Analysis of Submergence Tolerant Gene (*Sub-1*) on BC_2F_1 Population, Backcross of Selected Swamp Rice Genotipe Using Molecular Marker

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***Abstract*—Water fluctuation and poor water management at swamp area caused rice plant is susceptible to flooding and submergence stress during plant growth and development. Rice plant which is submerged for long period caused plant wilt and die. Marker Assisted Backcrossing (MABc) is one of the rapid solution for introgression the submerged tolerant gene (*sub*-1) from donor parents to the local swamp rice varieties. It is expected that new swamp rice genotype will be able to adapt to stress submerged. The purpose of this researches were to select the heterozygous progenies from BC_2F_1 generation based on backcrossing of 4 local parents of South Sumatera swamp rice genotypes i.e Siam, Pegagan, Pelita Rampak, and Payak Selimbuk with the donor of submergence tolerant gene (*sub-1*) of FR13A. Molecular analysis was studied by using foreground selection on *sub-1* gene as a gene target or QTL, using 2 flanking markers based on microsatellite or Simple Sequence Repeat (SSR) of RM 23805 and RM 23915. The results of molecular analysis indicated that there were 37 number of progenies from total population of BC_2F_1 generations. They were 8 numbers of BC_2F_1 Siam from 24 progenies, 9 numbers of BC_2F_1 Pegagan from 27 progenies, 10 numbers of BC_2F_1 Pelita Rampak from 39 progenies, and 10 numbers of BC_2F_1 Payak Selimbuk from 31 progenies which were identified as having *sub*-1 gene.**

***Index Terms*—swamp rice, FR13A, *sub*-1 gene, Marker Assisted Backcrossing (MABc.), microsatelite, heterozygous**

I. Introduction

South Sumatra has swamp area which is almost 228 thousand hectares. Improvement of paddy in the swamp area is to support national need in Indonesia [1]. But, in order to fulfill all the requirements there are still several factors encountered. One of the most caused the problem is flooding. Flooding is the most important factor which affect the growth of paddy in the swamp area which can cause 50.000 -100.000 ha of crop failure each year [2]. Using the variety which is tolerant to submergence is one solution to anticipate the cause of submergence which is always encountered by the farmers in swamp area. Using the variety which can adapt to that area such as Swarna *sub1* and Samba Mashuri have been used in India and Bangladesh since year of 2005 [3].

In order to develop paddy which can be adapted in swamp area, the use of adapted varieties which are tolerant to submergence stress have been used by using moleculer marker such as Marker Assisted Backcrossing (MABc) which can be shorten the selection of backcrossing. MABc is microsatellite probe (SSR) which can be used as moleculaer marker which can be accurate, practice, high polymorphism, and can be used to show a lot of marker [4]. SSR marker can be differenciate homozygote allel with heterozygote, multiallel and precise in almost all *Oryza indica* and *Japonica*.

The research of selection of *sub1* gene on population of paddy which is tolerant to submergence has been made by Septiningsih in Bangladesh such as stain of BR11-*sub*1, the result of backcross from donor of IR40931-33-1-3-2 and strain of BR11. From 763 strains which have been selected by using RM 23195 and RM 23805 marker, they were found that 375 individu which have *sub1* gene [5]. Microsatellite RM 23805 marker show that all strain which are originated from crossing of OM1490 and IR64-*sub1* which have been intrograted significantly have survival rate more higher compared to the parents. Base from the parent of OM1490 and tolerant parent of IR64-*sub1* are 240 bp and 230 bp appear simultaneously on the locus of RM 23805 marker which are heterozygote [6].

Submergence tolerant gene which has *sub1* gene will grow normal in submergence condition. In contrast, most of paddy which has no *sub1* gene will elongated faster during submergence condition in order to grow faster during submergence stress in order to make *aerenchima* on the top of water. This experiment want to find several backcrossing of paddy with FR13A which is tolerant to submergence in order to get the best submergence cultivars which can be grow during submergence stress in South Sumatra Palembang.

