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Evaluation of Genetic Diversity of QPM, Provit-A, and Elite Maize Inbreds Resistant to Downy Mildew Disease Using Simple Sequence Repeats

Evaluasi Keragaman Genetik Jagung QPM, Provit-A dan Inbrida Jagung Elit Tahan Bulai dengan Menggunakan Marka SSR (Simple Sequence Repeats)

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ABSTRAK

Jagung fungsional lebih rentan terhadap penyakit utama jagung, khususnya bulai. Ada beberapa jenis jagung fungsional seperti jagung QPM dan jagung Provit. Kandungan asam amino yang lebih tinggi dan beta karoten pada jagung fungsional menyebabkan lebih rentan terhadap penyakit bulai. Tujuan penelitian adalah untuk mengidentifikasi potensi heterotik di antara inbrida jagung QPM, Provit A, dan galur-galur elit yang tahan penyakit bulai menggunakan penanda SSR. Penelitian dilakukan pada bulan April-Juli 2017 di laboratorium biologi dan molekuler dan rumah kaca Balai Penelitian Tanaman Serealia, Maros, Sulawesi Selatan. Sebanyak lima inbrida QPM, 15 jagung lokal Provit A, dan 11 jagung tahan bulai digunakan dalam percobaan menggunakan 34 marka SSR. Hasil penelitian menunjukkan di antara 34 lokus SSR yang dianalisis, variasi panjang alel berkisar antara 74-500 bp. Dihasilkan 125 alel, mulai dari dua hingga sembilan alel per lokus dengan rata-rata 3,68 alel. Variasi genetik yang cukup luas di antara karakter dapat diidentifikasi. Profil pita DNA menunjukkan penanda primer nc130 menghasilkan PIC tertinggi, lebih dari 0,83 dan nilai alel 8,00. Analisis jarak genetik menghasilkan 21 kelompok heterotik dengan jarak genetik lebih dari 0,65.

Kata kunci: Jagung, QPM, Provit-A, penyakit bulai, SSRs.

ABSTRACT

Functional maize tends to be more susceptible to major maize diseases, particularly Downy mildew. Among the functional maize are Quality Protein Maize (QPM) and Provit A maize. The presence of higher amino acid and beta carotene in functional maize might have caused these types of maize more susceptible to Downy mildew disease. The objective of the research was to identify the heterotic pairs among maize inbreds i.e. QPM, Provit A, and local maize varieties resistant to Downy mildew disease using Simple Sequence Repeats (SSR) marker. The research was conducted from April to July 2017 at the Molecular Biology Laboratory of Indonesian Cereals Research Institute. A total of five QPM inbreds, 15 Provit A inbreds, and 11 Downy mildew resistant local varieties of maize were used in the experiment using 34 SSR markers. Results indicated that among 34 SSR locus analysed, variation of allele lengths ranged from 74 bp to 500 bp. A total of 125 alleles ranging from two to nine alleles per locus with an average of 3.68 alleles were generated. The data indicated wide genetic variations among characters. DNA band profile showed that nc130 marker produced the highest PIC (over 0.83) and allele value (8.00). Genetic distance analysis found a total of 21 heterotic genotypes with genetic distance exceeds 0.65.

Keywords: Maize, QPM, Provit-A, downy mildew, SSRs.

INTRODUCTION

QPM and Provit-A population as functional maize containing high protein and beta carotene, originated from the introgression breeding program. Those genotypes are more susceptible to diseases, especially downy mildew diseases that attack during the early growth of the plant (Rashid *et al*. 2013). Yield losses due to downy mildew varied between 50-100%, particularly on susceptible varieties (Lukman *et al*. 2013). According to CIMMYT (2012), some strain of the pathogen causing downy mildew could be transmitted through seeds, although the transmission was only limited to fresh seeds with high moisture content.

QPM trait is controlled by opaque-2 (o2) and floury-2 (fly2) genes, which affect the maize endosperm composition. The opaque-2 gene increases lysine and tryptophan level in the maize endosperm. Ordinary maize (non-QPM) may be converted into QPM maize by back crosses method, introgressing the o2 gene from QPM maize material into the maize genome. After three back crosses the ordinary maize was converted into QPM variety (Yasin *et al*. 2010).

The important characteristic of the Provit-A maize is the presence of the Phytoene Synthase-1 (Ps-1) gene

that plays a role in the carotenoid formation. The Ps-1 gene is located at about 300 bp (base pair). Carotenoids are natural pigments that give a yellow, orange, or red color of the grains with an estimated wavelength between 430-480 nm. Carotenoids are essential for photosynthesis and photo-protection processes that have the potential to improve crop yield and nutritional quality (Juhriah *et al*. 2012).

