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Kata Pengantar

Jurnal Agro*Biogen* Volume 11 Nomor 2 berisi lima naskah primer tentang pemanfaatan marka SSR dan STS pada kentang, pembuatan set marka SSR untuk identifikasi kedelai, identifikasi gen *RB* pada kentang transgenik, teknik simpleks dan dupleks untuk identifikasi GMO, dan evaluasi galur mutan cabai terhadap CVMV.

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Balai Besar Penelitian dan Pengembangan Bioteknologi dan Sumber Daya Genetik Pertanian
Jalan Tentara Pelajar 3A, Bogor 16111, Indonesia
E-mail: jurnal.agrobiogen@gmail.com
Telepon: (0251) 8339793, 8337975
Faksimili: (0251) 8338820

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Development of SSR Marker Set to Identify Fourty Two Indonesian Soybean Varieties

(Pengembangan Set Marka SSR untuk Identifikasi Empat Puluh Dua Varietas Unggul Kedelai Indonesia)

Andari Risliawati*, Eny I. Riyanti, Puji Lestari, Dwinita W. Utami, and Tiur S. Silitonga

Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Jl. Tentara Pelajar 3A, Bogor 16111 Indonesia
Phone (+62-251) 8337975; Fax. (+62-251) 8338820; *E-mail: boendar@yahoo.co.id

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ABSTRAK

Profil marka molekuler atau sidik jari DNA dapat digunakan dalam kegiatan identifikasi varietas, pengawasan kemurnian genetika plasma nutfah, dan pelengkap dokumen perolehan hak kekayaaan intelektual. Analisis sidik jari DNA tanaman kedelai di BB Biogen-Balitbangtan telah dilakukan sejak tahun 2004 dengan menggunakan marka *simple sequence repeat* (SSR) yang diautomatisasi dengan mesin *genetic analyzer* CEQ 8000 berbasis sistem elektroferesis kapiler. Metode ini telah menghasilkan profil sidik jari DNA dari sebagian besar varietas yang diuji, namun set markanya belum pernah dikembangkan untuk mengidentifikasi varietas secara efisien. Tujuan penelitian adalah mengembangkan set marka SSR untuk identifikasi varietas unggul kedelai Indonesia. Penelitian menggunakan 42 varietas unggul kedelai yang dianalisis dengan 14 marka SSR berflouresen yang bersifat acak. Sebanyak 168 alel diperoleh dari analisis polimorfisme dengan rerata nilai *polymorphic information content* (PIC) tiap lokusnya sebesar 0,7337. Berdasarkan parameter tingkat reproduksi marka, nilai PIC, jumlah alel jarang, frekuensi alel dominan, dan tingkat keberhasilan deteksi fragmen SSR oleh *genetic analyzer,* teridentifikasi lima marka SSR, yaitu Satt414, Satt147, Satt308, Satt009, dan Satt516, sebagai set marka identifikasi. Set marka identifikasi ini dapat digunakan untuk menyusun identitas (ID) dari 42 varietas unggul kedelai Indonesia.

Kata kunci: Sidik jari DNA, set marka identifikasi, kedelai, SSR.

ABSTRACT

Profile of molecular marker can be used for variety identification, genetic purity monitoring of germplasm and additional requirement in proposing intellectual property protection. DNA fingerprinting of soybean had been applied at the ICABIOGRAD-IAARD since 2004 using simple sequence repeat (SSR) markers which were run automatically by CEQ 8000 Genetic Analyzer platform based on capillary electrophoresis system. This method had produced unique DNA fingerprints of the varieties tested, but the marker set to efficiently identify the varieties had not yet been developed. This study aimed to develop a set of SSR markers as a tool to identify the Indonesian soybean varieties. Fourty two soybean varieties were analyzed using 14 random SSR markersA total of 168 alleles that were obtained from the polymorphism analysis. The average of polymorphic information content (PIC) value observed was 0.7337 per SSR locus. Based on marker reproducibility rate, PIC value, number of rare alleles, frequency of dominant alleles, and percentage of SSR fragment detected by genetic analyzer, we identified five SSR markers i.e. Satt414, Satt147, Satt308, Satt009, and Satt516 as a SSR marker set to be used for soybean variety identification purposes. This marker set was used to develop the identity (ID) of the 42 Indonesian soybean varieties.

