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APPLICATION OF MARKER ASSISTED BACKCROSSING TO SELECT THE INDIVIDUAL PLANTS OF BC_3F_1 POPULATION CARRYING THE QTL/GENE TO IMPROVE YIELD OF KD18

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Abstract:

Rice (Oryza savita L.) is the most important food crop, and staple food for many people in the world. However, the pressure of rapid population growth, adverse effects from climate change and limited areas of rice growing in the country due to the urbanization and industrialization that need to urgently enhance rice yield to meet the increasing demands of food – consuming. Molecular breeding such as Marker – Assisted Backcrossing (MABC) is one of the efficient methods to transfer the specific quantitative trait loci (QTL) or gene into the elite varieties. In this study, MABC was applied to transfer QTL/gene which is responsible for trait of increasing number of grains per panicle from donor (KC25) to recipient plant (KD18). The results have shown that the successfully selected individual No 14 in BC_3F_1 population carrying QTL/gene and attained the highest genetic background of the recipient plant up to 100% were made.

Keywords: *Marker – assisted backcrossing (MABC), QTL/gene, KD18, KC25.*

1. Introduction

Rice (*Oryza sativa* L.) is the most important food crop in Vietnam, and is also the main source of food for more than half of the world wide population. Nowadays, with the rapidly growing population, the significant reduction of agricultural land and the extreme effects of climate change have caused adverse impacts on rice productivity. In some recent years, with the rapid development of modern biotechnology, numerous molecular markers linked to the important traits of rice have been identified. Scientists and breeders have paid much attention to those molecular markers for rice breeding programs. Marker assisted backcrossing (MABC) is a power tool and proposed for rapid and effective in plant breeding. As a fore mentioned issues, the application of molecular markers and backcrossing to introgress and pyramid the high yield QTLs/genes into traditional and elite rice varieties is imperative work [1], [5], [6]. Therefore, the main objective of this research is to improve the yield of some rice lines/varieties by use of molecular marker.

2. Materials and methods

2.1. Materials

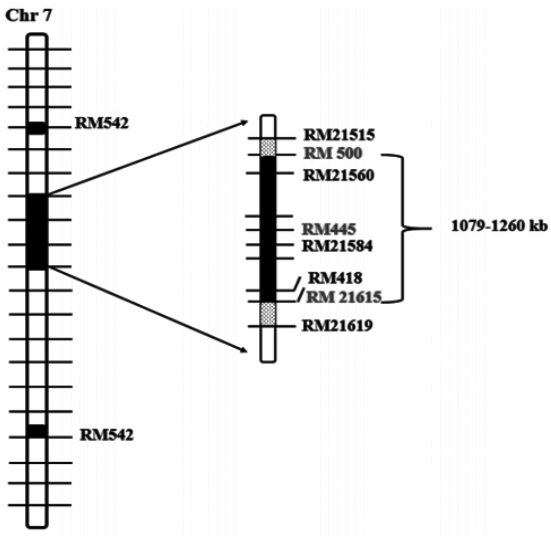

Figure 1. The position of the yd7 QTL/gene increased the number of seeds per panicle located on chromosome

- KC25 donor lines carrying *yd7* QTL/gene which controlling the increase grains per panicle were used as the donor plant materials in this study.

- The selected recipient plants were Khang Dan 18 variety. This variety is being cultivated in the Red River Delta.

- Individuals 59 and 61 are BC_2F_1 individuals that have been determined to carry QTL/gene controlling the trait of increasing seed number per panicle and have the highest genetic background of the transgenic plants, inherited from previous studies [2].

- Three SSR molecular markers linked to the target *yd7* QTL/gene RM445, RM500, RM21615 [4].

- Sixty-two polymorphic molecular markers spread evenly on 12 chromosomes between KD18 and KC25.

2.2. Methods

- Methods of DNA extraction and purification under the improved CTAB method based on the method of Shagai - Maroof et al (1984)

- PCR method with SSR primer

- Electrophoretic method on agarose gel 0,8%; 3,5%

- Statistical analysis: Data in field were analyzed by use of the IRRISTAT 5.0 program; Excel version 2007. Data collection and analysis techniques in the laboratory were analyzed by using Graphical Genotypes 2 (GGT 2.0) software and other necessarystatistical methods.

3. Results and discussion

3.1. Use of polymorphic markers to select the individual plants carrying target QTL/gene of BC_3F_1 population

Individuals plants in BC_2F_1 population carrying QTLs/genes were used for checking their genetic background on 12 chromosomes. The result showed that individual plant number 61 and 59 carrying QTL/genes increased the number of seeds per panicle and retained the highest genetic background, were 91,8% and 92,3%, respectively [2].

