BIOVALENTIA: BIOLOGICAL RESEARCH JOURNAL

e-ISSN: 2477-1392

Vol. 8 No. 2, Nov 2022

Development of Non-Tidal Adaptive Rice Varieties: Molecular Marker Assisted Selection of BC_2F_1 Progenies

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Peer review under responsibility of Biology Department Sriwijaya University

Abstract

Submergence stress and drought stress are main abiotic constraints encountered in South Sumatra swampland rice cultivation area. The development of new abiotic stress tolerant varieties through the introgresion of tolerance genes, such as the Sub1 gene (submergence tolerance) by using Marker-assisted Backcrossing (MABC method) is one of an ideal solution to obtain an adaptive rice variety for those conditions. The study was carried out at Faculty of Agriculture, Sriwijaya University, Indonesia. In this study, BC2F1 used as the plant materials derived from backcross performed in BC_1F_1 and the recipient parent, Inpago 5. Previously, BC_1F_1 obtained from backcrossed progenies from Inpara 8 (inherited Sub1A gene) and Inpago 5 as recipient parent, a drought tolerant variety. The main objective of this study was to analyze the Sub1 introgressed plants in backcrossed progeny BC_2F_1 that closest similarity to recipient parent. The result showed that Sub1 introgression was confirmed by a tightly linked Sub1 gene marker, SUB1C173 marker. Out of 47 plants, 20 plants were selected based on foreground selection. Those plants were further analyzed on background selection by using 13 SSR markers. Based on the foreground and background selection, two plants, viz, plant no. 41 and 44, were selected and will be used for further study.

Keywords : Dual tolerance; marker assisted backcrossing, Sub1 gene

Received: November 21, 2022, Accepted: December 21, 2022

1. Introduction

Rice, an important staple food, is widely cultivated in the most of Asian countries, including Indonesia. Total rice production in Indonesia was recorded as much as 31.6 tons in 2020 [1]. Approximately 2.98 million ha of swamp land is located in South Sumatra and 298,198 ha has been utilized for agriculture activities [2],[3]. On the other hand, population growth increment causes a high demand for rice. Utilization of swamp land is considered to increase rice productivity by developing an adaptive rice variety [4]. Unpredictable of water level in swamp land causes an unorganized of hydrotopography [5]. In South Sumatra, rice cultivation starts at the end of rainy season and causes submergence stress and drought stress on vegetative and generative phase.

Development of non-tidal adaptive rice variety can be initiated by *Sub1* (Submergence 1) gene introgression to a rice cultivar that has drought tolerant [6]. *Sub1* is a gene on rice chromosome 9 [7]. The gene will express the quiescence strategy to

survive during submerged condition. Xu et al. [8] reported that *Sub1* locus contains three genes viz. *Sub1A*, *Sub1B*, *Sub1C*, but only *Sub1A* gene was found in submerged tolerant cultivar, FR13A. Inpara 8 has the *Sub1A* gene inherited by FR13A [9]. *Sub1A* will slackens the ethylene production and GA responsiveness during submerged condition, maintains carbohydrate reserves, and significantly elongates endurance [10].

Several studies have been widely carried out to obtain high-yielding cultivars as well as adaptive to submerged condition during vegetative phase through the insertion of the *Sub1* gene [11],[12]. A study conducted by Iftekharuddaula et al. [13], obtained a dual tolerant cultivar by using MABC (Marker-Assisted Backcrossing) method. Previous study by Suwignyo et al. [14], reported that Inpago 5 was an high-yielding rice variety under drought conditions and can be inserted by *Sub1* gene from Inpara 8 to survive in the non-tidal swamp. This study aimed to identify *Sub1* gene and investigate backcrossed progenies (BC_2F_1) from Inpara 8 and Inpago 5 by using MABC method to obtain a dual tolerant rice cultivar.