Keywords: DNA fingerprinting, marker set for identification, soybean, SSR.

INTRODUCTION

Soybean as an edible legume crop which contains high protein and vegetable oil is the third main crop in Indonesia. During the past 30 years its breeding program achieved a significant progress and more than 70 varieties have been released to farmers. Around twenty of the released varieties have been adopted by the farmers in soybean central production areas of Indonesia (Krisdiana, 2014; PPVT, 2015). In order to preserve the genetic diversity of the Indonesian soybean, the ICABIOGRAD genebank coordinated under the IAARD have conserved them together with other local varieties (BB Biogen, 2010). However, managing a large number of germplasm collections need a huge effort particulary in preventing germplasm from seed mixture and collection duplication.

It is well known that the use of molecular marker for marker assisted selection (MAS) have shortened time period in a breeding program and increased the precision of obtaining gene target. Meanwhile in germplasm research area, some studies showed that the application of molecular marker can generate phylogeny tree more precisely and the possibility to predict evolution existed among species or germplasm (Priolli *et al*., 2002; Vu *et al*., 2013). This advantage can be utilized to discard redundant collection and decrease management cost for germplasm conservation. Moreover, molecular marker can be used for variety identification as complementary tool for distinctness, uniformity and stability (DUS) tests in releasing a new variety (Gunjaca *et al*., 2008; Hudcovicova and Kraic, 2003; Narvel *et al*., 2000; Song *et al*., 2004).

SSR is a type of molecular markers that has been used widely in genetic diversity analysis of some important crops such as rice, soybean, tomato, potato, wheat, and sweet potato (Bredemeijer *et al*., 2002; Corbett *et al*., 2001; Luce *et al*., 2001; Plaschke *et al*., 1995; Zhang *et al*., 2000; Yoon *et al*., 2009). Therefore, since 2004 ICABIOGRAD has applied SSR markers for genetic diversity analysis of soybean (Chaerani *et al*., 2009; Santoso *et al*., 2006; Septiningsih *et al*., 2004). SSR marker is highly reproducible, codominant, able to detect high variance of allele polymorphism, only few amount of sample needed and is free from environmental influences (Agarwal *et al*., 2008). Therefore, SSR marker is considered a better marker in distinguishing genotypes having a close genetic relationship.

The use of SSR marker can be automated by using a genetic analyzer platform based on capillary electrophoresis system (Diwan and Cregan, 1997). The running time also can be shortened though multiplex panels by labelling the 5' ends of the primers using different fluorescent colors (Chaerani *et al*., 2009). Another importance feature of this platform is that it can read DNA fragments differences up to 1 base pair (bp). The accurate reading is necessary for variety identification since most of the release varieties were genetically close and is very difficult to be distinguished phenotypically. The flexibility of adjusting the multiplex panel also eases the user to add the marker number anytime in order to increase the reliability of the analysis. Practically, this method can benefit the Center of Crop Variety Protection personel in assigning the uniqueness of a new variety. The DNA fingerprinting information can also equip phenotypic data from variety that is being registered for plant protection.

The aim of the study was to develop SSR marker set as a specific identification for the Indonesian soybean varieties. The marker set was developed based on genetic diversity and relationship analysis among varieties tested. The developed SSR marker set is expected to be able to assign specific profiles of soybean varieties. Furthermore this study is a part of barcode database development for Indonesian soybean varieties in term of completing the barcode from phenotypic data.

MATERIALS AND METHODS

Genetic Materials and SSR Markers

A total of 42 improved soybean varieties originated from Indonesia was used as the genetic materials in this study, which 23 of them had been used previously (Table 1). The soybean genomic DNA were amplified with 14 SSR markers those were selected based on the result of soybean genetic diversity analysis as previously reported (Santoso *et al*., 2006; Song *et al*., 1999). All markers were labelled using three different fluorescent colors (black, green, and blue) and run to five multiplex panels in a genetic analyzer platform (Table 2).

