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# POLYMORPHIC IDENTIFICATION OF SIMPLE SEQUENCE REPEAT (SSR) MARKER TO DEVELOP ALUMINUM-TOLERANCE UPLAND RICE

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***Abstract.*** *SSR marker is one of the genetic markers widely applied in plant breeding programs. The application of molecular markers in plant breeding is meant to accelerate the selection of cross-progeny. The research aimed to identify the SSR primers polymorphism between the parent and control that linked to Al tolerance and verify the cross-progeny of five crosses. The result gained from 37 SSR primers used in this study showed that only nine primers are polymorphic. These nine polymorphic primers are RM257, RM214, RM247, RM205, RM490, RM262, RM569, RM271 and RM19. The application of polymorphic markers on five cross-progeny which have shown the same band pattern as the parents and tolerant control on the use of 9 SSR primers recorded as follows: RM257 2 lines, RM214 5 lines, RM247 5 lines, RM205 lines, RM490 13 lines, RM262 5 lines, RM569 7 lines, RM271 4 lines and RM19 6 lines. The selected SSR primers linked to Al tolerance in this research can be used as a reference for molecular breeding strategies to develop new Al tolerance rice varieties in dryland conditions.*

***Keywords:*** *aluminum tolerance, cross-progeny, plant breeding, SSR primer, upland rice*

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## INTRODUCTION

Upland rice is a type of rice that requires less water for its growth and suitable to be planted on dry land. Dryland is defined as an area that has never been flooded throughout the year. Dryland in Indonesia is dominated by red and yellow acidic podzolic and potential land for development up to 25.09 million ha (Sukarman & Ritung, 2012; Purnamaningsih & Mariska, 2008). Besides drought resistant, upland rice is an annual crop that is also resistant to disease (Sudarmawan et al ., 2017). Upland rice is widely planted on sub-optimal lands such as acidic land, which has low nutrient and high aluminum (Al) content. Rice planted on sub-optimal land is generally abnormal growth and low in productivity. Low productivity is due to Al stress which leads to thick and short roots. This abnormal structure reduces the plant's ability to absorb water and nutrients (Purnamaningsih & Mariska, 2008).

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Rice needs aluminum as one of the micronutrients essential for their growth. Rice only needs this element in minor quantities. The high Al level in the soil can be poisoning to plant. Indrayani et al. (2017) stated that Al poisoned plants will grow stunted due to the lack of other nutrients such as N, P, Ca and Mg. The same statement expressed by Purnamaningsih & Mariska (2008), who said that poisoning Al will cause adverse effects on the plant's growth both directly and indirectly. The effect of Al stress is specific on each plant, even for those which belong to the same species. However, the root is part of a plant that is most susceptible to poisonous Al thus root character is frequently used to determine plant tolerant level to poisonous Al. (Hanum et al., 2007). The first symptom of Al infection in plants is the abnormal development of the root system as a result of inhibition of cell-extension. This is caused by the combination of Al and cell walls and cell division inhibition, which lead to obstruction of water and nutrients absorption.

Sub-optimal land with a high Al level is cultivated by planting Al tolerant rice varieties. Planting Al tolerant varieties in sub-optimal land is an effective and environmentally friendly solution. These varieties obtained through crossbreeding, mutation induction, somaclonal variation, in vitro selection and developed by insertion of Al resistant genes in rice. The crossbreeding of Al tolerant rice is obtained by crossing the parent of Al tolerant traits with the parent possessing other superior traits. Al stress tolerance mechanism is developed through physiological mechanisms such as a) the ability of plants to change the pH level in the rooting area, b) preference towards nitrate and ammonium absorption and c) increasing specific enzyme activity (Utama, 2010). Lestari et al. (2014) added that Al tolerant rice varieties can change the pH level around the roots and hold Al excess in the roots to maintain Al content in the plant canopy lower.

The identification of rice plants is possibly done using a genetic marker. SSR marker is one of the genetic markers widely applied in plant breeding programs (Nurdianawati et al., 2016). Additionally, the SSR marker is used in genetic-diversity studies (Nugroho et al., 2017), Al tolerance study (Zhang et al., 2016) and rice seed-purity test (Mulsanti et al., 2013). SSR Marker is DNA sequences composed of 1-6 nucleotide repeats (Miah et al., 2013; Tasma & Warsun, 2009). SSR marker is PCR based marker widely used in rice identification (Islam et al., 2008). The advantages of using SSR markers are high polymorph level, codominant, high accuracy and easy to be applied because of genome abundance (Vieira et al., 2016; Miah et al., 2013). SSR marker is useful to evaluate the allele inheritance of cross-progeny between the parent. SSR locus is flanked with conserved nucleotide sequences. Conserved sequences can be used as a specific primer. SSR marker is useful as a selected marker for the plant's inheritance (Lestari et al., 2017).

