January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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Backcrossing of soybean lines containing aluminium tolerance gene into superior soybean variety, Biosoy

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Saptowo J. Pardal, Amalia Prihaningsih, Suharsono, et al.

Backcrossing of Soybean Lines Containing Aluminium Tolerance Gene into Superior Soybean Variety, Biosoy

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Abstract. Two new superior varieties of soybeans, Biosoy 1 and Biosoy 2 have the advantage of high productivity and bigger seeds than favorite local variety Grobogan. These varieties have been planted in several provinces in Java and showed a good performance. However, these varieties are not so adaptive in acid soil with high aluminum content. Therefore, to expand the planting area of Biosoy varieties outside Java, which is generally an acidic land with high Al content, it is necessary to improve its tolerance to Al stress. Two transgenic soybean lines (GM2 and GM5) carrying the Al tolerance gene (*MaMt*2) have shown tolerance to acidic soils pH 3.7–4.8 based on evaluation in the Biosafety Containment. Those transgenic lines have been crossed with the Biosoy variety. F_1 soybean plants carrying the *MaMt2* gene were then backcrossed to the Biosoy variety to produce BC_1F_1 seeds. The aim of this research was to do molecular confirmation of *MaMt*2 gene insertion in the BC_1F_1 soybean plants and backcrossing the positive BC_1F_1 to Biosoy 1 variety. Materials used for the research were seven plants of BC_1F_1-GM2, six plants of BC_1F_1-GM5, and Biosoy 1 variety. The research works included PCR analysis of BC_1F_1 plants, backcrossing of BC_1F_1-*MaMt*2 plants with Biosoy 1, and harvesting of BC_2F_1 seeds. The PCR analysis of 13 samples of BC_1F_1 plant showed that all samples were positive carrying the *MaMt*2 gene (100%). BC_2F_1 seeds have been produced from the second backcross. These BC_2F_1 soybean seeds will be used as backcross material in the next studies.

INTRODUCTION

The Ministry of Agriculture has released two new superior soybean varieties, namely Biosoy 1 and Biosoy 2, in 2018. Both varieties have the advantage of larger seed sizes than the favorite local variety, Grobogan, and high productivity of 3.3–3.5 t/ha [1]. However, these two new elite varieties are less adaptive in acidic soil with high aluminum (Al) content [2]. Therefore, to be planted more widely on acidic lands outside Java which generally contain high Al, they need to be improved for the Al tolerance character.

Plant varieties that are tolerant to Al stress can be produced through conventional breeding (ordinary crosses) or modern breeding (genetic transformation). Several Al tolerant varieties of soybean have been produced through conventional breeding, such as Slamet [3], Demas, Anjasmoro, and Tanggamus [4, 5]. In addition, four transgenic soybean lines, namely GM2, GM5, GM10, and GM14 have been produced through the insertion of the *MaMt*2 gene, an Al tolerant gene, into Lumut variety with the help of *Agrobacterium tumefaciens* [6]. Lumut is a soybean variety that has a small grain (13 g/100-seed), low productivity (2.5 t/ha), and susceptible to acid soil and high Al contain [7, 8].

The *MaMt*2 gene is a gene encoding Metallothionein type 2 protein which has the ability to bind heavy metals, including Al [9]. Two *MaMt*2 transgenic soybean lines, namely GM2 and GM5, showed tolerance to acidic soil with

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a pH of 3.7–4.8 based on test at the Biosafety Containment of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) [8]. However, these soybean lines came from the Lumut variety which has small seed size, low productivity and is intolerant to acid soil. Therefore, they need to be crossed with the high yielding superior soybean varieties, Biosoy to produce soybeans with high productivity and tolerance to acidic land with high Al.

In previous research, transgenic *MaMt*2 soybean lines have been crossed with Biosoy varieties to insert the *MaMt*2 gene into Biosoy. Confirmation through PCR analysis using specific primers for the *MaMt*2 gene showed that most of the F_1 plants carried the *MaMt*2 gene. The positive F_1 soybean plants carrying the *MaMt*2 gene were then backcrossed to the Biosoy variety and produced the BC_1F_1 soybean seeds [2].