DNA Extraction and Amplification

Seeds of soybean varieties were planted in pots at ICABIOGRAD green house. Three to four weeks after planting the seeds, DNA of young leaves from a single vigourus plant from each variety was extracted based on basic protocol from Doyle laboratorium in miniprep scale with few modification using extraction buffer of CTAB. The FastStart program for PCR was carried out with condition as follows: 1 cycle of 94°C for 4 min, then 40 cycles of 95°C denaturation for 45 sec, 55–60°C annealing for 45 sec, and 72°C elongation for 30 sec, with a final extension cycle of 72°C for 5 min and incubation at 4°C.

Table 1. List of Indonesian soybean varieties used in the study.

Variety name	Year released	Pedigree
Anjasmoro	2001	Mass selection from Mansuria pure line
Argo Mulyo	1998	Introduction from Thailand (var. Nakhon Sawan I)
Baluran	2002	AVRDC crossing
Burangrang	1999	Natural segregant from Jember local variety
Cikuray	1992	No 630 x No 1343 (Orba)
Davros	1965	Lines selection from Garut local variety
Dempo	1984	Introduction from Colombia (var. Amerikana)
Detam 1	2008	Introduction line 9837 x Kawi
Detam 2	2008	Introduction line 9837 xWilis
Dieng	1991	Manalagi x Orba
Galunggung	1981	Davros (No 1248) x TK-5 (No. 1291)
Gepak Ijo	2008	Lines selection from Ponorogo local variety
Gepak Kuning	2008	Lines selection from Ponorogo local variety
Grobogan	2008	Lines selection of Malabar Grobogan local variety
Gumitir	2005	Introduction from Taiwan (GC 86019-190-IN)
Guntur	1982	TK-5 (Gm 26) x Genjah Slawi (Gm 14)
Ijen	2003	Backcross of Wilis x Himeshirazu
Kaba	2001	Diallel cross of 16 parents
Kawi	1998	Introduction line from Taiwan MSC 9050-C-7-2 (G 10050) x MSC 8306-1-M
Kipas Putih	1992	Lines selection of Aceh local variety
Leuser	1998	Pasuruan local var (MLG 2621) x mutant B-1682
Lokon	1982	TK-5 (Gm 26) x Genjah Slawi (Gm 14)
Lompobatang	1989	Sinyonya x No 1682
Lumajang Bewok	1989	Lines selection of Lumajang local variety
Malabar	1992	No 1592 x Wilis
Menyapa	2001	B-3034 x Lampung local variety
Merapi	1938	Lines selection of East Java local variety
Merbabu	1986	Orba x Sinyonya
Orba	1974	Davros x Shakti
Panderman	2003	Introduction from Taiwan
Pangrango	1995	Lampung local variety x Davros
Petek	1989	Lines selection of Kudus local variety
Rajabasa	2004	Mutant No 214 x 23-D (irradiation of Guntur var)
Ratai	2004	Wilis x No. 3465
Raung	1986	Darvos x Shakti
Rinjani	1989	Shakti x No. 1682
Sindoro	1995	Dempo x Wilis
Slamet	1995	Dempo x Wilis
Tambora	1989	Introduction from Philipines
Tanggamus	2001	Kerinci x No.3911
Tidar	1987	Mutant selection of B 1682 from Taiwan
Wilis	1983	Orba x No. 1682

*Source: Puslitbangtan, 2009; 2015; PPVT, 2015.

Table 2. Multiplex panel set of soybean SSR markers used in this study.

Multiplex panel	SSR markers*	Fluorescent color	Chromosome	Repeat motifs	Allele size (bp)
1	Satt131	D4-blue	18	(TAT)13	111–320
	Satt516	D3-green	13	(TAA)19	131–280
2	Satt009	D2-black	3	(AAAT)3(AAT)13	161–250
	Satt038	D3-green	18	(ATA)17	161–200
	Satt114	D4-blue	13	(AAT)17	71–120
3	Satt242	D2-black	9	(TTA)26	131–160
	Satt177	D3-green	8	(ATT)16	101–120
	Satt294	D4-blue	4	(TAT)23	251–300
4	Satt147	D2-black	1	(ATA)15	100–179
	Satt308	D2-black	7	(TTA)22	180–330
	Satt414	D4-blue	16	(ATT)23	160–380
5	Satt191	D4-blue	18	(TAT)19	191–240
	Satt534	D3-green	14	(TAT)30	101–230
	Satt373	D4-blue	19	(TAT)21	151–170

*Soybean primers sequence can be accesed at http://soybase.org/resources/ssr.php.