Maize resistance to diseases is controlled by more than one gene or often called as QTL (Quantitative Trait Loci), so the genetic material used for resistance would have the highest segregation on the F2 population. The use of fungicide to control the diseases in intensive maize areas in Indonesia has been less effective in susceptible varieties, particularly in the endemic environment (Muis *et al*. 2013). A comprehensive research had indicated that metalaxyl fungicide was unable to control maize downy mildew in a maize intensive cropping areas, such as in East Java, with the disease severity of 78% on plot treated with metalaxyl at the concentration of 7.5 g/kg seed as compared to disease severity of 83% on the untreated plot (Talanca *et al*. 2011). This finding indicated that resistance of the pathogen *Peronoscleropora* spp. to metalaxyl was gradually progressing. The alternative strategy applied in the endemic areas is by using resistant varieties. Therefore, it is necessary to increase the resistance of the new maize varieties to downy mildew disease (Pakki *et al*. 2018).

Muis *et al*. (2015) reported that screening large amounts of S1 maize lines against downy mildew produced only a few resistant lines (three lines). However, as the screening continues from S1 to S2, the probability of getting more resistant lines is higher. The resulted S2 lines can be selfed to develop downy mildew resistant S2 lines. Several lines that showed moderate resistant reaction were derived from local germplasm susceptible to downy mildew in the previous test.

In addition to genetic factors, supportive agroecosystem conditions and abundant inoculum sources cause changes in the level of resistance that was previously resistant to being susceptible. Furthermore, it is argued that changes in the level of resistance can also occur if the same variety is planted in different locations. Apart from these factors, the high and low incidences of downy mildew of the tested lines also depend on the source of the downy mildew inoculum. Hartatik (2007) results indicated that local maize varieties were more resistant to downy mildew than those of improved varieties. Observation of the phenotypic characters of local maize in East Nusatenggara (NTT) strengthens the results of previous studies that local maize is more resistant to downy mildew (Bani *et al*. 2017). Maize with special properties, such as QPM and Provit-A had been developed through cross breeding methods. Molecular genetics is a powerful tool to improve downy mildew resistance in maize breeding. Several quantitative trait loci (QTL) studies had identified downy mildew - resistance genes by linkage mapping in maize (Jampatong *et al*. 2012). Although the QTLs and population sample sizes were diverse, the QTL analysis had located downy mildew resistance related to genomic regions. Because the identification of QTLs so far was limited by high cost and poor resolution, a combined protocol of SSR method is a useful approach to identify resistant genes against downy mildew (Anderson *et al*. 2011; Phumichai *et al*. 2012; Franchel *et al*. 2013).

SSRs are the most widely used markers for genetic analysis, because their polymorphisms are high, replicable, low cost, and their ability to be automated. Furthermore, using SSR markers, researchers could learn about genetic diversity, add genetic link information from a population to more detail and create genetic maps (Sa *et al*. 2012; Hidalgo *et al*. 2013; Zheng *et al*. 2013). SSR markers are also frequently used to study the genetic diversity of maize, characterization of structures and genetic diversity of inbred maize lines (Suteu *et al*. 2014). It is necessary to evaluate the utilization of SSR markers as a selection tool to introduce the opaque-2 recessive mutant gene and phytoene synthase-1 in the already selected maize strains.

The objective of this study was to determine the genetic diversity of 31 inbred QPM, Provit-A, and downy mildew resistance elite maize lines, based on the SSR markers. Cluster analysis was performed to classify maize lines based on the genetic distance indicator, and to determine potential recombination pairs for hybrid development.

MATERIALS AND METHOD

The research was conducted from April to July 2017 at the Biology and Molecular Laboratory and the screen house of the Indonesian Cereals Research Institute (ICERI), Maros, South Sulawesi. Set of 31 maize varieties collected by ICERI were used as experimental materials in this study (Table 1). Marker information about Simple Sequence Repeats (SSRs) was collected from maize genetics and genomics database 2010 (www.maizegdb.org).

Table 1. List of maize inbred lines and their pedigree used in the study.