The research aimed to identify the SSR primers polymorphism between the parent and control that linked to Al tolerance. Furthermore, to verify the cross-progeny of several crosses with superior advantages trait such as Al tolerance on cross-progeny. The genetic information obtained from this research is useful to determine the cross-progeny that have desirable traits quickly and precisely. Moreover, the selected SSR primers can be used as a reference for a molecular breeding program to develop new upland rice varieties that are tolerant to Al.

## MATERIALS AND METHODS

### Experimental Time and Place

The research was conducted between 2017-2019 in Agronomy Laboratory to evaluate Biotechnology product, Biotechnology Research Centre of LIPI Cibinong Bogor.

### Genetic Material

The genetic material used for crosses in this study came from 7 parents which have different superior advantages (Table 1). Susceptible and tolerant control were ITA131 and IR60080-32. The cross-progeny of the five types of crosses are presented in Table 2.

Table 1. Parent and comparative controls used in the research

| Parents | Superiority | Information |
|---|---|---|
| TB368B–TB25–MR–2 | High productivity | Genotype |
| B11178G–TB–29 | Aluminum moderate | Genotype |
| Situ Patenggang | Blast tolerant, high productivity, aromatic | Listed varieties breeding* |
| B11930F–TB–2 | Aluminum tolerant | Genotype |
| Inpago 8 | Blast tolerant | Released varieties* |
| B11492F–TB–12 | High productivity | Genotype |
| Danau Gaung | Aluminum moderate, Fe tolerant, blast moderate, brown leaf spot moderate. | Released varieties * |
| | Comparative controls | |
| IR60080-32 | Aluminum tolerant | Genotype |
| ITA131 | Aluminum susceptible | Genotype |

Table 2. Cross-progeny used in this research

| | | | |
|---|---|---|---|
| | Danau Gaung X Situ Patenggang (A) | | |
| A1 | B14069H-Ng-78-(205-1)-1-1 | A4 | B14069H-Ng-50-(198-1)-1-1 |
| A2 | B14069H-Ng-69-(150-1)-1-1 | A5 | B14069H-Ng-64-(201-1)-1-1 |
| A3 | B14069H-Ng-40-(194-1)-1-1 | A6 | B14069H-Ng-4-(384-1)-1-1 |
| | Situ Patenggang X B11930F-TB-2 (B) | | |
| B1 | B14081H-Ng-25-(81-1)-1-1 | B9 | B14081H-Ng-17-(80-1)-1-1 |
| B2 | B14081H-Ng-26-(214-1)-1-1 | B10 | B14081H-Ng-16-(210-1)-1-1 |
| B3 | B14081H-Ng-30-(215-1)-1-1 | B11 | B14081H-Ng-13-(296-1)-1-1 |
| B4 | B14081H-Ng-38-(217-1)-1-1 | B12 | B14081H-Ng-41-(281-1)-1-1 |
| B5 | B14081H-Ng-44-(84-1)-1-1 | B13 | B14081H-Ng-1-(78-1)-1-1 |
| B6 | B14081H-Ng-23-(13-2)-1-1 | B14 | B14081H-Ng-40-(463-5)-1-1 |
| B7 | B14081H-Ng-17-(80-2)-1-1 | B15 | B14081H-Ng-40-(463-2)-1-1 |
| B8 | B14081H-Ng-1-(78-2)-1-1 | B16 | B14081H-Ng-48-(86-1)-1-1 |
| | B11492F-TB-12 X B11178G-TB-29 (C) | | |
| C1 | B14084H-Ng-32-(117-1)-1-1 | C3 | B14084H-Ng-39-(160-1)-1-1 |
| C2 | B14084H-Ng-104-(131-1)-1-1 | | |
| | Inpago 8 X B11930F-TB-2 (D) | | |
| D1 | B14087H-Ng-65-(188-1)-1-1 | D5 | B14087H-Ng-8-(310-1)-1-1 |
| D2 | B14087H-Ng-10-(312-1)-1-1 | D6 | B14087H-Ng-18-(92-1)-1-1 |
| D3 | B14087H-Ng-21-(314-1)-1-1 | D7 | B14087H-Ng-27-(403-1)-1-1 |
| D4 | B14087H-Ng-71-(191-1)-1-1 | | |
| | TB368B-TB-25-MR-2 X B11178G-TB-29 (E) | | |
| E1 | B14089H-Ng-90-(276-1)-1-1 | E3 | B14089H-Ng-95-(278-1)-1-1 |
| E2 | B14089H-Ng-10-(51-1)-1-1 | E4 | B14089H-Ng-2-(45-1)-1-1 |

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**The Genomic DNA Isolation**