Detection of SSR Fragment with CEQ8000 Genetic Analyzer

Procedure for sample preparation and running process in CEQ8000 Genetic Anyalyzer followed basic protocol of Thomson (Thomson, 2004). PCR products were diluted with sample loading solution (SLS) at ratio of 1:6 (v/v). For each multiplex panel set, the diluted PCR products from three different fluorescent SSR markers were loaded in the same well of CEQ sample plate. SLS liquid and 0.5 μl of CEQ internal standard size (400 bp) were added to the well until the volume reached 40 μl. To prevent the evaporation of sample-mixed during the fragment separation by CEQ8000, one drop of mineral oil was added to each well. In another plate (CEQ buffer plate), CEQ buffer was added up to three:quarters part of the well. Afterward, both plates were placed into CEQ8000 Genetic Analyzer platform and Frag-1 program was run with following condition: capillary temperature of 35°C, injection at 2.0 kV for 30 sec, denaturation at 90°C for 120 sec, and separation at 7.5 kV for 35 min. After 12 hours, DNA fragments can be seen in the monitor screen as peaks with different fluorescent color.

Data Analysis, Marker Set Development, and Varieties Coding

Allele sizes of each DNA fragment obtained from CEQ8000 Genetic Analyzer were analyzed by binning analysis using CEQ Fragment Analysis Software (Thomson, 2004). Binning is grouping the DNA fragments based on the number of repeat motifs of di-, tri-, or tetra- nucleotide repeats of SSR markers. Binning data then was analyzed by PowerMarker v3.25 program to obtain the genetic information and polymorphism rate of SSR markers, which can be accessed at http://statgen.ncsu.edu/powermarker/index.html (Liu and Muse, 2005). This information was used as selection criteria for SSR marker set candidate for variety identification.

Afterward we determined the number of groups and the group range of each SSR marker. The number of group was based on the repeat number of SSR motifs. As an example for Satt414 which has SSR motif of "(ATT)23", we assigned 23 group for this locus. Meanwhile, group range represented the range of allele size in each group and was determined based on following standard grouping formula (https://www.wyzant.com/).

$$Group\ range = \frac{Maximum\ allele\ size - Minimum\ allele\ size}{The\ number\ of\ repeat\ motif\ of\ SSR\ marker}$$

The last step was to transform the allele size of the varieties into appropriate group code number which set in two numeric codes started from "00". The transformation was done to all SSR marker set candidates and the final ID of a variety is a combination of two numeric codes from all SSR marker set candidates. Validation of possibly duplicated code or ID was conformed through cluster analysis using SPSS 21.0 statistical software.

RESULTS AND DISCUSSION

Polymorphism Analysis and SSR Characterization

Based on SSR fragment generated from genetic analyzer platform, each SSR marker from the multiplex panel resulted a variety of amplicons (Figure 1). Fluorescent signal from each marker was shown as different peak color and size that represented DNA fragment from each variety. Based on a general terminology of SSR marker, one marker represents one locus and fragments from a marker represent alleles within the locus (Agarwal *et al*., 2008). Therefore different sizes of peaks from a marker show polymorphism among genotypes that are being tested. This allele size can be assigned as a specific DNA fingerprinting band of the varieties and can be used as variety identification.

The characteristics of 14 SSR markers from allele calling and binning analysis of the 42 soybean varieties are shown in Table 3. Eleven markers among of them had fragments detection more than 85%. This can be used as an initial criterion to determine SSR marker candidate for variety identification. Total detected alleles observed from this study were 168 alleles with a range of 4–25 alleles per SSR locus. This was higher than the previous study that detected only 115 alleles with a range of 7–19 alleles per SSR locus (Santoso *et al*., 2006). The more diverse varieties tested, the higher polymorphism rate of the markers would be obtained.

Other criteria that can be used to select SSR marker candidate for variety identification are frequency of rare allele and PIC value. Rare allele is the allele whose presence less than 5% within a population. We identified three SSR markers that did not have rare alleles, i.e. Satt177, Satt242, and Satt373. These markers could not be used for variety identification since they will not be able to distinguish tested varieties (Karp *et al*., 1997).