Line	Type	Source population	Pedigree
QPM01	QPM	CIMMYT Mexico	MSQ(S1)CØ-2-1-3-1-1
QPM02	QPM	CIMMYT Mexico	MSQ(S1)CØ-81-1-3-4
QPM03	QPM	CIMMYT Mexico	MSQ(S1)CØ-14-4-3-1
QPM04	QPM	CIMMYT Mexico	MSQ(S1)CØ-171-1-1-3-#
QPM05	QPM	CIMMYT Mexico	CML169-1-1-#
Pro.A1	Provit-A	Lembah Palu (Central Sulawesi)	CLP-5-2-1
Pro.A2	Provit-A	Lembah Palu (Central Sulawesi)	CLP-6-2-3
Pro.A3	Provit-A	Lembah Palu (Central Sulawesi)	CLP-6-3-1
Pro.A4	Provit-A	Lembah Palu (Central Sulawesi)	CLP-6-4-2
Pro.A5	Provit-A	Lembah Palu (Central Sulawesi)	CLP-8-2-3
Pro.A6	Provit-A	Lembah Palu (Central Sulawesi)	CLP-11-5-1
Pro.A7	Provit-A	Lembah Palu (Central Sulawesi)	CLP-12-1-1
Pro.A8	Provit-A	Lembah Palu (Central Sulawesi)	CLP-17-1-#
Pro.A9	Provit-A	Lembah Palu (Central Sulawesi)	CLP-30-2-1
Pro.A10	Provit-A	Lembah Palu (Central Sulawesi)	CLP-33-4-5
Pro.A11	Provit-A	Lembah Palu (Central Sulawesi)	CLP-33-5-6
Pro.A12	Provit-A	Lembah Palu (Central Sulawesi)	CLP-39-1-1
Pro.A13	Provit-A	Lembah Palu (Central Sulawesi)	CLP-39-3-4
Pro.A14	Provit-A	Lembah Palu (Central Sulawesi)	CLP-40-1-1
Pro.A15	Provit-A	CIMMYT Thailand	B-16-1
LTB.2/45.Kandora	DM Resistant	Tana Toraja (South Sulawesi)	LK-35-21-33-12
LTB.9/30.Tongo	DM Resistant	Palu (Central Sulawesi)	LT-42-47-30
LTB.15/1.Bebo	DM Resistant	Tana Toraja (South Sulawesi)	LBE-27-41-39
LTB.23/12.Majene	DM Resistant	Majene (West Sulawesi)	LM-5-11-35
LTB.27/11.Entok	DM Resistant	Majalengka (West Java)	JE-1-11-39
LTB.83/50.Pensaijan(1)	DM Resistant	TTU (Nusa Tenggara Timur)	Pen-32-16-50
LTB.112/3L.K.O.Kalsel	DM Resistant	Tanah Laut (South Kalimantan)	JKK96A-29-3-3
LTB.135/29.Jole(48B)	DM Resistant	Donggala (Central Sulawesi)	Jole48A-33-29-40
LTB.141/41BL.Sulut(37A)	DM Resistant	Central Sulawesi	SU37A-21-46-21
LTB.188/39Jg.K.Kalsel	DM Resistant	Tanah Laut (South Kalimantan)	JKK96A-36-34-18
LTB.54/30P.S.Bone	DM Resistant	Bone (South Sulawesi)	PSB-2-3-50

QPM = Quality Protein Maize, Provit-A = Provitamin-A, DM = downy mildew

DNA Extraction and SSR Analysis

DNA extraction was carried out by taking 8-10 leaves from each line at the age of 10-15 days. Sample of 0.4 g of fresh leaves of each line was bulk for the DNA isolation. DNA extraction procedure followed the protocol recommended by George *et al*. (2004), with minor modification by replacing liquid nitrogen with cetyltrimethylammonium bromide (CTAB) buffer. The number of DNA samples was measured by using Nano Photometer TM (Implen, Munich, Germany), and the DNA quality was estimated by using 0.9% agarose gel electrophoresis.

The process of staining and visualizing of DNA banding pattern followed the protocol where each DNA sample was genotyped using 43 SSR primers dispersed through the genome representing at least one microsatellite marker from chromosomes.

The polymerase chain reaction was carried out in 10 μL reaction volume consisted of 10 ng of DNA, 0.2 μM of each primer, 1X PCR buffer containing 10 mM Tris-HCl pH 8.3, 5 mM KCl, 0.001% gelatin, 1.5 mM MgCl2, 0.125 dNTPs, and one unit of Taq DNA Polymerase. Amplifications were performed in thermocycler Biometra Professional Standard 96 with the following touch-down program: initial denaturation at 95°C/five minutes, by 15 cycles each of denaturation at 95°C/30 s, annealing at 63.5°C/one minutes (-0.5°C/cycle) and extension at 72°C/one minutes; another 22 cycles of 95°C/30 seconds, 56°C/one minutes, and 72°C/one minutes were performed. Final elongation was at 72°C for four min. The amplified fragments were resolved by electrophoresis on 8% polyacrylamide gel, with 100 bp ladder as a marker. Gels were run on a small format (7.3 x 10 cm) vertical gel system (Mini Protean Tetra-Cell BioRad) at 40 mA for 1.5 hours. After staining with 0.5 ìg/ìL ethidium bromide they were photographed under UV light on BioDoc Analyse Biometra.

Amplification was carried out for 30 cycles, according to CIMMYT protocol (2004), ie denaturation two minutes at 94°C, continued 30 seconds at the same temperature, one minutes at 65°C and one minute at 72 °C. The annealing temperature was lowered from 1°C, every two cycles to the end when annealing was

reached. The second stage was repeated 29 times and ended with an elongation cycle at 72°C and cooling at 4°C, then the reaction was stopped. The PCR results were separated by a vertical electrophoresis process using a Dual Mini-Verticals Complete System MGV-202-33 with 8% polyacrylamide gel.