Therefore, these two individuals were used to backcross with Khang Dan 18 to create BC_3F_1 population. Hybrid seeds are planted to develop the BC_3F_1 population. After the rice was about 20 days old, the leaf samples were collected. The collected sample was extracted and checked for DNA quality.

In this study, polymorphism markers including RM445, RM500, RM21615 at the target *yd7* QTL/gene were used. Some screening images are shown in Figure 2; Figure 3 and Figure 4.

The result in Figure 2 showed that: There were 50 individuals showing heterozygote genotypes including plant numbers: 1, 3, 4, 5, 6, 7, 9, 10, 11, 13, 14, 15, 16, 19, 21, 22, 24, 25, 28, 29, 30, 34, 35, 37, 38, 41, 43, 46, 48, 51, 53, 57, 59, 61, 67, 69, 70, 73, 74, 76, 77, 79, 82, 85, 87, 90, 92, 93, 94 and 95.

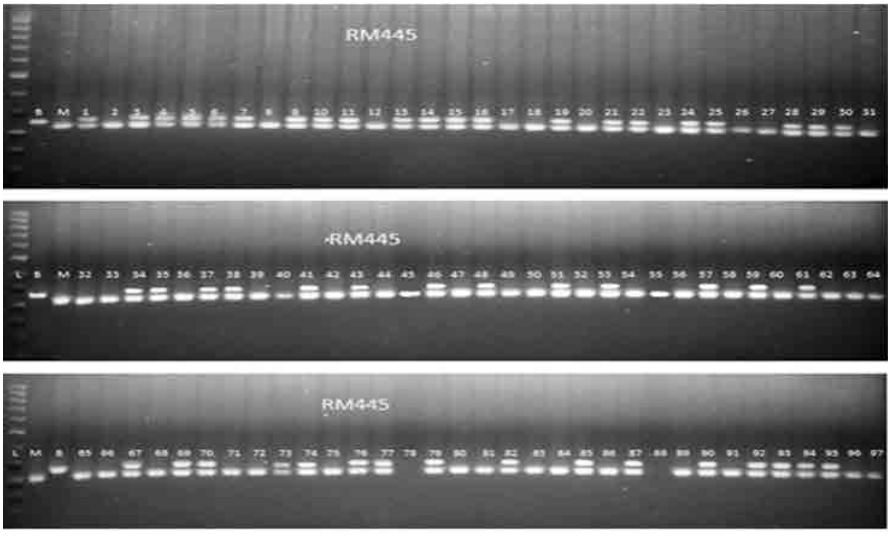

Figure 2. Electrophoresis to examine the individuals carrying the target gene in BC_3F_1 population by use RM445 marker

L: 50bp ladder; M: KD18; B: KC25;1-95: Individual BC_3F_1

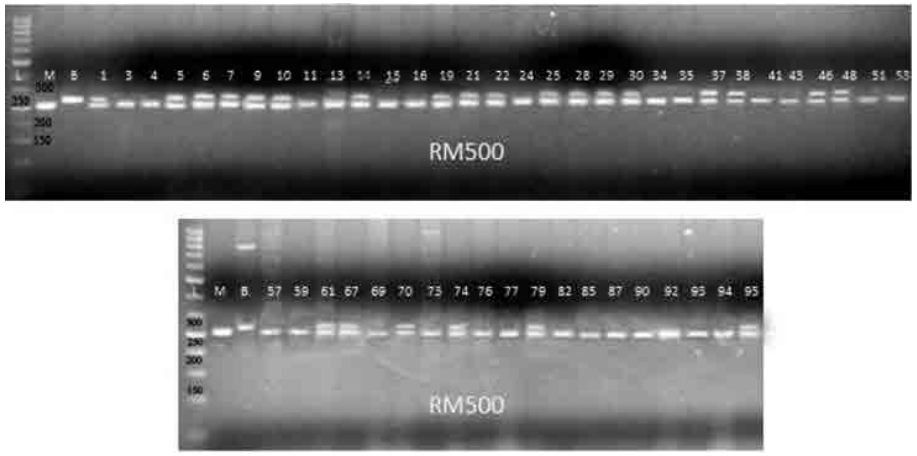

Figure 3. Electrophoresis to examine the individuals carrying the target gene in BC_3F_1 population by use RM500 marker
L: 50bp ladder; M: KD18; B: KC25; 1-95: Individual BC_3F_1