Isolation of DNA was carried out using the CTAB method (Doyle & Doyle, 1987) by adding 2% of PVP and 1% mercaptoethanol. The isolation was started by weighing the 0.1 g of fresh leaf samples, which then put into 2 mL tube and crushed using liquid nitrogen and pestle until they changed into powder. Then CTAB buffer was added into the tube as much as 500 µL with an additional 2% (v/v) of PVP and 1% (v/v) of mercaptoethanol. The tube was then incubated in the incubator machine for 60 minutes at 65°C. After incubation, the 24:1 of chloroform: isoamyl alcohol was added into the tube. The tube was then centrifuged at 12,000 rpm for 15 minutes. After that, the supernatant was transferred to a new 1.5 mL tube with and additional isopropanol and 3M NaOAC. The DNA were then stored in the freezer of -20°C for 12 hours. The precipitated DNA pellet was washed using ethanol 70% twice. The washed pellet was aerated at room temperature for 2-3 hours. The DNA pellet was then dissolved in TE buffer with the addition of RNAse (10 mg/mL) and incubated for 60 minutes at 37°C. Next, the isolated DNA's quality and quantity were tested using the *Nano Photometer Implan* machine. The DNA resulted from this process was ready for the PCR running or storage in a freezer at a temperature of -20°C.

**Polymerase Chain Reaction (PCR)**

Amplification of isolated DNA from the leaves was carried out using a pair of forward and reverse primers. The PCR reaction was performed using *MyTaq RedMix Bioline* with a total of the reaction of 12.5 µl consisted of 6.25 µl *MyTag RedMix* buffer, 2 µl DNA, 0.5 µl primer forward, 0.5 µl primer reverse and 3.25 µl $dH_2O$. The PCR program was pre-denaturation at 94°C for 5 minutes, followed by 30 cycles which consist of denaturation temperature at 94°C for 1 minute, annealing 55-60°C (Table 3) for 1 minute and a 72°C of synthesis for 3 minutes. The primer elongation was 72°C for 10 minutes. PCR was performed using *Thermal Cycler GTC96S*, *Cleaver Scientific Ltd*, *UK*. The examination of DNA amplification performed by electroporation activity was carried out using the Cleaver machine. The PCR product was run on 2% agarose gel and colored using 4% *SYBR DNA Stain*. The machine was run at 50-volt power for approximately 1.5 hours. The DNA amplification result was visualized under *Syngene G: Box Gel Image Analysis System machine*.

**Data Analysis**

The molecular data for polymorphic band patterns were analyzed by comparing the pattern between the crossing parents and the control, which then continued to the progeny of its results. The band patterns which share the same patterns with Al susceptible control labeled with A. While those identical to Al tolerant control are B and the AB label for the progeny of the crossing result, which has two band patterns.

Table 3. Primer SSR used in the development of upland rice aluminum tolerance (Gramene.org)