In this study, the PIC value represents high variations of alleles. From 14 SSR markers tested four markers demonstrated high PIC value of more than 0.8, i.e. Satt131, Satt147, Satt414, and Satt516. As previously reported, molecular marker applied in fingerprinting system must reflect the genotype information, having high ability to discriminate

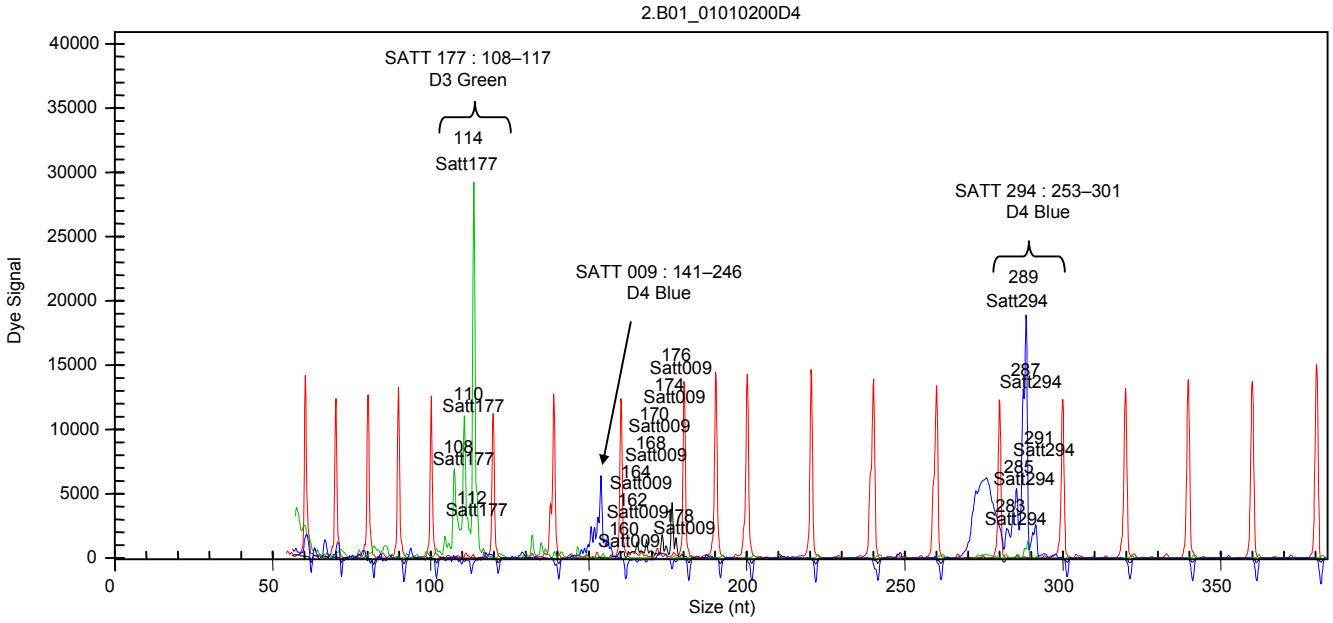

Figure 1. Fluorescent signal of 42 soybean varieties in one overlay graph from three SSR markers (SATT 177, SATT 009, SATT 294) of multiplex panel number one, generated from capillary electrophoresis of CEQ8000 Genetic Analyzer.

Table 3. Allele characteristics of 14 SSR markers used in DNA fingerprinting studies of 42 Indonesian soybean varieties.

SSR Markers	Chromosome	Fragment detection rate* (%)	Number of alleles detected	Frequency of dominant allele** (%)	Number of rare alleles detected	PIC value	Marker reproducibility***
Satt009	3	100.00	12	35.71	9	0.7817	3 times
Satt038	18	85.71	7	36.11	2	0.7277	3 times
Satt114	13	100.00	8	59.52	3	0.5794	3 times
Satt131	18	100.00	25	9.52	18	0.9466	once
Satt147	1	80.95	17	17.65	12	0.9089	3 times
Satt177	8	100.00	4	39.29	0	0.6589	3 times
Satt191	18	95.24	13	62.50	11	0.5819	once
Satt242	9	100.00	5	46.43	0	0.6728	3 times
Satt294	4	100.00	11	58.33	5	0.6195	2 times
Satt308	7	78.57	12	39.39	9	0.7856	3 times
Satt373	19	100.00	6	44.05	0	0.6411	once
Satt414	16	85.71	22	36.11	17	0.8354	3 times
Satt516	13	83.33	18	18.57	12	0.8848	once
Satt534	14	100.00	8	51.19	3	0.6480	once