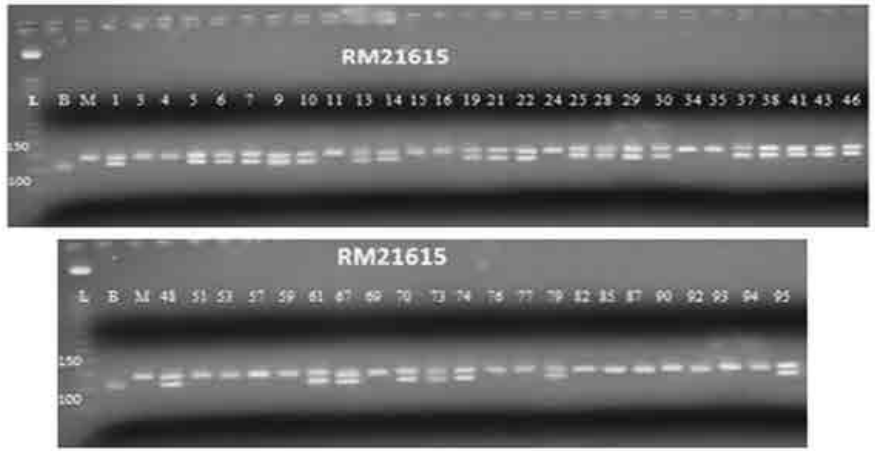

Figure 4. Electrophoresis to examine the individuals carrying the target gene in BC_3F_1 population by use RM21615 marker
L: 50bp ladder; M: KD18; B: KC25;1-95: Individual BC_3F_1

This heterozygous plants were screened by RM500 marker. The results of the BC_3F_1 hybrid test for the RM500 marker were showned in Figure 3.

The result in Figure 3 showed that: There were 24/50 individuals showing heterozygote genotypes by use of RM500 marker including plant numbers: 1, 5, 6, 7, 9, 10, 14, 19, 21, 22, 25, 28, 29, 30, 37, 38, 46, 48, 61, 67, 70, 74, 79 and 95.

Continue screening for fifty heterozygous BC_3F_1 individuals at the RM445 marker using RM21615 marker. This marker is the flanking markers on the both QTL/gen sides. The results were showed in Figure 4.

Polymorphism markers including RM445, RM500, RM21615 at the target *yd7* QTL/gene were used. The results demonstrated that 24 individuals carrying the target QTL/gene were the plant number as following: 9, 10, 14, 19, 21, 22, 25, 28, 29, 30, 37, 38, 46, 48, 61, 67, 70, 74, 79 and 95.

3.2. Identification of BC_3F_1 hybrids carrying target QTL/gene with the highest genetic background of the recipient plant

Further analyze of the 24 individuals carrying QTL/genes indicated by use of 62 polymorphic markers evenly distributed on 12 chromosomes was made, the data were analyzed using the Graphical Genotyper 2 (GGT2) program. Among them, the plant number 14 carrying target gene, simultaneously had the highest background which are similar with Khang Dan 18 (approximately 100%).