In this study, the BC_1F_1 soybean seeds were planted in a screen house of the Biosafety Containment for molecular confirmation of *MaMt*2 presence, then the positive BC_1F_1 soybean plants carrying the *MaMt*2 gene were backcrossed to Biosoy 1 to produce BC_2F_1 seeds.

MATERIALS AND METHODS

Plant Materials

Plant materials used were BC_1F_1 and Biosoy 1 soybean seeds. The research was conducted at the screen house of the Biosafety Containment, ICABIOGRAD, in January–May 2021.

Molecular Confirmation of BC_1F_1 Plants

The research included molecular confirmation of BC_1F_1 soybeans plants, backcrossing of BC_1F_1-*MaMt*2 with Biosoy 1, and harvesting of BC_2F_1 soybean seeds. Twenty-four soybean seeds of BC_1F_1-GM2, 21 soybean seeds of BC_1F_1-GM5 and Biosoy 1 seeds were planted in 5 kg bucket pots with 1:1 soil and compost mixed media and then maintained in the screen house. Planting was carried out in stages with 2–3 days of intervals. The male parents were planted first to attain the same flowering time. Plants were fertilized with NPK according to the recommended dosage for soybeans, and pest and disease control was carried out when necessary.

The molecular analysis using PCR technique was carried out to determine the success of backcross. The positive plants carrying the *MaMt2* gene indicated plants were the result of crossing, not the result of self-pollination. Molecular analysis began with the extraction of BC_1F_1 plant genomic DNA using the Extract-N-Amp plant kits on leaf samples. The concentration of extracted DNA was measured by NanoDrop at the absorbance of α-260 and α-280. DNA samples were then carried out for PCR analysis with MyTaq^{TM} HS Red Mix PCR kits. The 5 µg template DNA was inserted into the PCR tube then was added with 1× PCR mix, 2 µl ddH_20, 0.5 mM of forward and reverse primers specific for *MaMt*2 gene (SMt2F-SMt2R), and vortexed until evenly distributed. The PCR program was pre-heating 94°C for 5 min, 25 cycles of 94°C for 60 sec, followed by annealing at 58°C for 60 sec, and ended with an extension at 72°C for 1 min 40 sec. The last stage was post-extension at a temperature of 72°C for 5 min. The cooling stage was carried out at a temperature of 10°C for 30 min [10].

The amplicons were visualized on 1.5% gel agarose electrophoresis. The 5 µl DNA sample was electrophoresed at 70 volts with a current of 300 mA for 60 min. The gel electrophoresis was immersed in ethidium bromide, then visualized with UV transilluminator (λ = 320 nm).

Backcrossing of BC_1F_1-*MaMt*2 × Biosoy 1

Backcrossing was carried out between the Biosoy 1 (female parents) with BC_1F_1-*MaMt*2 (male parent). The flowers from the crosses were maintained and observed. Pods produced were then maintained and observed until they were ready to be harvested. The BC_2F_1 seeds were then collected and processed properly for further research.

The data analysis for the results of the PCR analysis was the presence or absence of DNA bands with a size equivalent to the *MaMt*2 gene (positive control). Meanwhile, the observation variables for the result of backcrossing was the number of flowers that were crossed, the number of fallen flowers (unsuccess crosses), the number of flowers that succeeded (did not fall), the number of pods resulting from the crosses that were formed and the number of BC_2F_1 seeds produced.

RESULTS AND DISCUSSIONS

Molecular Confirmation of BC_1F_1 plants

Fig. 1 showed that not all of the BC_1F_1 soybean seeds from GM2 and GM5 grew well in the screen house. Only seven plants from 24 BC_1F_1-GM2 soybean seeds planted and six plants from 21 BC_1F_1-GM5 soybean seeds planted grew well in screen house. Molecular analysis by PCR was carried to determine the presence or absence of the *MaMt*2 gene. Table 1 showed the data of all thirteen BC_1F_1 soybeans.

TABLE 1. The BC_1F_1 soybean plants that grew well in the screen house of Biosafety Containment, ICABIOGRAD.

