Diversity of 33 Genotypes of Potato Revealed by Simple Sequence Repeats Markers

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***Abstract*—Genetic diversity analysis is essential for developing newly high-yielding potato varieties along with other important traits. Simple Sequence Repeats (SSR) markers can be robust tools to assess the genetic diversity of this plant species because they are abundant in the genome, codominant, and more accurate than morphological markers. This study was carried out to quantify the genetic divergence of 29 elite potato varieties in Indonesia and 4 clones that originated from the United States using the 12 SSR markers and identify the potential genotypes for potato breeding programs. A total of 136 gene alleles were detected from 12 SSR markers. The number of alleles per marker ranged from 2 to 22, with an average value of 12.8. All SSR markers showed Polymorphism Information Content (PIC) of 0.66-0.92, with an average of 0.80, and an average value of genetic diversity of 0.82, indicating their high suitability for potato diversity studies. Clustering and principal coordinate analysis classified 33 genotypes into three groups with a coefficient of similarity of 0.76, indicating their high genetic variability. All clones originating from the United States belonged to the same group and separated from the other genotypes. This study gives an overview of the genetic diversity of the Indonesian potato and provides an initial basis of selection for appropriate parents to assist breeders efficiently in developing newly potato varieties with desired agricultural traits in Indonesia.**

***Index Terms*—genetic diversity, molecular, SSR markers, genotype, potato**

I. Introduction

Potato is one of the most essential non-cereal food crops globally [1]. Potato can be cultivated in several world regions with a wide range of environmental conditions due to its diversity and resourcefulness [2]. However, cultivated potatoes in Indonesia have still produced lower yields than other potato-producing countries. Its supply is not sufficient due to a large increased Indonesian population. Several factors may limit potato productivity in Indonesia, i.e., conversion of agricultural land to non-agriculture, low soil fertility levels, and biotic stresses such as pests and plant diseases [3]. Demand for potatoes in Indonesia from 2015 to 2019 continued to increase, as evidenced by the average growth of potato consumption by households of 6.06%. In addition, the potato processing industry also contributes to an increase in potato demand by producing 20-30 t/day [4]. High demand for potatoes as a result of the modern lifestyle in Indonesia. However, the rise in demand for potatoes was unbalanced with an increase in potato productivity, whose average growth only increased by 2.28% from 2015 to 2019. Unbalancing consumption demand and production of potatoes will force the government to continue importing potatoes. Therefore, the development of superior potato varieties with high productivity, disease resistance, and suitability for cultivation in Indonesia needs to be implemented as a current breeding strategy [5].

The availability of plant genetic resources is a significant prerequisite in assembling new superior varieties [6]. Efforts to increase that availability can be made through the introduction of varieties abroad, mutation, crossing, exploration, and genetic engineering. Information on genetic diversity from each germplasm collection can be used as the basis for breeders in developing new varieties because it provides information on the population structure, patterns of genomic differentiation, which are important for plant breeding applications [7]. Estimating genetic diversity value is also important in the evaluation, conservation, and utilization of genetic resources. The plant genetic diversity had been investigated using several markers, including morphological, biochemical, and molecular. The approach using morphological markers has weaknesses, requiring a long time, relatively expensive, environmental influences, and limited diversity. To overcome those drawbacks, molecular markers are used. Currently, molecular markers

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are the most widely used for genetic resource characterization due to their rapidness and quality data produced, including in potato [6]

Estimating genetic diversity value using molecular markers allows us to quantify and determine the genetic variation rate more precisely and can be used to distinguish individual genotypes [8]. Simple Sequence Repeats (SSR) is one of the most common and powerful polymerase chain reaction-based molecular markers used for genetic diversity study. SSR markers can efficiently facilitate the establishment of genetic linkages due to their high polymorphism level, codominant inheritance, high allele diversity level, randomly wide distribution in the genome, multi-allele, and experimentally reproducible and transferable among related species [9], [10]. Some molecular markers have been applied to the genetic diversity study on potatoes in Indonesia [5], [11]-[15], but only few reported with SSR markers. Therefore, this present study was aimed to quantify the genetic diversity among 29 elite potato varieties in Indonesia and 4 clones that originated from the United States using the 12 SSR markers and to identify the potential genotypes for potato breeding programs.