2. Materials and Methods

Plant Materials and Crossing Scheme

Inpara 8, a high-yielding rice cultivar is widely cultivated and grows well in tidal swamp of South Sumatra [15]. Inpara 8 derived from a double cross of Cinglonik/IRBB7//Memberamo/IR64 [9] was used as *Sub1* donor parent for submergence tolerance. In our previous study [14], the recipient parent Inpago 5 showed drought tolerance in generative phase and high-yield under non-tidal cultivation. Figure 1 shows crossing scheme, where in previous study, Inpara 8 was crossed with Inpago 5 to obtain F_1 seeds. Then, the F_1 was backcrossed with Inpago 5 (recipient parent) to obtain BC_1F_1 seeds that confirmed *Sub1* introgression [14], [16]. BC_2F_1 seeds was obtained from BC_1F_1 backcrossed with Inpago 5 (recipient parent).

Molecular Marker Analysis

Young leaves of 14-day-old plants were extracted using a protocol of Wizard® Genomic Purification Isolation Kit (Promega, USA). A total of 15 SSR markers were used for selection (foreground and background). Samples were amplificated in a single 96-well PCR (Biologix) with a total volume of 12.5 µL reactions containing of 0.5 µL template DNA from Inpara 8, Inpago 5, and BC_2F_1 generations; 0.5 µL the forward and reverse primers; 4.75 µL of MyTaq DNA polymerase (Bioline, BIO); and 6.25 µL of ddH_2O. After pre-denaturation at 94°C for 5 min, the amplification was followed a procedure by Adriansyah et al. [12] with 34 cycles; each cycle comprised 1 min of denaturation at 94°C, 1 min of annealing at 55°C, 2 min of extension at 72°C, and 2 min of final extension at 72°C. The PCR product was mixed with loading dye gel and separated by electrophoresis on 1% agarose gel in 1x TBA with 100 bp DNA ladder (Promega, USA) to determine the amplicon size. The gels were stained with 1 µl of DNA dye (GelRed, Biotium Inc., USA). The images were taken using Kodak Gel Logic 112 (Carestream, USA).

Selection Approach

A tightly-linked SSR marker, SUB1C173 [17] used for foreground selection (Table 1). The foreground marker used in this study also used by Septiningsih et al. [18], Iftekharaddaula et al. [13], Mojulat et al. [19], and Adriansyah et al. [12] to identify *Sub1* gene introgressed. In foreground selection, the heterozygous plants were selected, then proceed to background selection. A total of 13 SSR markers, un-liked to *Sub1* covering 12 rice chromosomes were used for background selection (Table 2).

Table 1: *Marker of foreground selection*

Chr.	Marker	Sequence
9	SUB1C173	F: AAC GCC AAG ACC AAC TTCC R: AGA GGC TGT CCA TCA GGT

Analysis of Molecular Data

The marker data were scored as A (homozygous recipient allele), H (heterozygous allele), and B (homozygous donor allele) for foreground selection and background selection. The Mendelian segregation ratio of markers was analyzed following the formula $\chi^2=(O-E)^2/E$, where O was the observed value and E was the expected value by Popgene software [20]. All the data of background selection were analyzed using Graphical Genotyper (GGT 2.0) software [21].