No.	Code of BC_1F_1 plants	Transgenic events
1	BC_1F_1 [Bio1 X (F1Bio1 × GM2-11) 1-1]	GM2
2	BC_1F_1 [Bio1 X (F1Bio1 × GM2-16) 1-1]	GM2
3	BC_1F_1 [Bio1 X (F1Bio1 × GM2-16) 1-4]	GM2
4	BC_1F_1 [Bio1 X (F1Bio1 × GM2-16) 2-3]	GM2
5	BC_1F_1 [Bio1 X (F1Bio1 × GM2-16) 3-6]	GM2
6	BC_1F_1 [Bio1 X (F1Bio1 × GM2-16) 4-2]	GM2
7	BC_1F_1 [Bio1 X (F1Bio1 × GM2-16) 4-5]	GM2
8	BC_1F_1 [Bio1 X (F1Bio1 × GM5-11) 1-1]	GM5
9	BC_1F_1 [Bio1 X (F1Bio1 × GM5-6) 1-1]	GM5
10	BC_1F_1 [Bio1 X (F1Bio1 × GM5-6) 2-1]	GM5
11	BC_1F_1 [Bio1 X (F1Bio1 × GM5-6) 2-2]	GM5
12	BC_1F_1 [Bio1 X (F1Bio1 × GM5-6) 3-1]	GM5
13	BC_1F_1 [Bio1 X (F1Bio1 × GM5-6)]	GM5

FIGURE 1. Performance of Biosoy 1, BC_1F_1-GM2, and BC_1F_1-GM5 soybean plants in the screen house of Biosafety Containment, ICABIOGRAD (3 weeks after planting).

The PCR analysis of 13 DNA samples of BC_1F_1 plant from GM2 and GM5 (sample no. 1–13) showed that all samples amplified 280 bp DNA fragment which is expected to be part of *MaMt2* gene (Fig. 2). The sample no. 5 showed very thin DNA band which may indicate very small amount of the gene was amplified. Meanwhile, the PCR result of the negative control (sample N) did not produce DNA bands of the *MaMt2* gene. These results indicate that the backcrossing-1 process was successful (100%) in still carrying the gene of interest.

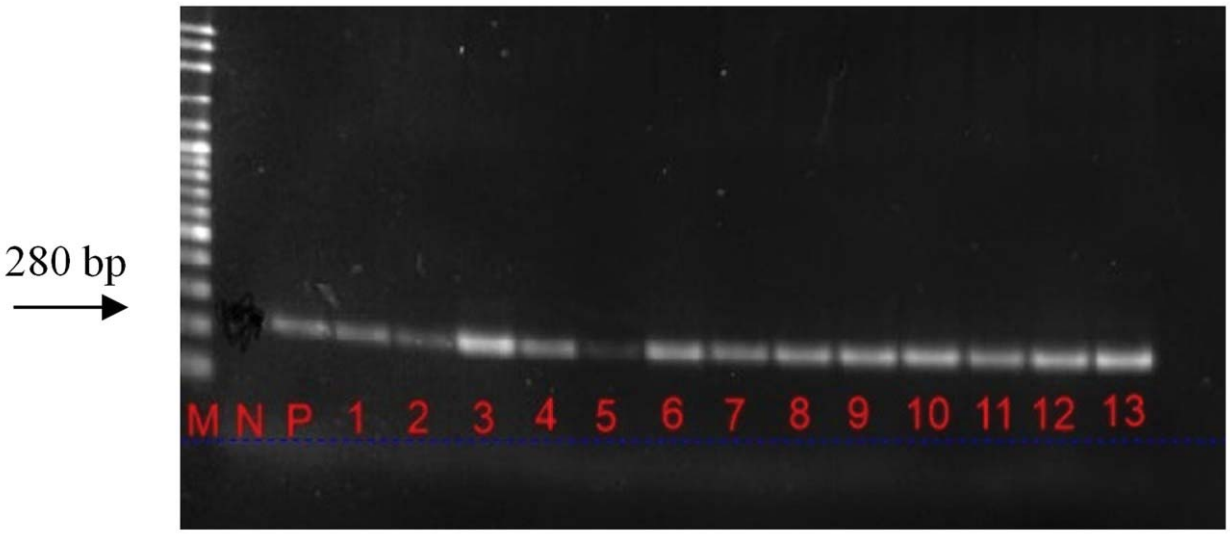

FIGURE 2. Visualization of PCR amplicon of thirteen BC_1F_1 soybean samples on 1% gel electrophoresis. M = 1 kb marker, N = negative control, P = positive control, 1–13 = samples.