| Primers | Sequences | Repeat motif | Chromosom | Allele size (bp) | TM (ºC) | Ref |
|---|---|---|---|---|---|---|
| RM4 | F:ttgacgaggtcagcactgac<br>R:agggtgtatccgactcatcg | (GA)16 | 12 | 159 | 60 | 4 |
| RM19 | F:caaaaacagagcagatgac<br>R:ctcaagatggacgccaaga | (ATC)10 | 12 | 226 | 55 | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| RM21 | F: acagtattccgtaggcacgg<br>R: gctccatgagggtggtagag | (GA)18 | 11 | 165 | 56 | 4 |
| RM23 | F:cattggagtggaggctgg<br>R:gtcaggcttctgccattctc | (GA)15 | 1 | 145 | 60 | 1 |
| RM25 | F:ggaaagaatgatcttttcatgg<br>R:ctaccatcaaaaccaatgttc | (GA)18 | 8 | 146 | 50 | 4 |
| RM105 | F: gtcgtcgacccatcggagccac<br>R:tggtcgaggtggggatcgggtc | (CCT)6 | 9 | 134 | 60 | 4 |
| RM126 | F:cgcgtccgcgataaacacaggg<br>R:tcgcacaggtgaggccatgtcg | (GA)7 | 8 | 171 | 60 | 2 |
| RM130 | F: tgttgcttgccctcacgcgaag<br>R: ggtcgcgtgcttggtttggttc | (GA)10 | 3 | 85 | 56 | 1 |
| RM147 | F:tacggcttcggcggctgattcc<br>R:cccccgaatcccatcgaaaccc | (TTCC)5(GGT)5 | 10 | 97 | 60 | 4 |
| RM168 | F: tgctgcttgcctgcttccttt<br>R: gaaacgaatcaatccacggc | T15(GT)14 | 3A | 116 | 56 | 2 |
| RM171 | F: aacgcgaggacacgtacttac<br>R: acgagatacgtacgcctttg | (GATG)5 | 10 | 328 | 55 | 4 |
| RM172 | F:tgcagctgcgccacagccatag<br>R:caaccacgacaccgccgtgttg | (AGG)6 | 7 | 159 | 60 | 4 |
| RM188 | F:tccgcctctcctctcgcttccc<br>R:gcaacgcacaaccgaaccgagc | (CA)8 | 5 | 210 | 60 | 4 |
| RM205 | F:ctggttctgtatgggaccag<br>R:ctggcccttcacgtttcagtg | (CT)25 | 9 | 122 | 55 | 4,5 |
| RM211 | F: ccgatctcatcaaccaactg<br>R: cttcacgaggatctcaaagg | (TC)3A(TC)18 | 2 | 161 | 56 | 2 |
| RM214 | F: ctgatgatagaaacctcttctc<br>R: aagaacagctgacttcacaa | (CT)14 | 7 | 112 | 60 | 4 |
| RM222 | F: cttaaatgggccacatgcg<br>R: caaagcttccggccaaaag | (CT)18 | 10 | 213 | 56 | 4 |
| RM232 | F: ccggtatccttcgatattgc<br>R: ccgacttttcctcctgacg | (CT)24 | 1 | 158 | 56 | 2 |
| RM247 | F:tagtggggatcgatgtaacg<br>R:catatggtttgacaaagcg | (CT)16 | 9 | 131 | 60 | 2,3,4,5 |
| RM254 | F:agccccgaataaatccacct<br>R:ctggaggagcatttggtagc | (CT)24 | 11 | 147 | 55 | 4 |
| RM257 | F:cagttccgagcaagagtactc<br>R:ggatcggacgtggcatatg | (CT)24 | 12 | 147 | 60 | 2,3,5 |
| RM262 | F:cattccgtctcggctcaact<br>R:cagagcaaggtggcttgc | (CT)16 | 2 | 154 | 60 | 4 |
| RM271 | F:tcagatctacaattccatcc<br>R:tcggtgagacctagagagcc | (GA)15 | 10 | 101 | 50 | 2 |
| RM277 | F:cggtcaaatcatcacctgac<br>R:caaggcttgcaagggaag | (GA)11 | 12 | 124 | 60 | 2 |
| RM279 | F: gcgggagagggatctcct<br>R: ggctaggagttaacctcgcg | (GA)16 | 2 | 174 | 56 | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| RM319 | F: atcaaggtacctagaccaccac<br>R: tcctggtgcagctatgtctg | (GT)10 | 1 | 134 | 56 | 3 |
| RM442 | F:cttaagccgatgcatgaagg<br>R:atcctatcgacgaatgcacc | (AAG)10 | 3A | 257 | 55 | 2 |
| RM473 | F: acaccaaccagatcagggag<br>R: tgctcgtcaatggtgagttc | (TCTA)14 | 5 | 97 | 56 | 2 |
| RM490 | F:atctgcacactgcaaacacc<br>R:agcaagcagtgctttcagag | (CT)13 | 1 | 101 | 60 | 2 |
| RM506 | F: cgagctaacttccgttctgg<br>R:gctacttgggtagctgaccg | (CT)13 | 8 | 123 | 56 | 2 |
| RM509 | F: tagtgagggagtggaaacgg<br>R: atcgtccccacaatctcatc | (TC)11 | 5 | 141 | 56 | 2 |
| RM514 | F:agattgatctcccattcccc<br>R:cacgagcatattactagtgg | (AC)12 | 3 | 259 | 55 | 1 |
| RM520 | F:aggagcaagaaaagttcccc<br>R:gccaatgtgtgacgcaatag | (AG)10 | 3A | 247 | 60 | 2 |
| RM555 | F:ttggatcagccaaaggagac<br>R:cagcattgtggcatggatac | (AG)11 | 2 | 223 | 55 | 2 |
| RM566 | F: acccaactacgatcagctcg<br>R: ctccaggaacacgctctttc | (AG)15 | 9 | 239 | 55 | 2 |
| RM569 | F:gacattctcgcttgctcctc<br>R:tgtcccctctaaaaccctcc | (CT)16 | 3 | 175 | 55 | 1 |
| RM580 | F:gatgaactcgaatttgcatcc<br>R:cactcccatgtttggctcc | (CTT)19 | 1 | 221 | 55 | 2 |
| RM6333 | F:agagaagacacggtggatgg<br>R:caaactcctcatttcgctcc | (GAA)8 | 1 | 102 | 60 | 3 |

Note: 1: (Akhmad, 2008); 2: (Prasetiyono et al., 2003) ; 3: (Famoso et al., 2011); 4: (Yuniarini, 2013); 5: (Anggraheni & Mulyaningsih, 2017)

## RESULTS AND DISCUSSION

The polymorphism analysis of SSR primers linked to Al tolerance in crossing parents and cross-progeny needs to be conducted. Prasetiyono et al. (2008) added that polymorphism among parents is needed to determine the primers used for progeny selection of each cross. The SSR primer was used for the selection of cross-progeny, which has the same band pattern with parents and Al tolerant control. The monomorphic primers cannot be used for selection activities because they cannot distinguish the contribution of alleles in one locus. According to Surahman (2002), the marker (primer) is effectively used if it can distinguish among parents with different genotypes and the marker must inherit to their cross-progeny. IR60080-32 and ITA131 are genotypes used as control. They have different Al tolerant level comparing to each other. IR60080-32 has a tolerance to Al, whereas ITA131 was susceptible to Al. Apriliani et al. (2017), in their study about the germination of upland rice against Al stress, showed that at 20 ppm Al concentration, IR60080-32 has a germination rate higher than genotype ITA131. Besides, for the long root-relative (RPA), IR60080-32 is the longest root compared to other genotypes. Wirnas et al. (2002) mentioned that the roots from Al tolerance plant could develop normally, the root can reduce the acidic around the rooting area, Al translocation into the upper canopy is less be-

cause Al is retained by roots and they are no hindrance absorption of Ca, Mg, P and K nutrients.