Data Scoring

For statistical analyzes of DNA ribbon scaling patterns, the emerging alleles were labeled based on the relative positions of the bands against the φX174/Hinf1 fragments. To obtain a binary matrix, the SSR mark was scored as one (1) if there the band appears and zero (0) if no tape. The resulting matrix data were statistically analyzed using Powermarker V 3.25 + Treeview and NTSYS_pc 2.1 programs.

Data Analysis

Polymorphism or Polymorphic Information Content (PIC) levels are calculated for each SSR marker (Smith *et al.* 1997). The value of PIC is used in measuring the diversity of alleles at one locus by the formula:

$$PIC = 1 - \sum_1^n f_i^2, \text{i} = 1,2,3,.....n$$

where i = allele frequency.

The degree of genetic similarity is the level of character similarity, in this case, the collected ribbon fragments of the identified genotypes. The genetic similarity (GS) level is estimated from the data of allele numbers using the Jaccard coefficient (Rohlf 2000) with the formula:

$$GS = \frac{m}{(n+u)}$$

where: m = the same number of DNA bands of position, n = total DNA band, u = unequal amount of DNA band (allele) of position.

The genetic distance matrix can be obtained from the results of genetic similarity analysis (Lee 1998) with the formula:

$$S = 1 - GS$$

where: S = genetic distance, GS = genetic similarity.

Cluster analysis is conducted by grouping according to the matrix of genetic similarity with UPGMA (Unweighted Pair Group Using Arithmetic Average) method using the Jaccard coefficient. Distance and dendrogram matrices are formed using NTSYS-pc (Numeral Taxonomic System) version 2.1 (Rohlf 2000) and Manual Power Marker V3.25 (http://www.powermarker.net).

RESULTS AND DISCUSSION

Genotype Analysis Based on SSR Markers

The 34 SSR primers used in this study were all amplified. The SSR-based molecular characterization used against the QPM maize inbred and Provit-A was selected with homosigosity> 80% (Andayani *et al*. 2016). Among the 31 genotypes analyzed with SSR markers, four QPM maize lines were obtained, nine Provit-A maize lines, and eight local downy mildew resistant maize, with homozygosity levels above 80% and missing data below 15%. A list of the 34 primers used in the analysis was given in Table 2.

Detection of Opaque-2 (o-2) Gene DNA Markers

DNA bands appeared in all 31 maize genotypes analyzed using SSR marker umc1066 o-2 gene marker (Figure 1). The emerging DNA bands indicated that all genotypes studied were detected containing the o-2 gene. The SSR marker of the opaque-2 gene marker has been studied previously by molecular researchers of maize plants, the gene located on chromosome 7 (bin 7.01) designed from the opaque-2 gene sequence region itself with a product amplification of about 140-160 bp. CIMMYT designed opaque 02-gene specific SSR primers viz. phi 057 and phi 112 which were located as internal repetitive elements within opaque-2 gene at the short arm of chromosome 7. Among these, phi 057 was reported to be co-dominant while phi 112 was a dominant marker16 (Devraj and Tripathy 2017). Hence, the utilization of molecular markers as a selection tool (MAS = Marker Assisted Selection) is very helpful for researchers in detecting individual opaque-2 recessive homozygote genotype.

Detection of gene DNA markers Carotenoid Phytoene Synthase (Psy1)

The DNA bands appeared in 31 maize genotypes analyzed using marker of the SSR marker umc1196 Psy1 gene marker (Figure 2). However, there was one genotype that did not show the DNA band (missing data) that was found in the genotype to-10. Mark markers of the Psy1 gene used in this study include phi109275 (bin 1.03), phi423796 (bin 6.01), and umc1196 (bin 10.07).

As reported previously, the magnitude of the resistance level of maize to downy mildew pathogens was quite diverse, depending mainly on genetic variability, phenotypic variability, and interactions between genetic and the environment (Azrai *et al*. 2006). In developing hybrid maize varieties, inbred parents with high homozygosity and wide genetic distance were more

Table 2. List of SSR primer names, Bin number, annealing, types of repeat, and size range.

Primer	Bin number	Annealing (ºC)	Repeat type	Size range
phi109275	1.03	54	AGCT	104,5-151
phi109642	2.00	54	ACGG	134,5-185,9
phi96100	2.00-2.01	54	ACCT	249-500
phi083	2.04	52	AGCT	114,4-165
phi101049	2.09	54	AGAT	209,8-311
phi374118	3.03	54	ACC	249-398
phi102228	3.04-3.05	54	AAGC	104,5-140
phi072	4.01	52	AAAC	140-186,6
phi079	4.05	60	AGATG	140-165
phi093	4.08	60	AGCT	283,4-427
nc130	5.00	54	AGC	134,5-206,1
phi109188	5.00	54	AAAG	129-212,3
phi331888	5.04	58	AAG	126,3-167,3
umc1153	5.09	54	(TCA)4	100-129
umc1143	6.00	54	AAAAT	74-89,2
phi423796	6.02	54	AGATG	123,5-167,3
phi452693	6.06	52	AGCC	122,4-163,3
phi299852	6.08	58	AGC	109-151
umc1066	7.01	63	(GCCAGA)5	135-151
phi112	7.01	56	AG	147-162
phi057	7.01	54	GCC	140-164
phi034	7.02	56	CCT	123,5-165,7
phi420701	8.01	58	CCG	256,8-349,7
umc1304	8.02	54	(TCGA)4	131,2-137,8
phi233376	8.03	54	CCG	133,4-159,2
phi080	8.08	60	AGGAG	187,8-290,3
umc1279	9.00	54	(CCT)6	87,9-97,0
phi065	9.03	54	CACTT	170,6-249
phi448880	9.05	54	AAG	187,8-208,9
phi041	10.00	56	AGCC	193,9-224,5
phi96342	10.02	54	ATCC	236,8-249
phi050	10.03	56	AAGC	77,6-97,8
umc1061	10.06	52	(TCG)6	94-107,2
umc1196	10.07	54	CACACG	145,5-186,6