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II. Material and Methods

FR13A that has sub-1 gene and four local varieties from South Sumatra such as Siam, Pegagan, Pelita Rampak and Payak Selimbuk will be used in this study. BC_1F_1 has been conducted and this experiment will continue to get the BC_2F_1 from crossing of four local genotypes will be selected and INPARA 5 and IR 42 were used as controls. Molecular marker which is used in this research was RM 23805 and RM 23915 which are the markers which have tolerant sub-1 gene (Table I).

TABLE I. Molecular Marker of SSR Which Will Be Uses for *Foreground Selection*

Marker	Chromosome	Long of Bases	Sequence
RM 23805	9	271 bp	F : CACATAGTTTCCATGTCCGTTCAC R : GGTAGAATCCATGACCGTCTCATC
RM 23915	9	262 bp	F : TACATTGGAAGGAAATTCAGCTCC R : CATGCAGATATGACCAAGAACCTG

Paddy leaves after 2 weeks submergence are collected 5-10 cm long using Dellaporta method [7]. All leaves which have been ground were put in 2 ml of micro tube. Extraction buffer of 800 µl was used and incubate for 15 minutes in temperature of 65°C. After that 800 µl of Chisam was added and homogenized by using vortex. After that the solution was centrifuge for 15 minutes at 12.000 rpm. 400 µl of supernatant will be transfer to 1.5 ml microtube. NaoAc of 40 µl (1/10 volume) was added together with absolut ethanol (isopropanol) of 800 µl (2x volume) and then were centrifuge with 12.000 rpm for 10 minutes. DNA was washed by using 70% *ethanol*. The DNA was dried for one night and after that 10 µl RNAse + dd H_2O were added.

DNA amplification was done by using PCR Biorad with 20 µl solution on each PCR reaction with the composition as follow: 2 µl DNA, 2 µl mix dNTP, 1 µl F-Marker, 1 µl R-Marker, 0.2 µl enzim *Taq Polymerase*, 2 µl *polymerase*, and 10.8 µl *MiliQ-water*. PCR program are as follow: denaturation for 5,45 minutes on temperature of 94 °C, annealing on 52 °C for 0,45 minutes, extention (DNA Polimerase synthesis) on the temperature of 72 °C for 1,45 minutes which are repeated 34 times. Amplification of DNA was done by using *Poly Acrilamic Gel Electroforesis* (PAGE) and visuilized by s*ilver staining*.

III. Result and Discussion

Molecular marker which is used in foreground selection is used during first selection in order to get the result of crossing of progeny during background selection. Foreground selection in this study will be used to identify and select each individu which contains submergence tolerant gene (Sub-1) on population of BC_2F_1. Molecular probe which is used during selection is SSR. Marker of RM 23805 and RM 23915 which are located in chromosome number 9 was known correlated to sub-1 which is located at chromosome number 9 and which are known correlated to sub-1 gene showed in Fig. 1.

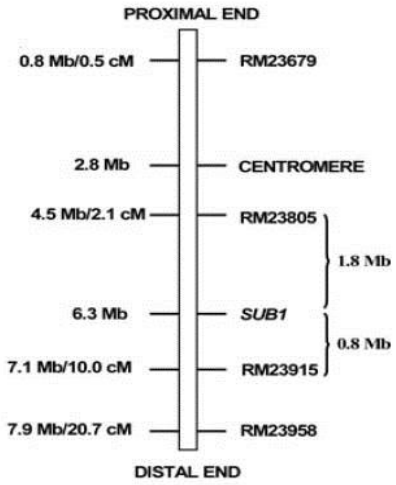


Figure 1. Position of RM 23805 and RM 23915 markers on chromosome number 9 [5].