Electrophoregram PCR result of parent cross with control tolerant and susceptible as the comparison has shown that from 37 of SSR primers used nine primers showed polymorphism band pattern (Figure 1). The nine primers were then used for band pattern analysis on cross-progeny. Table 4 showed the result of band pattern analysis between parents and control. Band pattern analysis of crossing parent compared with control using 9 SSR primers showed that parents of B11930F-TB-2 and B11492F-TB-12 have the same band pattern to tolerant control of IR60080-32. Parents of TB368B-TB-25-MR-2, B11178G-TB-29, Situ Patenggang and Inpago 9 have the same band pattern with susceptible control ITA131 in the use of 9 selected SSR primers. Parents of both Inpago 8 and Danau Gaung have the same band pattern with tolerant control of IR60080-32 at the use of RM569 primer (Inpago 8) and RM490 (Danau Gaung) and for the use of other primer showed the same band pattern with susceptible control ITA131. Furthermore, parents who have the same band pattern with IR60080-32 tolerant control were analyzed furtherly.

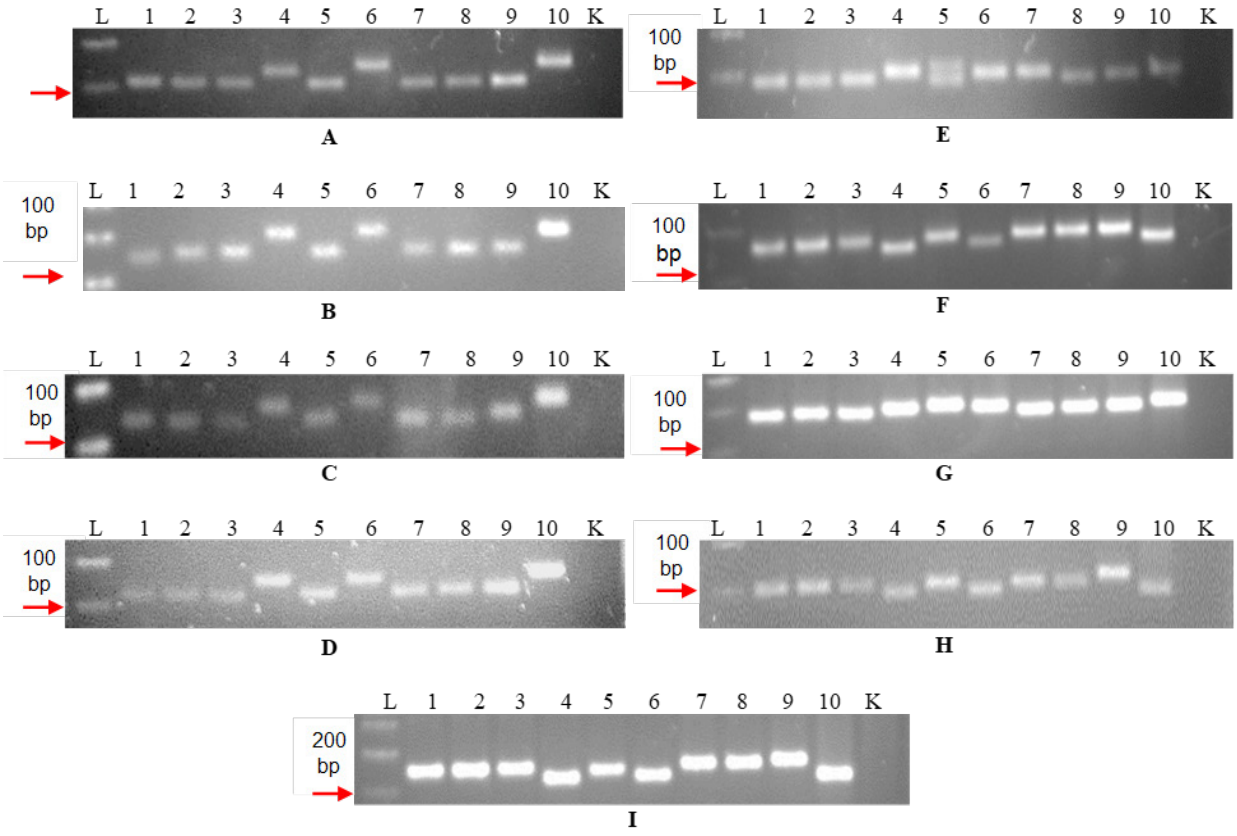


Figure 1. Electrophoregram polymorphism analysis of crossing parents and comparative controls. A: RM214, B: RM257, C: RM247, D: RM205, E: RM490, F: RM262, G: RM569, H: RM271 and I: RM19. Note: 1: TB368B-TB-25-MR-2, 2: B11178G-TB-29, 3: Situ Patenggang, 4: B11930F-TB-2, 5: Inpago 8, 6: B11492F-TB-12, 7: Danau Gaung, 8: Inpago 9, 9: ITA 131, 10: IR60080-32, K: control and L: Ladder

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Table 4. Polymorphism scoring analysis of crossing parents with selected SSR primers

| Parent | SSR primers | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | RM214 | RM257 | RM247 | RM205 | RM490 | RM262 | RM569 | RM271 | RM19 |
| TB368B-TB-25-MR-2 | A | A | A | A | A | A | A | A | A |
| B11178G-TB-29 | A | A | A | A | A | A | A | A | A |
| Situ Patenggang | A | A | A | A | A | A | A | A | A |
| B11930F-TB-2 | B | B | B | B | B | B | B | B | B |
| Inpago 8 | A | A | A | A | A | A | B | A | A |
| B11492F-TB-12 | B | B | B | B | B | B | B | B | B |
| Danau Gaung | A | A | A | A | B | A | A | A | A |
| Inpago 9 | A | A | A | A | A | A | A | A | A |
| ITA 131 | A | A | A | A | A | A | A | A | A |
| IR60080-32 | B | B | B | B | B | B | B | B | B |

Note: A: Band pattern same with susceptible Al control (ITA131) and B: Band patterns same with tolerant Al control (IR60080-32).

The selection of parents is highly important to be considered in plant breeding activities. The use of different parent among crosses produces cross-progeny that have different traits as well. According to Prasetiyono et al. (2003), in their research on the identification of microsatellite markers linked to Al in the crossing of DUPA X ITA131 stated that the use of different parent would affect the Al tolerance mechanism of the plant because many genes influence Al tolerance. The same statement was expressed