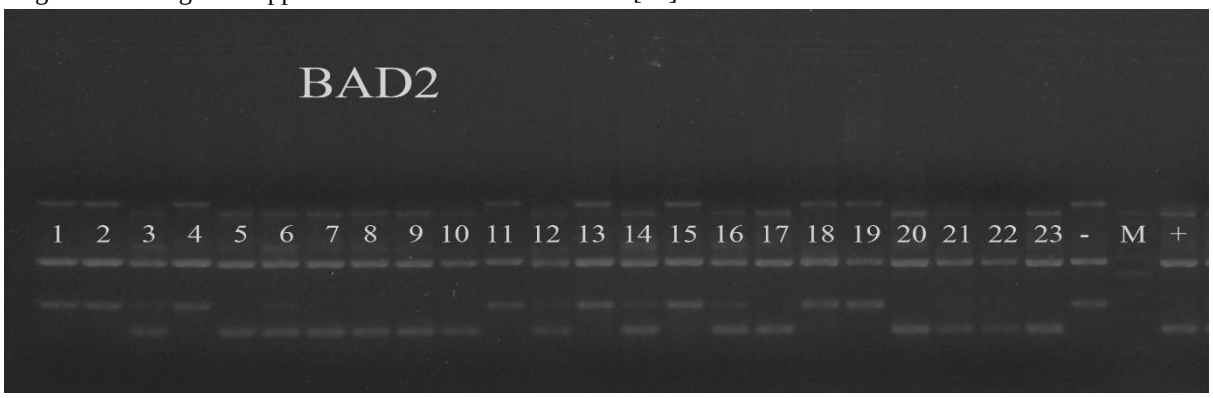

Figure 4: Electrophoresis of the F2 individual plants by use BAD2 primers
1- 23(F_2); + (control +); - (control -); L (marker 100bp)

In F3 population, the BAD2 was also used to identify the F3 individual plants. Eleven plants had two DNA bands at 585bp and 257bp as shown in Figure 5.

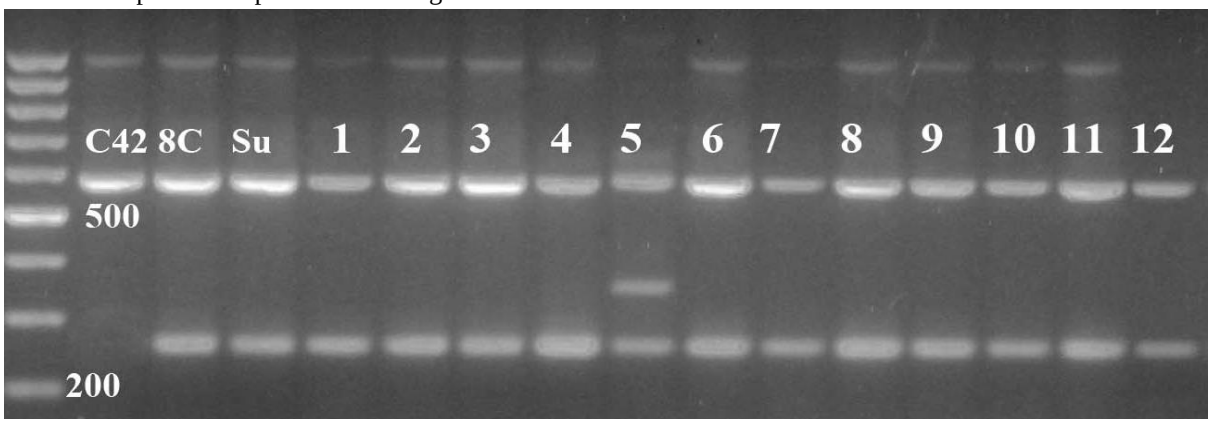

Figure 5: Electrophoresis of the F3 individual plants by use BAD2 primer
First band on the left: marker 100b; C42, 8C, Su, and number 1-12: individual plants of F3 population

In F3 populations, we have similarly screened the F3 individual plants. The results have shown that the total of 40 individual plants carrying both amylose contents (amylose ≤15.6%) and aromatic gene was identified. The potential lines are being grown in the field to further evaluate the important agronomic traits.

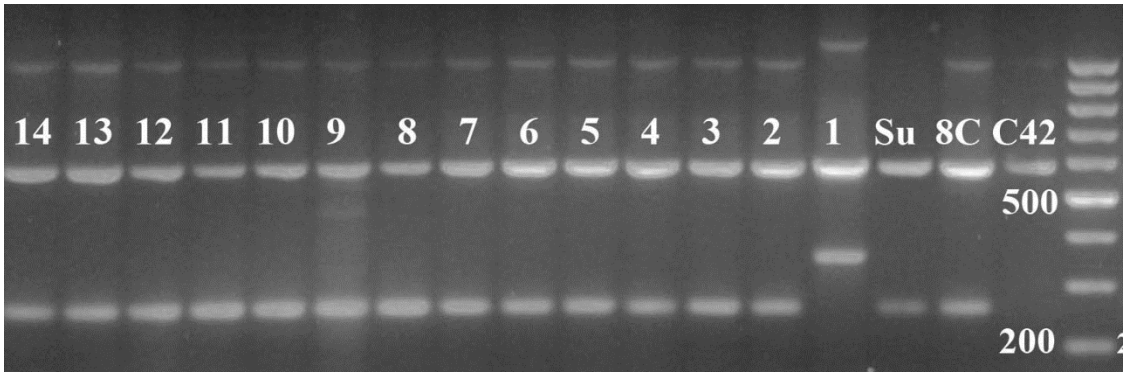

Figure 6: Electrophoresis of the F4 individual plants by use BAD2 primer

First band on the right: marker 100b; C42, 8C, Su, and number 1-14: individual plants of F4 population

In the F4 population, sample number 1 (band number 1) including 10 individual plants carrying the BAD2 gene at the 585bp và 257bp position. Other samples were not found to possess BAD2 because, the DNA bands were at 585bp, 385bp and 257 bp, and it showed two bands at 585bp and 385, was non-aromatic property (Figure 6)

In conclusion, we have successfully improved the quality of salt tolerance rice lines by introgressing the waxy and BAD2 genes by use of marker-assisted selection methods.

Acknowledgements

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