*The percentage of succesfull detection of DNA fragment from 42 varieties by genetic analyzer platform; **the frequency of dominant allele throughout 42 varieties that are being tested; ***the number of repeated assays of using the same SSR locus in DNA fingerprinting analysis.

genotypes within the population, and user friendly for application and data analysis (Gale *et al*., 2005). Therefore based on the PIC value, these four SSR markers were qualified as candidate marker for variety identification.

Development of SSR Marker Set for Variety Identification

The goal of development a marker set for variety identification is to maximize ability in distinguishing the varieties with a number of markers within the set as minimum as possible (Jones *et al*., 2010). Therefore some factors have to be considered when selecting marker set composition for variety identification. Some approaches were applied by other researchers abroad such as linear integer (Gale *et al*., 2005), multivariate (Song *et al*., 1999) and genetic algorithm (Jones *et al*., 2010).

In our study we considered some factors in developing the marker set. This includes: (1) criteria to select candidate for marker set, (2) method to assign ID for genotypes tested, and (3) method to validate the chosen marker set. In selecting a marker candidate for variety identification, we used some criteria such as detection rate of the SSR fragment, the presence of rare allele, and PIC value. Reproducibility of SSR markers can be considered as well, as a criterion to

select a marker candidate for variety identification. From 14 SSR markers tested in this study, nine markers have been used 2–3 times in 2004, 2008, and 2011 (Table 3). The reproducibility means the use of same markers in different year of DNA fingerprinting analysis. Therefore considering those criteria, we selected four SSR markers as first candidates for variety identification, i.e Satt414, Satt147, Satt308, and Satt009.

Following step was determining the method for assigning variety ID that could be either original allele size-based or coding-based methods. Original allele size-based method can be performed qualitatively through DNA band image from gel electrophoresis and quantitatively in the form of allele size generated from genetic analyzer platform. The disadvantage of this method is difficulties in distinguishing alleles those have 1–3 bases differences. Whereas, coding-based method means the original allele size will be coded in another numeric value. To do so, series of groups have to be developed to distinguish alleles among the varieties and group range is calculated using a specific formula.

We determined the number of group for each SSR marker candidates based on their SSR motif repeat number. We calculated group range based on the formula in the materials and methods. This represented the allele size range within each group of each SSR marker candidates (Table 4). Afterward based on this code table, we transformed the allele size into codes and combine codes from all SSR marker candidates as the final ID of the varieties. As an example, Argomulyo variety had allele sizes of 296, 146, 190, and 213 for locus of Satt414, Satt147, Satt308, and Satt009, respectively. Based on the code table, this allele would be coded as 14, 09, 04, and 10 for Satt414, Satt147, Satt308, and Satt009 loci respectively. Therefore, the variety ID for Argomulyo would be "14090410". The same procedure then was applied to other varieties until all of them had specific ID. For those that did not succesfully amplified in a particular marker, would be assigned as "**".

Afterward we checked the possible variety ID duplication among varieties tested through clustering analysis. We found that two soybean varieties had same ID, i.e. Rinjani and Slamet (Figure 2). Therefore we added another SSR marker into the marker set to distinguish these two varieties. We checked their allele sizes in the remaining SSR markers that have not been used in the set. We found only one marker (Satt516) that can discriminate these two varieties. We then applied the same coding procedure for Satt516 to all varieties and added two numeric codes of Satt516 in the last position of variety ID that were being assigned previously. Therefore the final marker set for soybean varieties identification consisted of five markers (Satt414, Satt147, Satt308, Satt009, and Satt516) and variety ID was comprised of 10 numeric codes (Table 5).

Assigning ID or numeric code for a variety using a marker set is an effort to give a spesific identity that

Table 4. Codes for allele size range of five SSR markers of final set marker identification.