by Miftahudin et al. (2005), Al tolerance in rice plants is controlled by many genes/QTL. Therefore, according to Nguyen et al. (2001), assembly of Al tolerant plants should contain many Al genes. In this study, 7 parents were used and 3 parents (B11178G-TB-29, B11930F-TB-2 and Danau Gaung) having the advantage of Al tolerance. Among the 37 SSR primers linked to Al tolerance from previous studies, 9 SSR primers were obtained, which showed polymorphism. The 9 primary SSRs are on chromosome 1 (RM 490), chromosome 2 (RM262), chromosome 3 (RM569), chromosome 7 (RM214), chromosome 9 (RM 205, RM247), chromosome 10 (RM271) and chromosome 12 (RM 19, RM 257).

The application of SSR in the identification of cross-progeny with crossing parents obtained precise results due to the absence of environmental factors. Surahman (2002) stated that the principle of genetic linkage determines the relationship between DNA markers. This is based on a view that a genome is organized and transmitted as a linear unit, a chromosome. Chromosomes are part of linkages of several locus positions in a linking group (Sobir & Syukur, 2015). Efforts to determine the locus and position of genetic markers are called genetic mapping that has essential effects in the study of organism genetics. Sobir & Syukur (2015) stated that genetic mapping is needed to study the function of genes and their position in chromosomes, the evolution of genes and the formation of new varieties. The visualized amplification band is considered as an allele. DNA bands that having the same rate movement is assumed as the same locus (Nugroho et al., 2019). A cross-progeny having the same band pattern with the parents and controlled

Al susceptible named by the letter A whereas that which has the same band pattern with the parents and controlled Al tolerant assigned by the letter B. This letter was used to facilitate in cross-progeny tracing which was expected to have the same character as their parents. In this case, the expected progeny is the crossed-progeny that is Al tolerant. Genetic knowledge in controlling locus plant tolerance against Al poisoning is the key to successful plant breeding programs (Tasma, 2015).

The analysis was carried out by applying selected primers on the cross-progeny (Figure 2) as electropherogram results. Applications of selected primers to cross-progeny lines with selected parents showed the same band pattern as IR60080-32 control (Table 5). The pattern of RM257 at Inpago 8 X B11930F-TB-2 cross indicate only 1 line with the AB's band pattern and in B11492F-TB-12 X B11178G-TB-29 cross with 1 line of B band pattern. At the same time, in the Situ Patenggang X B11930F-TB-2 cross did not obtain a progeny line that had a band pattern of tolerant control. In RM214 out of 12 lines, only 1 line had AB band pattern, which is in Situ Patenggang X B11930F-TB-2 cross, and in Inpago 8 X B11930F-TB-2 cross obtained four lines, e.g., AB, B, AB and AB crossing patterns.