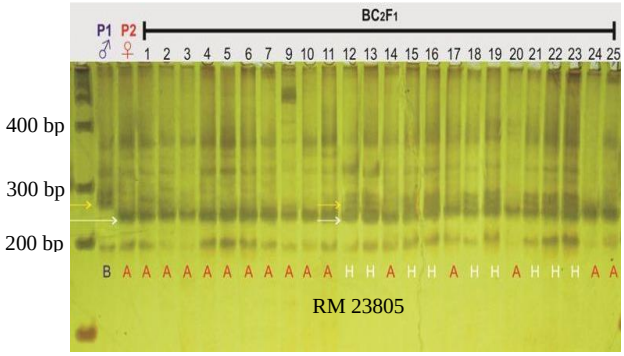
The result of molecular identification on foreground selection will be showed by Table II. and the visualization of DNA electrophoresis will be showed by Fig. 2 to Fig. 5.

TABLE II. Locus DNA on RM 23805 and RM 23915 Markers

Plant Population	Code	Numb of Plant	RM 23805 Marker				RM 23915 Marker			
			A (♀)	B (♂)	H	O	A (♀)	B (♂)	H	O
BC_2F_1 Siam	C1	24	15	0	9	0	16	0	8	0
BC_2F_1 Pgg	C2	27	9	9	9	0	9	9	9	0
BC_2F_1 P.Ra	C3	39	24	3	10	2	15	4	19	1
BC_2F_1 Py.Se	C4	31	11	6	14	0	13	5	12	1

Note: A (♀)= homozygote with recipient parent [♀], B = homozygote with parent as donor [♂], H = heterozigot (gene from parent as donor [♂] intrograted with recipient parent [♀]), O = No DNA.

BC_2F_1 Siam (C1)



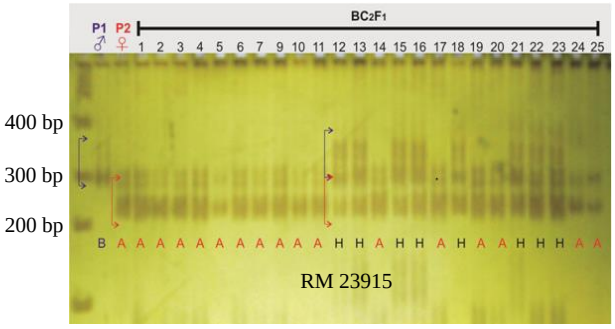


Figure 2. Confirmation introgression locus *Sub1* on BC_2F_1 Siam; P1 = donor parent, P2 = recipient parent, A = homozygote with recipient parent [♀], B = homozygote with donor parent [♂], H = heterozygote (gene from donor parent [♂] introgression to recipient parent [♀]), O = No DNA appear.

Fig. 2 showed the results of electrophoresis on population of BC_2F_1 Siam (C1) by using RM 23805 marker indicate that, there are 9 numbers which are located on the heterozygote locus (H) and there are 8 numbers which are heterozygote locus on the marker of RM 23915. After confirmation, there are 8 numbers which show heterozygote locus. They are BC_2F_1 Siam of number 12, 13, 15, 16, 18, 21, 22 and 23.

BC_2F_1 Pegagan (C2)

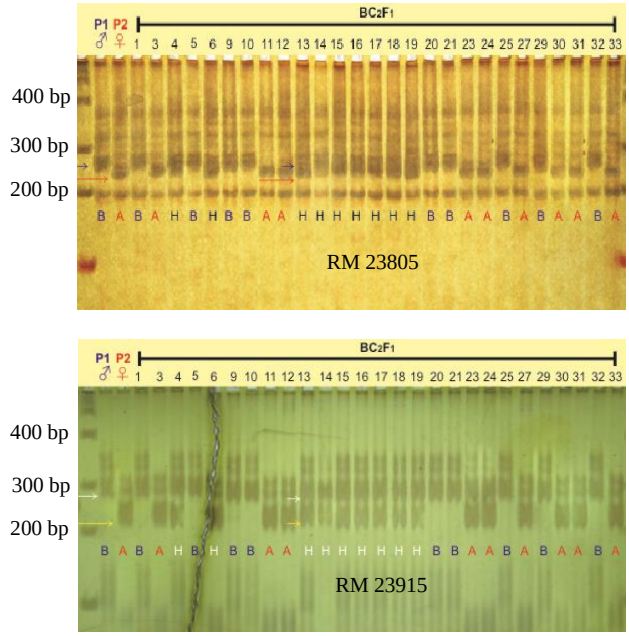


Figure 3. Confirmation of introgression locus *Sub1* on BC_2F_1 Pegagan; P1 = donor parent, P2 = recipient parent, A = homozygote with recipient parent [♀], B = homozygote with donor parent [♂], H = heterozygote (gene from donor parent [♂] introgression to recipient parent [♀]), O = No DNA appear.