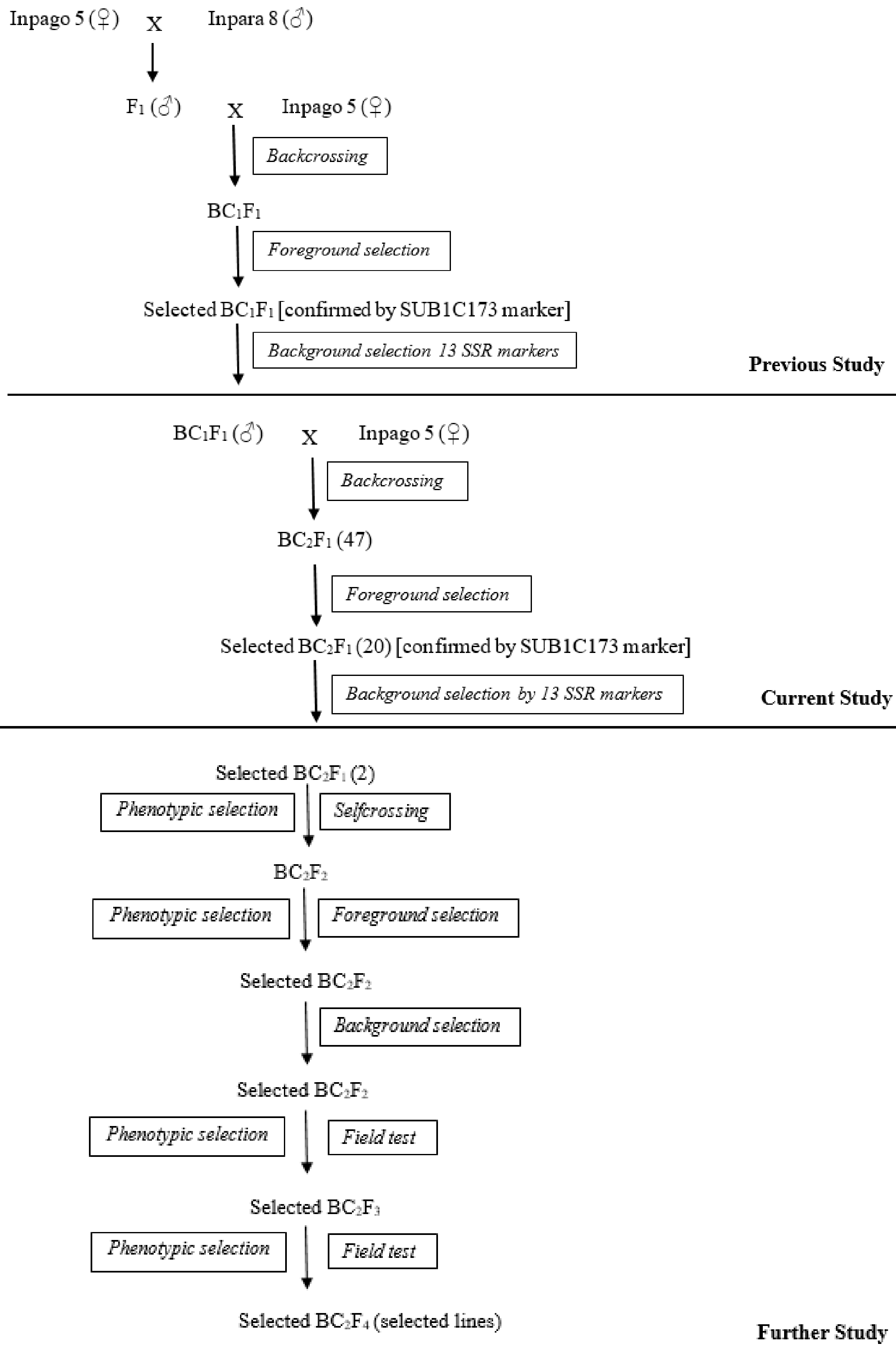

Figure 1. Scheme of crossing through marker-assisted backcrossing with details of markers used in each generation. The number in parentheses indicates the total number of plants.

Table 2: *Markers of background selection*

Chr.	Marker	Sequence
1	RM443	F: GAT GGT TTT CAT CGG CTA CG R: AGT CCC AGA ATG TCG TTT CG
2	RM262	F: TAG TTT AAC CAA GAC TCT C R: TAG TTT AAC CAA GAC TCT C
3	RM282	F: CTG TGT CGA AAG GCT GCA C R: CAG TCC TGT GTT GCA GCA AG
4	RM241	F: GAG CCA AAT AAG ATC GCT GA R: TGC AAG CAG CAG ATT TAG TG
5	RM164	F: TCT TGC CCG TCA CTG CAG ATA TCC R: GCA GCC CTA ATG CTA CAA TTC TTC
6	RM589	F: ATC ATG GTC GGT GGC TTA AC R: ATC ATG GTC GGT GGC TTA AC
7	RM234	F: ACA GTA TCC AAG GCC CTG G R: CAC GTG AGA CAA AGA CGG AG
7	RM 248	F: TCC TTG TGA AAT CTG GTC CC R: GTA GCC TAG CAT GGT GCA TG
8	RM3459	F: TCC TTG TGA AAT CTG GTC CC R: GTA GCC TAG CAT GGT GCA TG
9	RM219	F: ATG GAC TTT CGA GAA TGT TG R: GAG TAC GAA ATG AAG GCA AG
10	RM258	F: CGT CGG ATG ATG TAA AGC CT R: CAT ATC GGC ATT CGC CTG
11	RM3701	F: GAG CTA GAG GGA GGA GGT GC R: TTG ACT GAT AGC CGA TTG GG
12	RM1261	F: GTC CAT GCC CAA GAC ACA AC R: GTT ACA TCA TGG GTG ACC CC