Code	Satt414	Satt147	Satt308	Satt009	Satt516
00	166–174	100–104	160–166	171–174	141–147
01	175–183	105–109	167–173	175–178	148–154
02	184–192	110–114	174–180	179–182	155–161
03	193–201	115–119	181–187	183–186	162–168
04	202–210	120–124	188–194	187–190	169–175
05	211–219	125–129	195–201	191–194	176–182
06	220–228	130–134	202–208	195–198	183–189
07	229–237	135–139	209–215	199–202	190–196
08	238–246	140–144	216–222	203–206	197–203
09	247–255	145–149	223–229	207–210	204–210
10	256–264	150–154	230–236	211–214	211–217
11	265–273	155–159	237–243	215–218	218–224
12	274–282	160–164	244–250	219–222	225–231
13	283–291	165–169	251–257	223–226	232–238
14	292–300	170–174	258–264	227–230	239–245
15	301–309	175–179	265–271	231–234	246–252
16	310–318		272–278	235–238	253–259
17	319–327		279–285		260–266
18	328–336		286–292		267–273
19	337–345		293–229		274–280
20	346–354		300–306		
21	355–363		307–313		
22	364–372		314–320		
23	373–381				

Additional**DNA fragment was not successfully read by genetic analyzer.

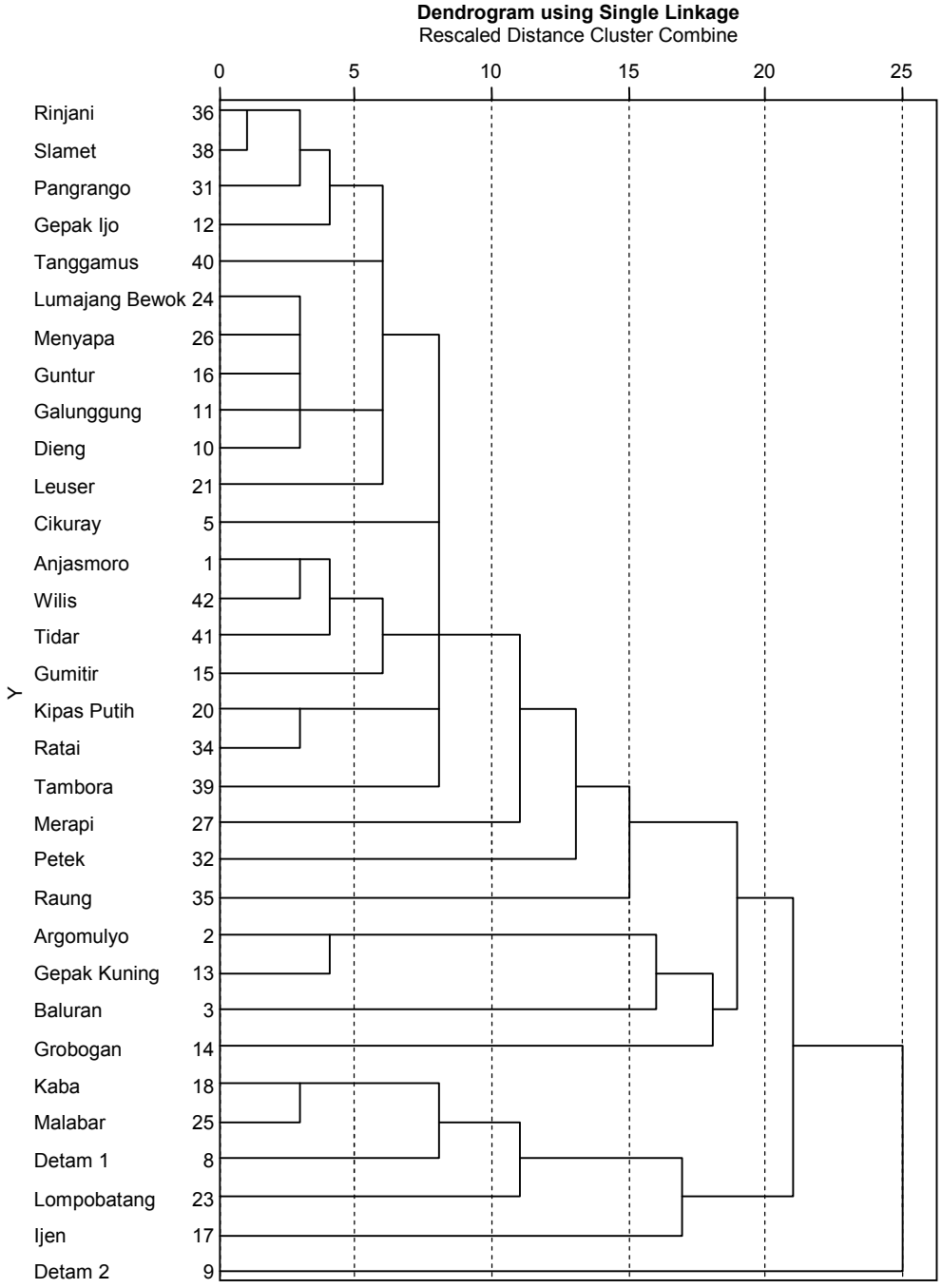

Figure 2. Dendrogram as a result of clustering analysis of soybean varieties analyzed with four SSR markers (Satt414, Satt147, Satt308, and Satt009).

can quantitatively differentiate them from others. It gives benefit in variety protection and breeder right, complement tools for DUS testing, and seed purity testing. In this study, 42 soybean varieties had unique profile ID and can be distinguished each other (Table 5). Two closest variety for example Rinjani and Slamet had ID of "1406040116" and "1406040117", respectively. The two only showed differences at the last two numeric codes since one marker identifier (Satt516) was added to the marker set. The original allele sizes of Rinjani and Slamet for locus Satt516 were 258 and 265, respectively. Based on code table, these two alleles were coded as 16 and 17, respectively.

Table 5. Profile code of 42 soybean varieties done based on marker set comprised of 5 SSR markers.

Variety name	Profile Code#	Variety name	Profile Code#
Anjasmoro	14**0401**	Lokon	******0900
Argomulyo	1409041000	Lompobatang	0409040217
Baluran	1309041617	Lumajang Bewok	1510040103
Burangrang	1206**16**	Malabar	**09040116
Cikuray	1513040116	Menyapa	1410040116
Davros	02****0200	Merapi	1113040003
Dempo	14****0202	Merbabu	14**0409**