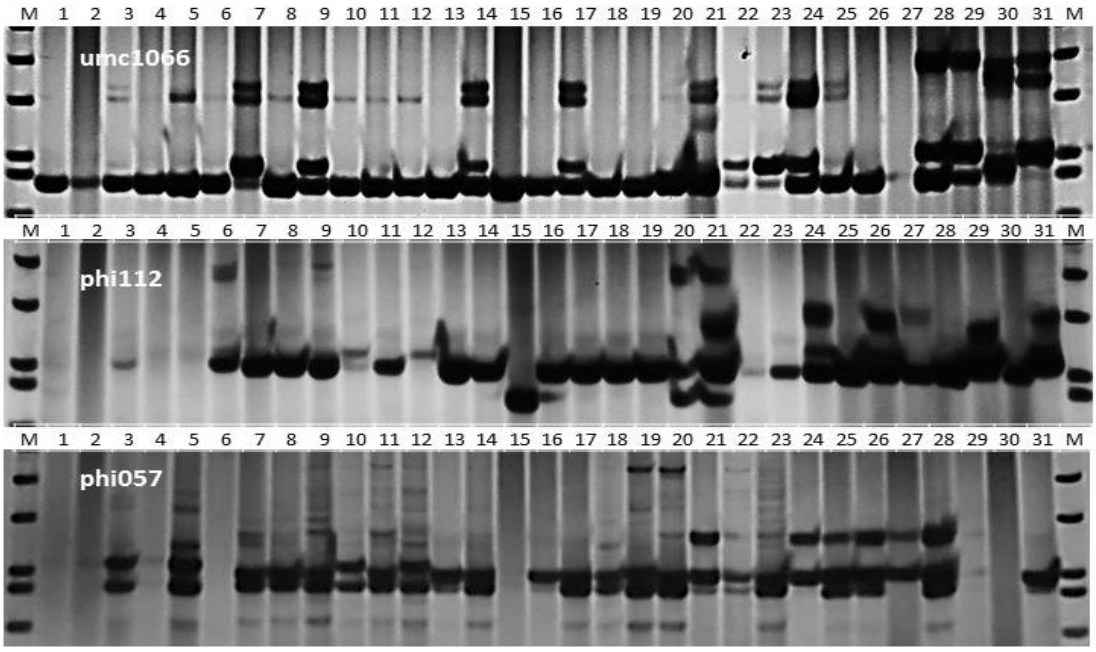

Figure 1. Gel profiles showing the amplification of SSR pimers umc1066, phi112, and phi057 (Bin 7.01) with all 31 genotypes. The number above the line indicates the genotype number as in Table 1.