3. Results and Discussion

Foreground Selection

Foreground selection is the first step in the Marker-assisted Backcrossing method. The purpose of foreground selection is to identify whether the *Sub1* gene has been inserted into backcrossed plants [5]. In this study, 47 plants were obtained from backcrossing and the DNA samples were amplified by using SUB1C17317 along with donor parent (Inpara 8) and recipient parent (Inpago 5). Fig.2 shows the banding pattern (using the SUB1C173 marker) of the several progenies BC_2F_1. The recipient parent had 150 bp and 121 bp; the donor parent had in 178 bp and 121 bp; and the heterozygous plants had in 178 bp, 150 bp, and 78 bp. The result fitted the expected 1:1 ratio of this generation (χ^2 value of 0.3, $P > 0.05$). There are 21 plants were recorded with 'A' score and 6 plants were recorded with 'B' score. There are 20 plants were recorded with 'H' score, indicating that the *Sub1* gene had been introgressed into the progenies. They are plant no. 2, 3, 8, 9, 10, 12, 13, 14, 15, 16, 19, 20, 34, 35, 36, 37, 41, 42, 44, and 47. The heterozygous plants were selected and subjected to background selection. The SUB1C173 marker is widely used to verify the introgression of *Sub1* into various rice cultivars, such as 'Swarna-*Sub1*' [22], 'Ciherang-*Sub1*' [17], and 'Pegagan-*Sub1*' [12].

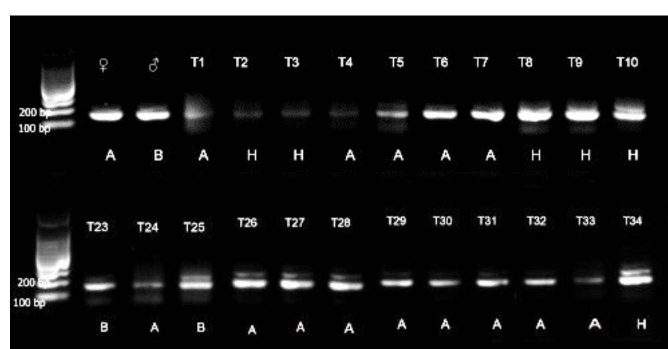

Figure 2. The DNA banding pattern of several progenies BC_2F_1 in foreground selection with SUB1C173 marker. ♀: Inpago 5, recipient parent; ♂: Inpara 8, donor parent; T1-T34: plant number

Background Selection

In the BC_2F_1 generation, a total of 20 plants were selected based on foreground selection. Background selection was carried out over the 20 selected plants using 13 background markers that cover all chromosomes of rice (12 chromosomes) to recover the recipient genome. Each backcrossed plant was scored as an A', 'B', and 'H' score considering the DNA band of each markers used. The scoring data were calculated by using GGT 2.0 software. The percentage of an 'A' score (homozygous recipient alleles) was ranked and shown in Table 3. The highest percentage of recipient alleles (A) was obtained in plant number 41 (57.1%), followed by plant number 44 (50%), plant number 12, 34, 36, 42 (42.9%), plant number 9, 13, 19, 20, 35, 47 (35.7%), plant number 3, 8, 10 (28,6%), plant number 2 (21,4%), and plant number 14,15,16,37 (14.3%). Fig. 3 shows the highest recipient parent genome recovery was 57.1%, found in plant no. 41. The result of background selection (Table 3) recommended plant number 41 and 44 for further planting materials. Selected plants had 6 homozygous recipient alleles. The chromosomal condition of individuals indicated the plants closest to recipient genome (Inpago 5) and carry *Sub1* gene.