Electrophoresis result for population of BC_2F_1 Pegagan (C2) to 27 numbers which are molecular identify in Fig. 3 showed that there are 9 numbers which have heterozygote locus in RM 23805 marker and 9 numbers on RM 23915 marker. Those two markers show heterozygote locus on the same individues so there are 9 numbers are found in population of BC_2F_1 Pegagan which are number 4, 6, 13, 14, 15, 16, 18, 19, and 20.

BC_2F_1 Pelita Rampak (C3)

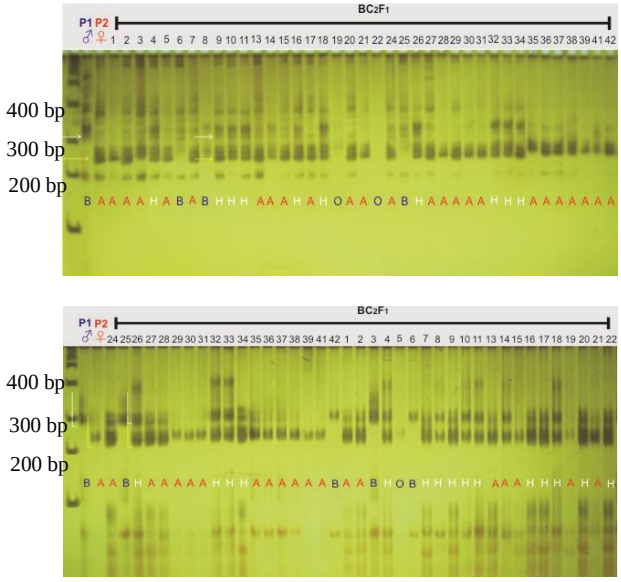


Figure 4. Confirmation introgression of *Sub1* locus on BC_2F_1 Pelita Rampak; P1 = donor parent, P2 = Recipient parent, A = homozygote with recipient parent [♀], B = homozygote with donor parent [♂], H = heterozygote (gene of donor parent [♂] Introgression to recipient parent [♀]), O = No DNA appear.

Electrophoresis results on population of BC_2F_1 Pelita Rampak (C3) to 39 numbers on RM 23805 marker show that there are 10 numbers have heterozygote locus (H), while on RM 23915 marker there are 15 numbers which show heterozygote locus (H) is showed by Fig. 4. After confirmation there are 10 number which show heterozygote locus (H) on the two markers i.e BC_2F_1 Pelita Rampak. They are numbers 4, 9, 10, 11, 16, 18, 26, 32, 33, and 34.

BC_2F_1 Payak Selimbuk (C4)