Detam 1	**6040116	Orba	16**220616
Detam 2	2302**01**	Panderman	******0204
Dieng	1410040016	Pangrango	1406040217
Galunggung	14090401**	Petek	1400060701
Gepak Ijo	1407050117	Rajabasa	0009**0216
Gepak Kuning	1410040900	Ratai	1104060116
Grobogan	1006000717	Raung	1604110216
Gumitir	1403040217	Rinjani	1406040116
Guntur	1509040118	Sindoro	0002**0117
Ijen	**01080117	Slamet	1406040117
Kaba	**10040117	Tambora	1101050216
Kawi	14****0117	Tanggamus	1405060117
Kipas Putih	1104050116	Tidar	14000502**
Leuser	1509060201	Wilis	1401040117

Profile code from left to right is for Satt414, Satt147, Satt308, Satt009, and Satt516.

The allele uniqueness of molecular marker for identification has been given specific identity of elite variety in some species. As reported by other researchers, 13 SSR loci were able to differentiate 66 American soybean varieties, 4 SSR markers differentiated 66 apple cultivars, 12 SSR markers differentiated 48 wheat cultivars, and 6 SSR markers differentiated 400 potato varieties (Galli *et al*., 2005; Reid and Kerr, 2007; Song *et al*., 1999). In Japan, identification marker consisted of random amplified polymorphic DNA (RAPD) was applied to protect Koshihikari rice from commercial seed forgery (Ohtsubo and Nakamura, 2007). Several japonica and indica rice have been identified as well using identification marker related to flavored rice quality trait (Lestari *et al*., 2009; 2012).

Therefore assigning an ID using a marker set can be implemented for releasing superior varieties in Indonesia. It can be applied as well for protecting local indigenous varieties of some crops. Marker composition within the marker set should be flexible in line with variety addition or alteration. However, method and technique used in developing the marker set should be consistent.

CONCLUSIONS

According to polymorphism and clustering analysis, a marker set for identification of Indonesian soybean varieties was successfully developed. This marker set comprised of five SSR markers (Satt 414, Satt147, Satt308, Satt009, and Satt516) and can differentiate 42 improved varieties of the Indonesian soybean. This marker set can be used as a complementary tool in DUS test and can be continuously adjusted in line with the release of new soybean varieties.

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