II. Material and Methods

A. Genetic Materials

A total of 33 genotypes of potato consisting of 29 elite potato varieties in Indonesia and 4 clones that originated from the United States obtained from Indonesian Vegetable Research Institute, Indonesian Agency for Agricultural Research and Development were used in this study. The 29 elite potato varieties were predominantly improved varieties in Indonesia (22 genotypes) and the remaining were introduced from Germany, Peru and the United States. The details of each genotype are described in Table I.

TABLE I. List of 66 Genotypes of Potato Used in This Study

No	Genotype	Status	Pedigree/Country
1	Medians	IL	Atlantik x 393284.39
2	Andina	IL	391580.30 x 385524.9
3	2015-66	IL	-
4	Cipanas	IL	Thung 1510 x Desiree
5	Spudy Agrihorti	IL	Atlantik x Repita
6	Granola L	I	Germany
7	Maglia	IL	Atlantik x 391058.175
8	GM 08	IL	Granola x Michigan Ping
9	Sangkuriang Agrihorti	IL	Granola x Katahdin
10	Atlantik Malang	I	United States
11	Kastanum	IL	393077.54 x 391011.17
12	AR 08 Agrihorti	IL	Atlantik x Repita
13	Vernei	IL	391011.17 x 385524.9
14	Papita Agrihorti	IL	Atlnatik x Granola
15	Oilimpus Agrihorti	I	-
16	Kikondo	I	CIP Peru
17	Amudra	IL	Shepody x Ritex
18	Erika	I	-
19	Margahayu	IL	Hertha x FLS-17
20	Amabile	IL	Atlantik x 393280.64
21	Merbabu 17	IL	IP 81001-1 x MF-1
22	Dayang Sumbi Agrihorti	IL	Granola x Katahdin
23	Tedjo MZ	IL	Granola
24	GM 05	IL	Granola x Michigan Ping
25	Repita	I	CIP Peru
26	Tenggo	I	CIP Peru
27	Ping 06	IL	Granola x Michigan Ping
28	PM 77.1 Granola	IL	Granola
29	Cosima	I	West Germany
30	MS 1.1	I	United States
31	MS 1.2	I	United States
32	MS 2.1	I	United States
33	MS 2.2	I	United States

I = Introduction, IL = Improved line

B. Genomic DNA Extraction and Amplification Using SSR Markers

Total genomic DNA was extracted from young fresh and healty leaves using the modified Cetyl Trimethyl Ammonium Bromide (CTAB) extraction protocol [16]. The grinded fresh tissues of leaves were put on 2 ml micro tube, followed by the addition of the extraction buffer to adjust up to 1 ml. The samples were incubated at 65°C for 15 minutes, extracted twice using chloroform: isoamyl alcohol solution (24:1), and centrifuged at a speed of 12,000 rpm for 10 min at 20°C. The supernatant was transferred to the new micro tube. Furthermore, 3M sodium acetate pH 5.2 was added as many as 1/10 of supernatant volume and followed by the addition of cold isopropanol as much as one supernatant volume. After incubating the mixture at -20°C for one hour, it was centrifuged at 12,000 rpm for 10 min at 20°C. The DNA pellets were then washed using 70% ethanol and dried with DNA Speed Vac Concentrator (ThermoScientific, USA). The dried pellets of DNA were dissolved with TE buffer and added with RNAase for eliminating RNA contaminant. The quality and quantity of DNA were determined using NanoDrop™ 2000 spechtrophotometer (Thermo Scientific, USA).

The genomic DNA of samples were subjected to PCR amplification using 12 SSR markers collected from Indonesian Agricultural Genome Center database (https://genom.litbang.pertanian.go.id) as primers (Table II). The PCR amplification was performed in 10 µL of reaction mixture, containing 2 µL of 10 ng/µL DNA template, 5 µL of 2x MyTaq HS (Bioline, UK), 0,5 µL of each primer, and sterilized ddH_2O. PCR profiling was set

up in a T1 thermocycler (Biometra, Germany) with initial denaturation temperature of 95°C for 5 min followed by 35 cycles of denaturation (94°C for 30 s), annealing (55°C for 1 min), extension (72°C for 1 min), and final extension (60°C for 15 min). The PCR products together with a 100 bp DNA ladder (Thermo Scientific, USA) were then separated on 6% vertical polyacrylamide gel electrophoresis in a tank containing 1 × Tris borate EDTA (TBE) buffer at 80 V for 1.5 h. Furthermore, that polyacrylamide gel was stained using ethidium bromide and observed under UV light using a UV Transilluminator (Biorad, USA).