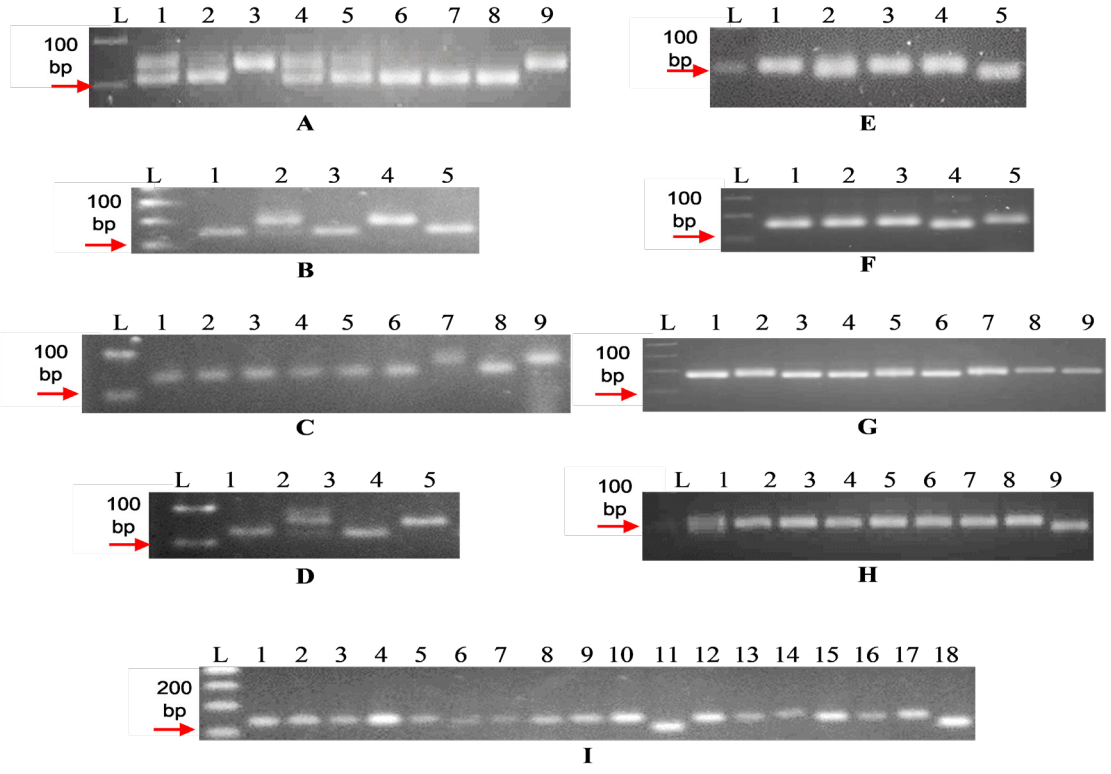


Figure 2. PCR electroporegram in cross-progeny using selected primers. A) RM214 applied in cross-progeny Inpago 8 X B11930F-TB2, B) RM257 applied in cross-progeny B11492F-TB-12 X B11178G-TB-29, C) RM247 applied in cross-progeny Inpago 8 X B11930F-TB-2, D) RM205 applied in cross-progeny B11492F-TB-12 X B11178G-TB-29, E) RM490 applied in cross-progeny B11492F-TB-12 X B11178G-TB-29, F) RM262 applied in cross-progeny B11492F-TB-12 X B11178G-TB-29, G) RM569 applied in cross-progeny Inpago 8 X B11930F-TB-2, H) RM271 applied in cross-progeny Inpago 8 X B11930F-TB-2 and, I) RM19 applied in cross-progeny Situ Patenggang X B11930F-TB-2. Note: L: ladder and two last number are comparative controls

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Table 5. Selected primers application in cross-progeny with the selected parents