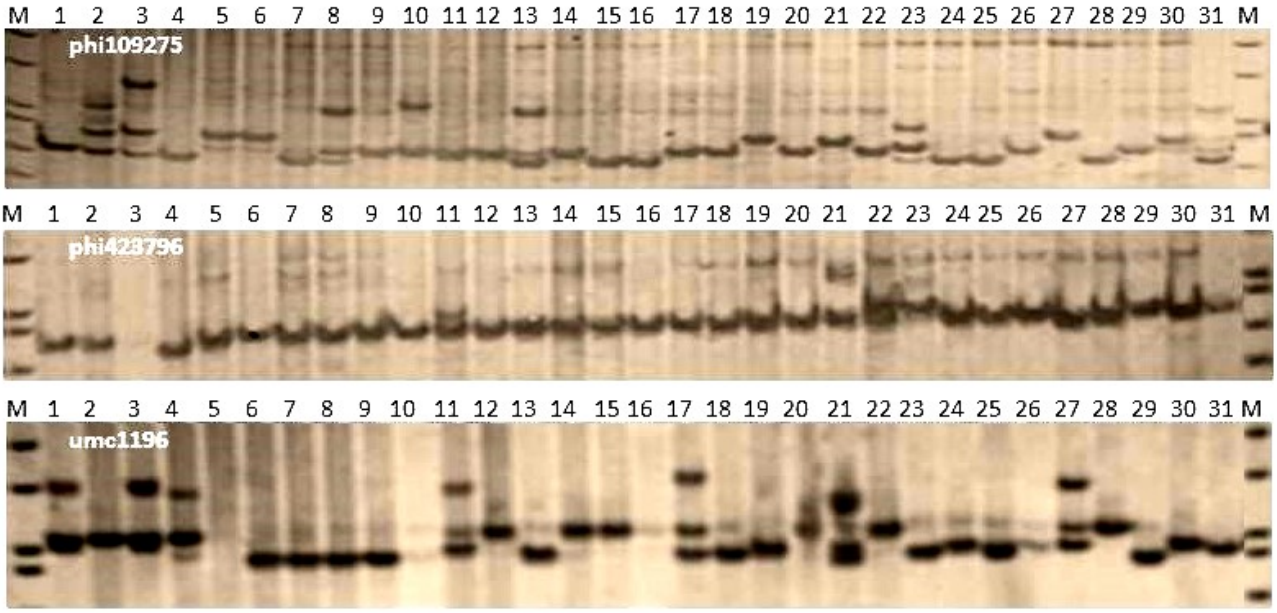

Figure 2. Gel profile showing the amplification of SSR primers umc1196 (Bin 10.07) with all 31 genotypes. The number above the line indicates the genotype number as in Table 1. Biology and Molecular Laboratory of the Indonesian Cereals Research Institute, April to July 2017.

preferred. The efficiency of the hybrid combination could be improved by grouping the inbreds into heterotic groups (Tandzi *et al*. 2015).

Polymorphism Analysis (PIC) on 34 SSR Largest

All inbreds of QPM maize, Provit-A, and level of resistance of local varieties could be distinguished based on the type of SSR primers used. The data profiles of 34 SSR markers of characters were shown in Table 3. The 34 SSR loci analyzed had a relative range of base pairs between 74-500 bp. Of the 21 test inbreds, there were 125 alleles with the range of 2-9 alleles per locus, averaging 3.68 alleles/SSR locus. These data indicated that the average genetic diversity in each character was quite high. The number of alleles obtained from this study ranged from two alleles/locus (phi102228, phi423796, phi420701, umc1304, phi233376, phi448880, phi96342, phi050) to nine alleles/locus (nc130). The value of PIC was greatly determined by the allele frequencies, the values of each marker ranging from 0.12 (phi102228) to 0.76 (nc130). The average PIC value for all markers was 0.38. The frequency of the major alleles in this study ranged from 0.33 (nc130) to 0.98 (phi423796) with an average of 0.68.

The SSR marker analysis indicated the average number of genetic diversity was 0.43. The lowest value of genetic diversity was found in phi423796 (0.05) and the highest was at nc130 (0.79). High genetic diversity indicated that genetic variability was large enough. Where the number of alleles was high then the value of PIC was also high, so the ability to distinguish samples tested was higher. High PIC values indicated codominant properties, high accuracy, abundant genomes, and spread throughout the chromosome. High levels of polymorphism indicated that the genetic variation of each character of the inbred analyzed was high. The high genetic variability led the flexibility in choosing parents for recombination in the improvement of varieties. Similar studies have also been conducted by Pfunde *et al*. (2015).

The heterozygosity values range from 0.0 (umc1122, phi227562, umc1143, phi328175, umc1304, umc1279, phi96342) - 0.89 (nc130), with an average value of 0.14. From the result of the data profile, the mark nc130 had the highest PIC value (0.83) and the highest allele number (8.00). This fact indicated that the primer could produce a large number of different characters between the accessions studied.

Genotype Cluster Analysis Based on SSR Markers

The encoding genes of opaque-2 and Psy1 characters enable breeders to choose the parental line for the formation of prospective superior varieties of QPM maize and Provit-A resistant to downy mildew. The genetic variability among accessions of specific maize based on SSR was presented in the dendrogram (Figure 1).

Table 3. Statistical summary of 34 SSR markers observed on 21 maize accessions.