Backcrossing of BC_1F_1-*MaMt2* × Biosoy 1

The BC_1F_1 soybean plants carrying the *MaMt2* gene were maintained in the screen house together with Biosoy 1 plants. Backcrossing was carried out between BC_1F_1-*MaMt2* plants as male parents and Biosoy 1 variety as female parents. The results showed that not all backcrossed flowers could develop into soybean pods. Preliminary data showed that 30 pods (41.1%) and 59 BC_2F_1 seeds were obtained from 73 Biosoy 1 flowers crossed with flowers from BC_1F_1-GM2 plants, and 20 pods (37.0%) and 35 BC_2F_1 seeds were generated from 54 Biosoy 1 flowers crossed with BC_1F_1-GM5 plants (Table 2). The BC_2F_1 seeds obtained were dried and stored for the next backcrossing. This result was consistent with the statement that the percentage of successful crossing of soybeans is very low (less than 40%) [11]. The success rate of artificial pollination followed by fertilization is influenced by several factors, including the skill of the breeder, compatibility of the varieties, receptive time, and anthesis time and environmental factors [12]. Another reason of the low success rate of crosses is the improper crossing time [13].

The pods obtained from the crosses were maintained until ripe, harvested, and stored for further studies (Fig. 3).

TABLE 2. Data of backcrossing BC_1F_1-*MaMt2* × Biosoy 1 plants in the screen house of Biosafety Containment, ICABIOGRAD.

Backcross	Number of Biosoy 1 bud	Number of succeed bud	Number of unsucceed bud	Number of pod	Number of BC_2F_1 seeds
BC_1F_1-GM2 × Biosoy 1	73	30 (41.1%)	43 (58.9%)	30 (41.1%)	59
BC_1F_1-GM5 × Biosoy 1	54	20 (37.0%)	34 (63.0%)	20 (37.0%)	35
TOTAL	**127**	**50 (39.4%)**	**77 (60.6%)**	**50 (39.4%)**	**94**

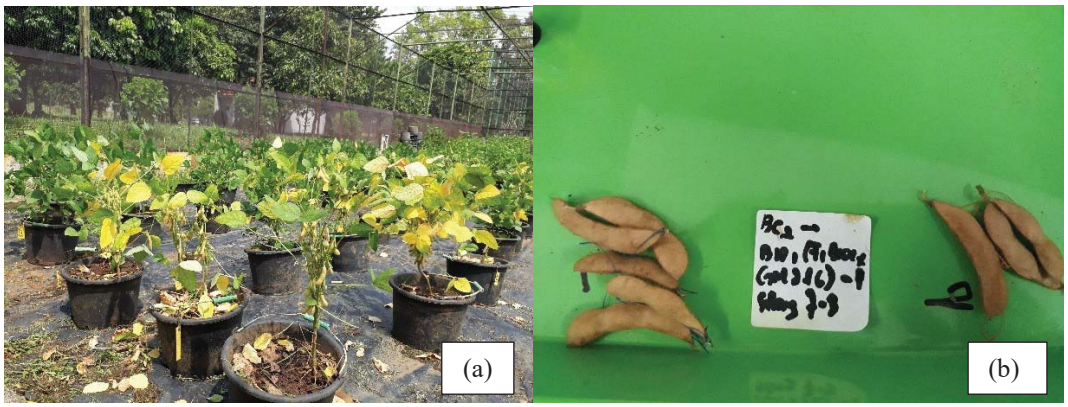

FIGURE 3. Result of backcrossed-2 of BC_1F_1-*MaMt*2 × Biosoy 1. (a) Biosoy 1 plants after backcrossed starting to mature. (b) Matured pods resulting from backcrossed-2.

CONCLUSION

The first backcrossing of soybean plants, F1-*MaMt*2 × Biosoy 1 variety, successfully produced thirteen BC_1F_1 plants containing the *MaMt*2 gene, confirmed by PCR analysis. The second backcrossing of BC_1F_1-*MaMt*2 × Biosoy 1 variety successfully produced BC_2F_1 soybean seeds with the success crossing percentage of 41.1% for BC_1F_1-GM2 and 30% for BC_1F_1-GM5.

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