| Primers | Cross | Cross-progeny | Band pattern |
|---|---|---|---|
| | Situ Patenggang X B11930F-TB-2 | B2 | AB |
| RM214 | Inpago 8 X B11930F-TB-2 | D1,D3,D4,D5 | AB,B,AB,AB |
| | B11492F-TB-12 X B11178G-TB-29 | - | - |
| | Situ Patenggang X B11930F-TB-2 | - | - |
| RM257 | Inpago 8 X B11930F-TB-2 | D5 | AB |
| | B11492F-TB-12 X B11178G-TB-29 | C2 | B |
| | Situ Patenggang X B11930F-TB-2 | B5,B6,B7,B12 | B,AB,AB,B |
| RM247 | Inpago 8 X B11930F-TB-2 | D7 | B |
| | B11492F-TB-12 X B11178G-TB-29 | - | - |
| | Situ Patenggang X B11930F-TB-2 | - | - |
| RM205 | Inpago 8 X B11930F-TB-2 | D8,D9,D12 | B,B,B |
| | B11492F-TB-12 X B11178G-TB-29 | C2 | B |
| | Situ Patenggang X B11930F-TB-2 | - | - |
| RM490 | Inpago 8 X B11930F-TB-2 | D1,D3,D4,D6,D7 | B,B,B,B,B |
| | B11492F-TB-12 X B11178G-TB-29 | C1,C2,C3 | B,B,B |
| | Danau Gaung X Situ Patenggang | A1,A2,A3,A4,A5 | B,B,B,B,B |
| | Situ Patenggang X B11930F-TB-2 | B1 | B |
| RM262 | Inpago 8 X B11930F-TB-2 | D5 | AB |
| | B11492F-TB-12 X B11178G-TB-29 | C1,C2,C3 | B,B,B |
| | Situ Patenggang X B11930F-TB-2 | - | - |
| RM569 | Inpago 8 X B11930F-TB-2 | D1,D2,D3,D4,D5,D6,D7 | B,B,B,B,B,B,B |
| | B11492F-TB-12 X B11178G-TB-29 | - | - |
| | Situ Patenggang X B11930F-TB-2 | B2,B5,B11 | B,B,B |
| RM271 | Inpago 8 X B11930F-TB-2 | D1 | AB |
| | B11492F-TB-12 X B11178G-TB-29 | - | - |
| | Situ Patenggang X B11930F-TB-2 | B11 | B |
| RM19 | Inpago 8 X B11930F-TB-2 | D1,D2,D4,D5 | B,B,B,B |
| | B11492F-TB-12 X B11178G-TB-29 | C1 | B |

Note: A: Band pattern same with susceptible Al control (ITA131), B: Band pattern same with tolerant Al control (IR60080-32) and AB: Band pattern same with susceptible and tolerant controls

Furthermore, the crossing of B11492F-TB-12 X B11178G-TB-29 did not obtain progeny lines that had tolerant control band patterns. In the primer application of RM247, two crosses were Situ Patenggang X B11930F-TB-2, which has four lines with the B, AB, AB, B band pattern and Inpago 8 X B11930F-TB-2 showed only 1 line with the B band pattern. In progeny lines that have tolerance band patterns using RM205 have been discovered three lines with B, B, B patterns in Inpago 8 X B11930F-TB-2 crosses and 1 line with B pattern in B11492F-TB-12 X B11178G-TB-29 crosses. For the use of RM490 from 4 types of crosses was gained three crosses that have a tolerant B pattern, namely: Inpago 8 X B11930F-TB-2 (5 lines), B11492F-TB-12 X B11178G-TB-29 (3 lines) and Danau Gaung X Situ Patenggang (5 lines).

While in primer application of RM262 crosses that have a tolerant band pattern are Situ Patenggang X B11930F-TB-2 as much as 1 line (B pattern), Inpago 8 X B11930F-TB-2 as much as 1 line (AB pattern) and B11492F-TB-12 X B11178G -TB-29 as much as 3 lines (B pattern). Then in RM569, there is only one cross, namely Inpago 8 X B11930F-TB-2, which has a tolerant band pattern as much as 7 lines with a B band pattern. Following primers, RM271, showed that in the crossing of Situ Patenggang X B11930F-TB-2, obtained three lines with band pattern B, and Inpago 8 X B11930F-TB-2 obtained 1 line with AB band pattern. The last primers, RM19 was applied to 3 crosses, which showed that the crossing of Situ Patenggang X B11930F-TB-2 and B11492F-TB-12 X B11178G-TB-29 obtained one line each with the B band pattern, and at the crossing of Inpago 8 X B11930F -TB-2 obtained four lines with B band pattern.

Yuniarini (2013), in her research on the exploration of microsatellite markers related to Al tolerance in rice, mentioned that based on the use of 47 SSR primers in cross-progeny Cabacu X IR64 showed 4 polymorphic SSR primers having the same band pattern between parents and the cross-progeny. The bulk 1 band (Al tolerant) follows the Cabacu parents (A pattern) and bulk 2 (Al sensitive) follows the IR64 parent band (B pattern). Moreover, Anggraheni & Mulyaningsih (2017) reported the use of 40 SSR primers linked to Al tolerance in 36 cross-progeny produced from Situ Patenggang X B11930F-TB-2 crossing obtained 3 polymorphic SSR primers. According to Waghmare et al. (2018), the objective of polymorphic genetic markers application in plant breeding activities, one of which is for parentage determination and another is to identify genetic markers and map loci affecting quantitative traits to monitor these loci during introgression breeding programs.

Rice is a staple food in Indonesia and needs essential attention to improve. The selected SSR primers linked Al tolerance in this research can be used as a reference for molecular breeding strategies to develop new Al tolerance rice varieties in dryland conditions. Also, nine selected polymorphic SSR primers are highly useful to enrich the varieties of marker that linked to Al in upland rice.

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