Marker	Major allele frequency	Allele number	Gene diversity	Heterozygosity	PIC
phi109275	0.5	6	0.69	0.14	0.66
phi109642	0.5	4	0.54	0.1	0.44
phi96100	0.64	3	0.48	0.05	0.38
phi083	0.74	4	0.42	0.05	0.37
phi101049	0.42	6	0.71	0.37	0.67
phi374118	0.48	5	0.59	0.45	0.51
phi102228	0.93	2	0.13	0.14	0.12
phi072	0.84	3	0.28	0.21	0.26
phi079	0.4	4	0.71	0.2	0.66
phi093	0.83	3	0.29	0.24	0.27
nc130	0.33	9	0.79	0.95	0.76
phi109188	0.52	6	0.58	0.29	0.5
phi331888	0.86	5	0.26	0.19	0.25
umc1153	0.67	3	0.47	0.1	0.4
umc1143	0.52	4	0.62	0.0	0.56
phi423796	0.98	2	0.05	0.05	0.05
phi452693	0.43	5	0.72	0.14	0.67
phi299852	0.75	3	0.41	0.0	0.37
umc1066	0.83	3	0.3	0.35	0.26
phi112	0.83	5	0.31	0.2	0.3
phi056	0.39	5	0.7	0.47	0.65
phi034	0.62	3	0.49	0.1	0.39
phi420701	0.88	2	0.21	0.24	0.19
umc1304	0.75	2	0.38	0.0	0.3
phi233376	0.63	2	0.47	0.0	0.36
phi080	0.76	3	0.37	0.05	0.32
umc1279	0.9	3	0.18	0.0	0.17
phi065	0.95	3	0.1	0.1	0.09
phi448880	0.9	2	0.18	0.1	0.16
phi041	0.58	3	0.58	0.05	0.51
phi96342	0.71	2	0.41	0.0	0.32
phi050	0.95	2	0.09	0.0	0.09
umc1061	0.79	3	0.35	0.05	0.3
umc1196	0.45	5	0.64	0.25	0.58
Mean	0.68	3.68	0.43	0.17	0.38

Phylogenetic analysis using 34 SSR markers illustrated the relationship between the 21 genotypes.

The coefficient values of genetic diversity among QPM, Provit-A and local downy mildew-resistant maize varieties ranged from 0.36 to 0.88, which were considered sufficiently wide. The wide genetic variability among the inbreds might be used to form hybrid between the heterotic groups. Recombination of inbreds with high-value genetic distance is expected to have the potential for heterotic effect for characters to be considered.

When a dendrogram line was set to a genetic similarity scale of 0.44, all QPM, Provit-A, and downy mildew resistant inbreds can be grouped into four clusters and two independent clusters. Cluster I consisted of five inbreds (QPM01, QPM03, QPM05, QPM04, and LTB112 /3). Cluster II consisted of seven inbreds (Pro.A12, Pro.A13, Pro.A15, Pro.A14, LTB23/12, LTB27/11, and LTB135/29). Cluster III consisted of five inbreds (Pro.A3, Pro.A8, Pro.A10, Pro.A11, and LTB9/30). Cluster IV consisted of two inbreds (LTB141/41 and LTB54/30). The remaining two independent clusters (non-cluster) inbreds were LTB188/39 (A) and Pro.A7 (B). These inbreds could be potentially crossed with all inbreds in the clusters, because of their considerable genetic distances. If the average value of the genetic distance in the cluster was smaller than that of the general average, then the cross between inbreds within the cluster should be avoided. Conversely, if the average value of genetic distance in the cluster is greater than that of the general average value, intercrosses of inbreds in the same cluster could be suggested (Pabendon *et al.* 2017).