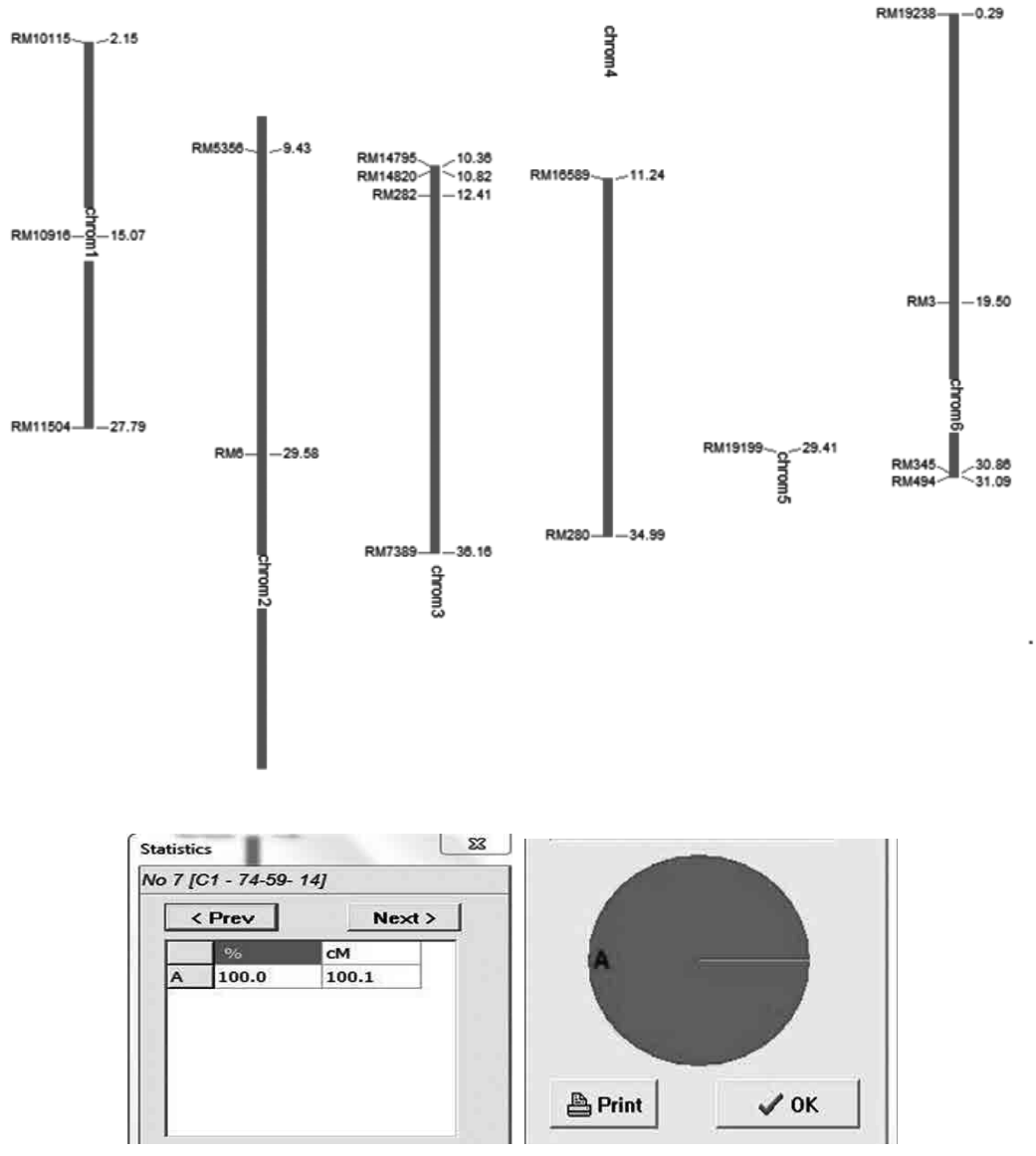

Figure 5. Genetic map of individual number14 BC_3F_1 population of breeding combination KD18/KC25

4. Conclusion

Successful application of MABC to transfer *yd7* QTL/gene controlling the trait of increasing seed number per panicle in some lines/varieties which will be helped to widely apply in rice breeding programe. According to Frisch research, in BC_4F_1 population can be selected individual with target QTL/gene and have a genetic background of approximately 100% of the recipient plants [3]. However, in this study, individual plant number 14 in BC_3F_1 population carrying QTL/genes increased the number of seeds per panicle and retained the highest genetic background, were 100%. This individual was selected to be used as the material for further research.

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ỨNG DỤNG CHỌN GIỐNG NHỜ CHỈ THỊ PHÂN TỬ KẾT HỢP LAI TRỞ LẠI CHỌN LỌC CÁ THỂ MANG QTL/GEN QUY ĐỊNH TÍNH TRẠNG TĂNG SỐ HẠT TRÊN BÔNG Ở QUẦN THỂ BC_3F_1 ĐỂ CẢI TIẾN NĂNG SUẤT GIỐNG LÚA KHANG DÂN 18

Tóm tắt:

Lúa (Oryza savita L.) là cây lương thực quan trọng ở nước ta và là nguồn lương thực chính của nhiều nơi trên thế giới. Trước những ảnh hưởng cực đoan từ biến đổi khí hậu cùng với quỹ đất trồng lúa bị thu hẹp do quá trình đô thị hóa đã làm năng suất lúa bị sụt giảm rõ rệt. Chọn giống nhờ chỉ thị phân tử và lai trở lại (MABC) là phương pháp thiết thực, hiệu quả để lai chuyển QTL hoặc gen vào dòng/giống ưu tú. Trong nghiên cứu này, nhờ ứng dụng MABC, đã lai chuyển thành công QTL/gen quy định tính trạng tăng số hạt trên bông từ dòng cho gen KC25 vào giống nhận gen (Khang dân 18). Ở thế hệ BC_3F_1 đã chọn lọc được cá thể số 14 mang gen và có nền di truyền cao nhất giống cây nhận gen đạt 100%.

Từ khóa: *Chọn giống nhờ chỉ thị phân tử kết hợp lai trở lại (MABC), QTL/gen, KD18, KC25.*