Table 3: *Results of the background selection of BC_2F_1 generation*

Plant No.	A	H	B	% A	Ranking Background
2	2	2	9	21.4	6
3	3	3	7	28.6	5
8	3	4	6	28.6	5
9	4	4	5	35.7	4
10	4	4	5	28.6	5
12	5	4	4	42.9	3
13	4	4	5	35.7	4
14	2	5	6	14.3	7
15	2	5	6	14.3	7
16	4	4	5	14.3	7
19	4	3	6	35.7	4
20	5	3	5	35.7	4
34	6	2	5	42.9	3
35	4	2	7	35.7	4
36	3	4	6	42.9	3
37	1	5	7	14.3	7
41	6	3	4	57.1	1
42	6	3	4	42.9	3
44	6	2	5	50.0	2
47	4	6	3	35.7	4

Note: Note: A = homozygous recipient allele(s); H = heterozygous allele(s); B = homozygous donor allele(s); %A = Percentage of recipient allele (A)

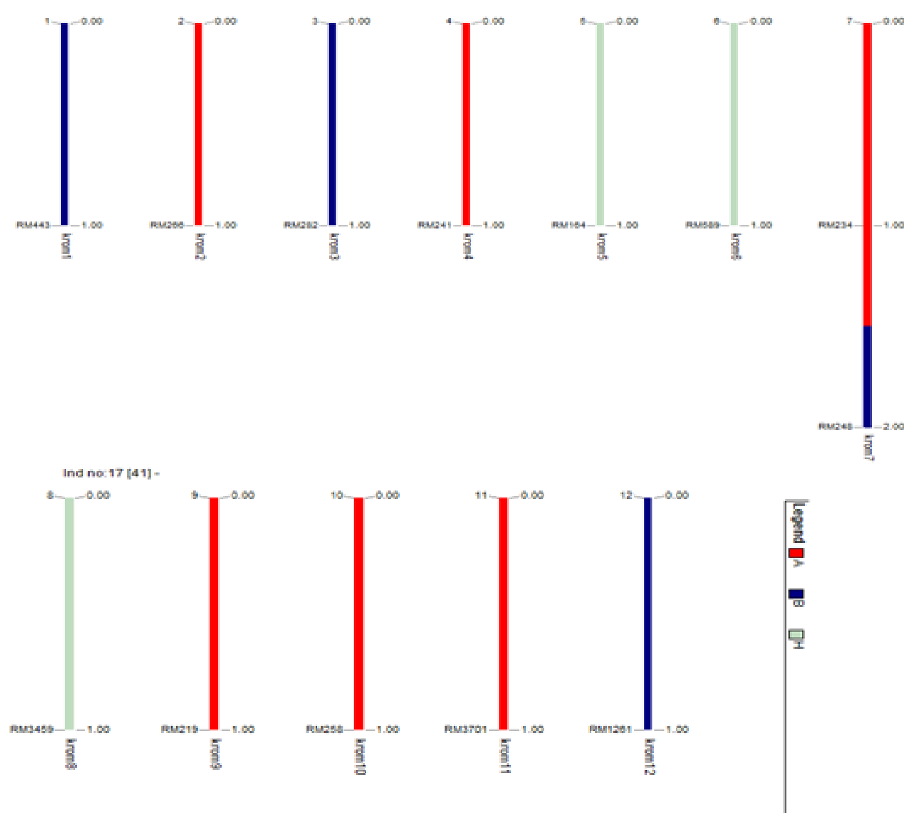

Figure 3. Positions of each polymorphic marker on 12 chromosomes in the population of BC_2F_1 progenies

4. Conclusion

Twenty out of 47 plants were found to be heterozygous by using the foreground marker, SUB1C173 marker. Two plants had a percentage of recipient alleles (A) greater than or equal to (≥) 50 % by using 13 background markers. They are plant no. 41 (57.1 %) and plant no. 44 (50 %). These plants were selected and will become plant materials for further study of submergence stress-field testing.

5. Acknowledgement

The authors would like to thank and appreciate the anonymous reviewers and editors of biovalentia journal for their meaningful comments and suggestions. we deeply appreciate the ministry of education, culture, research and technology who has funded this research under the research scheme of national competitive program research fiscal year 2022 no. 142/e5/pg.02.00.pt/2022 with DIPA No. SP DIPA 023.17.1.690523/2022.

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