TABLE II. LIST OF 12 SSR MARKERS WITH THEIR SEQUENCES

SSR markers	Sequences (5'-3')	Chr.	Ann. Temp. (°)
StSSR2.2	F-TCATGTGCAGTTTGATATGTG R-TCGGTACCAGGAATAGTCATA	2	52.1 51.0
StSSR3.1	F-GTGATCTTGATGTTGCCTAAC R-TCCTGGAGTTGCTCTATTATG	3	51.5 51.4
StSSR3.2	F-TGATACAAAAGTGTAGCATTCA R-TGTTTCCAGAAACCCTAGATT	3	50.0 51.3
StSSR4.2	F-TTATCTTCATTTAGCCACGAA R-CAATTGCTCTCATAAGTCCTG	4	49.4 51.0
StSSR5.1	F-AATTGAGTGACCATTGAGAAA R-GACTAATCAATTTCCATCTCG	5	49.9 48.7
StSSR6.2	F-TACTAGATGGCAAAACCGTAG R-CGCATATGCATAATCTCATTT	6	51.9 48.8
StSSR8.2	F-AAATTGATGACAGTGGAGATG R-TAGAACTCAATCAACCTTGGA	8	50.5 51.1
StSSR9.1	F-TTTCGACATAATCACACAACA R-ATTGTAGCATTGAGGGTCTTT	9	50.0 51.9
StSSR9.2	F-CAAGTTTAGGGATGTTTTTGA R-AGAAGGAGCACCTCACTTTAT	9	48.7 53.3
StSSR11.1	F-TTCAGAACCGTTAACTTCAAA R-AATAAAGCTGCTTGTGTATGC	11	50.0 51.5
StSSR12.1	F-TTTTGTTAAAAGAACGTCACC R-CCTGTACATCAATTCTGCACT	12	49.4 52.6
StSSR12.2	F-TAGATTTTGAGCAGGAAATTG R-CCATATAGGCGACACTGATTA	12	48.6 51.5

http://genom.litbang.pertanian.go.id

C. SSR Allele Scoring and Statistical Analysis

The unambiguous amplicon DNA products visualized of each SSR markers on all individual samples were scored according to their allelic band patterns by digital binary numbers depending on the numbers of fragments, and SSR allelic size was determined using Gel Analyzer [17]. Those assigned scores were used as genotype codes for genotyping different co-dominant alleles. Accordingly, all samples were then scored for each polymorphic SSR markers as a result of bi-allelic combination. Numbers of alleles detected, main alleles frequency, genes diversity, heterozygosity and Polymorphism Information Content (PIC) of each SSR markers were calculated using PowerMarker v. 3.25 software [18].

A data matrix file for clustering analysis was generated by NTedit version 1.04. Similarity coefficients based on Simple Matching (SM) coefficients were estimated using SIMQUAL module in NTSYS-pc v. 2.10e software [19]. Furthermore, a dendrogram of phylogenetic was constructed for clustering analysis using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) through Sequential Agglomerative Hierarchical Non-overlapping (SAHN) module in NTSYS-pc v. 2.10e software. Principal Coordinates Analysis (PCoA) was also carried out using XLSTAT 2020 trial version [20]. The PCoA analysis was used to visualize genetic relationship and to compare the conformity between clustering analysis result.

III. RESULTS AND DISCUSSION

A. Polymorphism Analysis of SSR Markers

All SSR markers showed polymorphism in all genotypes. One hundred forty-five alleles were detected by 12 SSR markers (Table III). Total alleles detected were higher than 46 alleles detected by Carputro *et al.* (2013) [21], and 60 alleles reported by Nugroho *et al.* (2015) [13]. However, this result was lower than Liao and Guo (2014) [22] reported, with 304 alleles. Increasing total genotypes and markers used in the study was followed by increasing total alleles detected and is in good agreement with previous study [23].

The number of alleles detected per locus ranged from 6 (StSSR4.2), to 22 alleles (StSSR12.1) with an average of 12.08 alleles per locus. The average number of alleles per locus observed in the current study was higher than 5.3 and 8.9 alleles per locus as reported by Rosa *et al.* (2010) [24] and Nugroho *et al.* (2019) [5], respectively. The variability in the number of alleles detected per locus is probably due to the use of different genotypes and molecular markers [8].