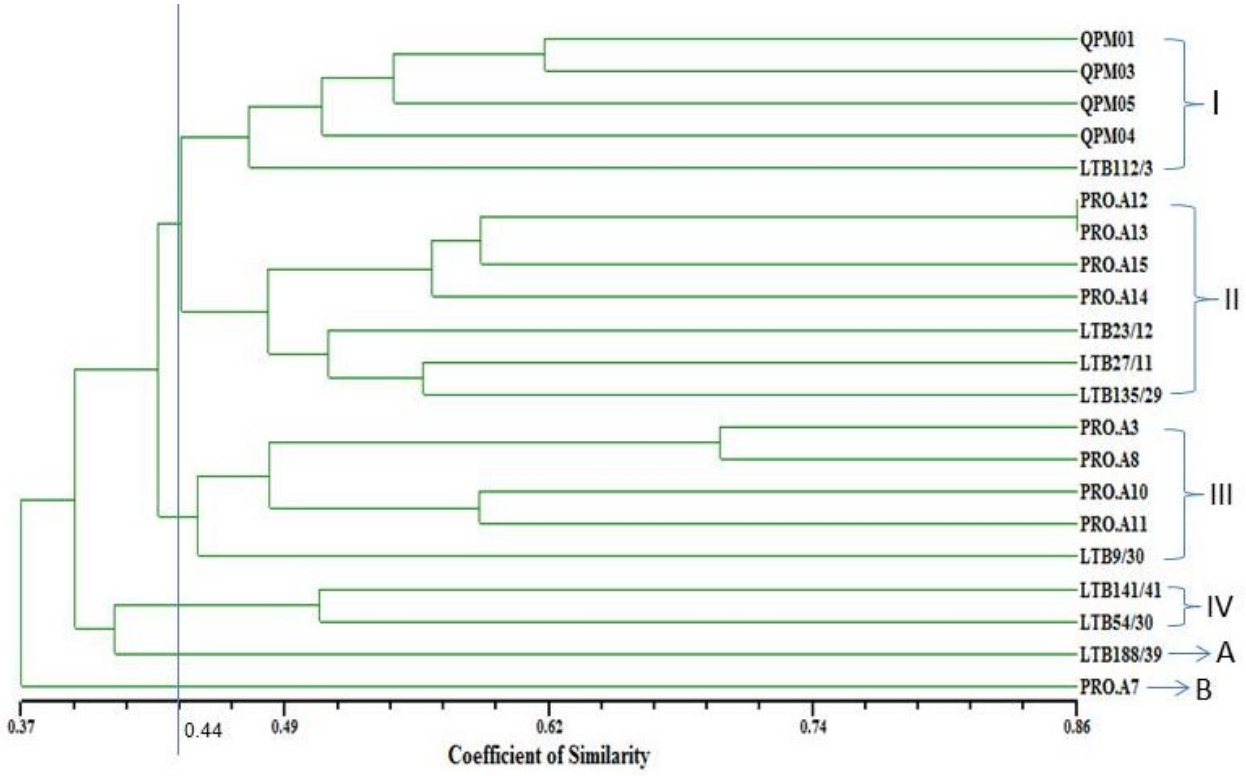

Figure 3. Dendrogram of 21 accessions based on similarity matrix from 34 SSR primers. Biology and Molecular Laboratory of the Indonesian Cereals Research Institute, April to July 2017.

Analysis of Genetic Distances and Opportunity for Crosses

Based on the ribbon pattern of the SSR locus, the matrix of genetic similarity of 21 QPM maize inbreds, Provit-A, and downy mildew resistant varieties could be obtained, which could be selected as parental crosses based on the Jaccard coefficient. Pair crosses with genetic distance values of ≥ 6.0 are likely to have high heterosis in the formation of hybrid maize. The genetic variabilities among 21inbreds were quite large, therefore the number of hybrid crosses opportunity between inbreds in this study should be selected based on the genetic distance ≥ 0.65. Twenty one pairs of crosses would have the possibility of heterosis (Table 4). The highest genetic distance values were obtained in the Pro.A7 crossed to 54/30P.S.Bone (0.76). Prior research indicated that genetic distance was positively correlated with heterosis (Nikolic *et al*. 2015). The present information suggested many more cross-combinations for downy mildew tolerant hybrids could be developed.

Table 4. List of 21 possible cross combinations of inbred maize based on their genetic distance values. Biology and Molecular Laboratory of the Indonesian Cereals Research Institute, April to July 2017.

Cross combination	Genetic distance	Cluster
QPM01 vs LTB.9/30.Tongo	0,67	K1 x K3
QPM01 vs LTB.141/41BL.Sulut(37A)	0,71	K1 x K4
QPM01 vs LTB.188/39Jg.K.Kalsel	0,65	K1 x A
QPM01 vs LTB.54/30P.S.Bone	0,72	K1 x K4
QPM03 vs LTB.141/41BL.Sulut(37A)	0,67	K1 x K4
QPM03 vs LTB.54/30P.S.Bone	0,67	K1 x K4
QPM05 vs LTB.141/41BL.Sulut(37A)	0,65	K1 x K4
QPM05 vs LTB.54/30P.S.Bone	0,65	K1 x K4
Pro.A3 vs LTB.141/41BL.Sulut(37A)	0,68	K3 x K4
Pro.A7 vs LTB.23/12.Majene	0,67	B x K2
Pro.A7 vs LTB.141/41BL.Sulut(37A)	0,73	B x K4
Pro.A7 vs LTB.188/39Jg.K.Kalsel	0,69	B x A
Pro.A7 vs LTB.54/30P.S.Bone	0,76	B x K4
Pro.A8 vs LTB.141/41BL.Sulut(37A)	0,69	K3 x K4
Pro.A8 vs LTB.188/39Jg.K.Kalsel	0,65	K3 x A
Pro.A8 vs LTB.54/30P.S.Bone	0,67	K3 x K4
Pro.A10 vs LTB.141/41BL.Sulut(37A)	0,65	K3 x K4
Pro.A10 vs LTB.188/39Jg.K.Kalsel	0,69	K3 x A
Pro.A11 vs LTB.27/11.Entok	0,67	K3 x K2
Pro.A11 vs LTB.188/39Jg.K.Kalsel	0,67	K3 x A
Pro.A11 vs LTB.54/30P.S.Bone	0,66	K3 x K4

K: Cluster

CONCLUSION

Analysis of genetic diversity using 34 SSR markers showed significant differences among maize inbreds. Based on the NTSYS pc analysis, as many as 31 inbreds maize, four clusters and two independent inbreds were identified. There were 21 potential heterotic pairs crosses that were identified from QPM, Provit-A and Downy Mildew resistant lines, where the genetic distance >0.65.

Based on the genetic distance of QPM maize, the highest heterotic pair was expected between QPM01 vs LTB.54/30P.S.Bone inbreds, with a genetic distance of 0.72. Among the Provit A maize, the highest heterotic pair was expected between Pro.A7 vs LTB.54/30P.S.Bone inbreds, with a genetic distance of 0.76.

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