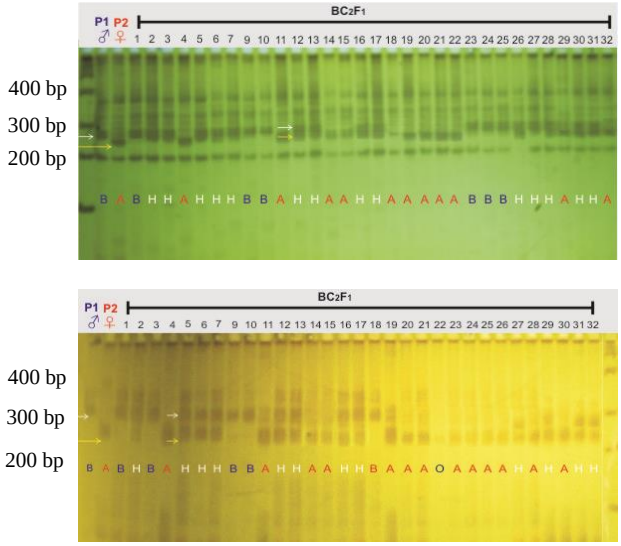


Figure 5. Confirmation introgression *Sub1* locus on BC_2F_1 Payak Selimbuk; P1 = donor parent, P2 = Recipient parent, A = homozygote with recipient parent [♀], B = homozygote with donor parent [♂], H = heterozygote (gene from donor parent [♂] Introgression to recipient parent [♀]), O = No DNA appear.

Fig. 5 showed the results of DNA on population of BC_2F_1 Payak Selimbuk to 31 numbers on RM 23805 marker show that there are 14 numbers which have heterozygote locus (H), while on RM 23915 marker, there are 12 numbers which show heterozygote locus (H). After confirmation from both markers, there are 10 numbers showed heterozygote locus which are number 2, 5, 6, 7, 12, 13 16, 17, 27, and 31.

After identify all locus and got some number from each plant, the percentage of foreground selection is showed by Table III and useful for selection effectivity requirement.

TABLE III. *FOREGROUND* SELECTION OF FOUR POPULATION OF BC_2F_1

Plant population	Crossing	code	Number of plant	Foreground selection	
				∑	%
BC_2F_1 Siam	♀Sm x SmF♂	C1	24	8	33%
BC_2F_1 Pgg	♀Pg x PgF♂	C2	27	9	33%
BC_2F_1 P.Ra	♀Ra x RaF♂	C3	39	10	26%
BC_2F_1 P.Se	♀Se x SeF♂	C4	31	10	32%
	Total		121	37	30,5%

IV. CONCLUTION

Foreground selection using *flanking marker* on RM 23805 and RM 23915 markers, they were found 8 numbers of BC_2F_1 Siam (33%), 9 numbers of BC_2F_1 Pegagan (33%), 10 numbers of BC_2F_1 Pelita Rampak (26%), and 10 numbers of BC_2F_1 Payak Selimbuk (32%) which are identified contain *Sub1* gene.

REFERENCES

[1] H. Ehara, Irmawati, R. A. Suwignyo, and J. Sakagami, "Swamp rice cultivation in South Sumatra, Indonesia: An overview," *Japanese Journal of Tropical Agriculture*, vol. 59, pp. 35-39, 2015.

[2] E. M. Septiningsih, A. M. Pamplona, D. L. Sanchez, C. N. Neeraja, G. V. Vergara, S. Heuer, A. M. Ismail, and D. J. Mackill, "Development of submergence tolerance rice cultivar: The Sub1 locis and beyond," *Annals Botany*, vol. 103, no. 2, pp. 151-160, 2009.

[3] C. N. Neeraja, "A marker-assisted backross approach for developing submergence-tolerant rice cultivars," *Theor. Appl. Gene.*, vol. 115, pp. 767-776, 2007.

[4] O. P. Yadav, S. E. Mitchell, A. Zamora, T. M. Fulton, and S. Kresovich, "Development of new simple sequence repeat markers for pearl millet," *Journal of Sat Agricultural Research*, vol. 3, no. 1, 2007.

[5] E. Septiningsih, A. Pamplona, D. Sanchez, and D. J. Mackill, "Rapid and high-precision marker assisted backcrossing to introgress the *Sub*1 QTL into BR11, the raindfed lowland mega variety of Bangladesh," *Euphytica*, vol. 178, pp. 83-97, 2011.

[6] N. T. Lang, N. V. Tao, and B. C. Buu, "Marker Assisted Backcrossing (MABC) for rice submergence tolerance in Mekong Delta," *Omonrice*, vol. 18, pp. 11-21, 2011.

[7] S. L. Dellaporta, J. Wood, and J. B. Hicks, "A plant DNA minipreparation: Version II," *Plant Molecular Biology Reporter* vol. 1, pp. 19-21, 1983.

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