The frequency of main alleles per locus varied from 0.16 (StSSR3.1) to 0.52 (StSSR9.2) with a mean of 0.28. Greater value of main alleles frequency produced by each SSR locus suggests the usefulness of SSR marker used for detecting genetic polymorphisms among varied genotypes [25].

TABLE III. SUMMARY STATISTICS OF 33 GENOTYPES OF POTATO USING 12 SSR MARKERS

SSR markers	Number of alleles detected	Main alleles frequency	Genes diversity	Heterozygosity	PIC
StSSR2.2	11	0.19	0.88	0.41	0.87
StSSR3.1	20	0.16	0.91	0.47	0.90
StSSR3.2	12	0.22	0.87	0.31	0.85
StSSR4.2	6	0.36	0.72	0.03	0.67
StSSR5.1	18	0.17	0.91	0.70	0.90
StSSR6.2	10	0.32	0.81	0.15	0.79
StSSR8.2	16	0.20	0.88	0.72	0.86
StSSR9.1	7	0.31	0.78	0.21	0.75
StSSR9.2	8	0.52	0.69	0.45	0.66
StSSR11.1	7	0.39	0.76	0.42	0.73
StSSR12.1	22	0.17	0.92	0.97	0.92
StSSR12.2	8	0.36	0.76	0.30	0.73
Average	12.08	0.28	0.82	0.43	0.80

Gene diversity of expected heterozygosity is important to be a parameter to estimate genetic variability within a population [26]. The average of gene diversity in this study

was 0.82 and ranged from 0.69 (StSSR9.2) to 0.92 (StSSR12.1), which was similar to gene diversity value reported by Nugroho *et al.* (2019) [5]. This can be attributed to the high exchange rate between germplasm collection sources.

All SSR markers used were able to detect heterozygous alleles with an average heterozygosity value of 0.43, which was higher than the average heterozygosity (0.05) reported by Nugroho *et al.* (2019) [5]. StSSR4.2 has the lowest heterozygosity value of 0.03, and StSSR12.1 has the highest heterozygosity value of 0.97. The PIC values of the 12 SSR markers used varied from 0.66 (StSSR9.2) to 0.92 (StSSR12.1) with a mean of 0.80, implying their high discriminating capability of the SSR markers. This also indicates that the selected microsatellites were highly informative in distinguishing different genotypes. Referring to Botstein *et al.* (1980) [27], who stated that markers with PIC>0.5 were considered to be highly informative and 0.25>PIC>0.5 were moderately informative. Highly informative markers can be considered for estimating the genetic diversity and genetic relationship [28].

B. Genetic Diversity Based on SSR Markers

The UPGMA clustering analysis based on the coefficient of genetic similarity grouped the 33 potato genotypes used in the study spread into three main distinct groups at a genetic similarity coefficient of 0.76 (Fig. 1). Group 1 consisted of 23 genotypes, group 2 comprised 8 genotypes, and group 3 composed of 2 genotypes. The grouping pattern in this study indicated the existence of genetic variability among genotypes based on their genetic background. For instance, Medians, Spudy Agrihorti, Maglia, AR 08 Agrihorti and Amabile were grouped together in group 1 with their female parent, Atlantik. However, Papita Agrihorti, whose female parent is also Atlantik, was grouped differently. Moreover, GM 05, GM 08, Tedjo MZ, Dayang Sumbi Agrihorti, and Sangkuriang also were grouped together in group 1 with their female parent, Granola L.

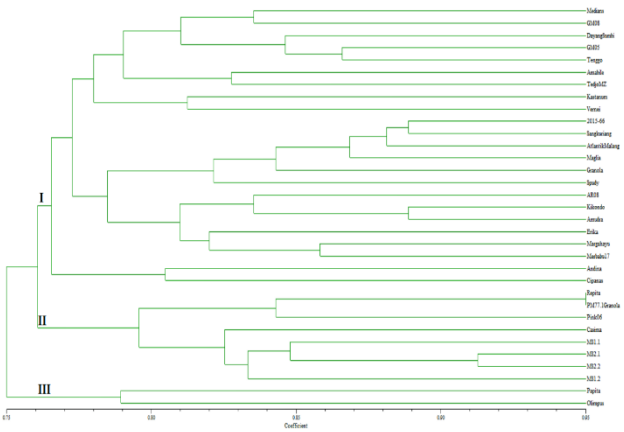

Figure 1. Dendrogram of genetic relationships of 33 genotypes of potato based on 12 SSR markers using NTSYS-pc v. 2.10e software.

The clustering analysis also revealed that SSR markers used were able to distinguish potato genotypes based on particular morphological characters. Medians (Atlantik x 393284.39), Maglia (Atlantik x 391058.175), AR 08 AR 08 Agrihorti (Atlantik x Repita), and Amabile (Atlantik x 393280.64), which grouped together having the similar white color of tuber as their female parent, Atlantik. Medians, Maglia, Spudy Agrihorti and Amabile, which also grouped together, were having the similar oval shape of tuber. On the other hand, this present study showed that characterization based on the molecular marker could support the efficiency of the breeding program at the breeding selection phase [29]. Furthermore, introduced clones from the United States were grouped into group 2 with Repita, PM 77.1 Granola, Ping 06, and Cosima.

Hereafter, SSR markers used could discriminate genotypes that have a close relationship, such as Tedjo MZ and PM 77.1 Granola with Granola L. Granola L has an off-type, Tedjo MZ [30]. Off-type is the result of pedigree errors at the breeding program, resulting in mislabeled accessions due to pollen contamination or accidental selfing, labeling mistakes, and nursery mixed-up [31].

C. Principal Coordinates Analysis

Principal Coordinates Analysis (PCoA), known as multidimensional scaling analysis, is used to determine the closeness between genotypes based on the similarity of characters through simplification of dimensions [32]. PCoA analysis will describe the relative position of each individual. PCoA can be an alternative to determine genetic similarity and diversity within a population [33]. PCoA revealed that potato genotypes spread and overlapped into four quadrants, with the first and second principal components explaining 49.35% of total variance in the population (Fig. 2).

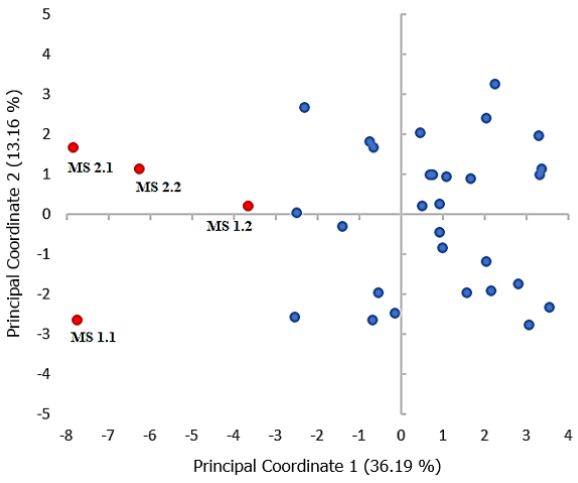

Figure 2. Principal coordinate analysis (PCoA) plots of 33 genotype of potato based on the 12 SSR markers.

Introduced clones from the United States are separated from other genotypes. The MS 2.1 was closely located to the MS 2.2, and the position of the MS 1.1 slightly separated from MS 1.2. Based on the value of genetic similarity, MS 2.1 and MS 2.2 have a relatively high value of 0.92, compared to MS 1.1 and MS 1.2 with only 0.83.

Similar with the UPGMA clustering analysis, PCoA results also revealed that introduced clones from the United States were grouped and separated from the other

genotypes. PCoA analysis has been used previously to assess genetic diversity on potato cultivars [34]. The previous study also has obtained the same results between phylogenetic analysis and PCoA analysis [35], [36]. Overall, the UPGMA clustering and PCoA analysis could work together to provide a comprehensive understanding of genetic diversity study of potato.

IV. Conclusion

Phylogenetic tree of SSR markers divided the 33 varieties into three major groups with a genetic similarity coefficient of 0.76. Group 1 consisted of 23 genotypes, group 2 comprised 8 genotypes, and group 3 composed of 2 genotypes. SSR markers used in this study were able to discriminate potato genotypes based on genetic background in support of morphological characters. All clones originating from the United States belonged to the same group and separated from the other genotypes. However, all the SSR markers used in this study were highly informative (PIC> 0.5), suggesting their potential use for genetic diversity study on potatoes. Similar with the UPGMA clustering analysis, PCoA results also revealed that introduced clones from the United States were grouped and separated from the other genotypes. Further genetic studies of using more diverse potato genotypes using these high informative SSR markers could be beneficial to assist breeding scheme in Indonesia.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All authors are contributed equally and had approved the final version.

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