Jurnal Agro*Biogen* 14(1):11–22

Genetic Diversity Analysis and F_2 Population Development for Breeding of Long Juvenile Trait in Soybean

(Analisis Diversitas Genetik dan Pembentukan Populasi F_2 untuk Pemuliaan Karakter Juvenil Panjang pada Kedelai)

I Made Tasma[1]*, N.P. Mega G. Yani[2], Rosliana Purwaningdyah[2], Dani Satyawan[1], Kristianto Nugroho[1], Puji Lestari[1], Kurniawan R. Trijatmiko[1], and Mastur[1]

[1]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: imade.tasma@gmail.com
[2]Department of Biochemistry, Bogor Agricultural University, Jl. Meranti, Dramaga Campus, Bogor 16680 Indonesia

Submitted: 26 December 2017; Revised: 18 May 2018; Accepted: 12 June 2018

ABSTRAK

Analisis diversitas genetik menggunakan marka molekuler merupakan langkah penting untuk memilih tetua yang tepat dalam program pemuliaan kedelai. Tujuan penelitian ini adalah (1) menganalisis diversitas genetik 29 genotipe kedelai menggunakan 27 marka SSR untuk memilih tetua yang tepat dan (2) membentuk populasi F_2 untuk digunakan dalam pemuliaan karakter juvenil panjang (JP) kedelai pada kondisi panjang hari pendek. Materi genetik yang digunakan terdiri atas 11 genotipe kedelai Indonesia dan 18 genotipe introduksi dari Amerika Serikat. Diversitas genetik ditentukan berdasarkan hasil analisis menggunakan 27 marka SSR yang lokasinya menyebar merata pada genom kedelai. Populasi F_2 telah dibentuk dengan menyilangkan Grobogan dengan tiga genotipe introduksi pembawa karakter JP. Nilai PIC 27 marka SSR berkisar antara 0,87–0,96. Hasil penelitian menunjukkan 29 genotipe kedelai yang diuji terbagi menjadi tiga klaster pada koefisien kesamaan 0,76. Lima genotipe dengan karakter JP dan sembilan genotipe kedelai Indonesia menunjukkan diversitas genetik tinggi dan tentunya akan bermanfaat sebagai pasangan tetua untuk digunakan dalam program pemuliaan. Keragaan progeni F_1 hasil persilangan tersebut jauh melebihi keragaan fenotipe kedua tetuanya. Tiga populasi F_2 telah dibentuk dengan menyilangkan genotipe berkerabat jauh. Populasi F_2 tersebut telah diverifikasi menggunakan marka SSR dan bersegregasi 1:2:1 yang mengonfirmasi segregasi marka SSR kodominan. Populasi F_2 hasil penelitian ini tentunya bermanfaat untuk pemuliaan karakter JP kedelai di negara tropis dengan derajat lintang rendah seperti Indonesia dengan panjang hari sekitar 12 jam sepanjang tahunnya.

Kata kunci: Kedelai, diversitas genetik, marka SSR, populasi F_2, juvenil panjang.

ABSTRACT

Genetic diversity analysis using molecular markers is an important step for selecting appropriate parents in a soybean breeding program. The aims of this study were to (1) analyze genetic diversity of 29 soybean genotypes assessed with 27 SSR markers for selecting appropriate parents and (2) develop F_2 populations to be used for breeding long juvenile (LJ) trait in soybean to be cultivated in short photoperiod condition. The soybean genotypes used consisted of 11 Indonesian soybean genotypes and 18 genotypes introduced from the USA. F_2 populations were developed by crossing Grobogan with three introduced genotypes carrying LJ character. The PIC values of the 27 SSR markers ranged from 0.87 to 0.96. Cluster analysis resulted in three main clusters at coefficient similarity of 0.76. The five LJ introduced accessions and the nine Indonesian genotypes showed high genetic distances and are useful as parent pairs for developing breeding populations. The F_1 progeny phenotypic performances of the cross far exceeded the performaces of both parents. Three F_2 populations were developed by crossing the distantly related soybean genotypes. The F_2 populations were verified by using SSR markers and it was found that they segregated in a 1:2:1 ratio confirming the segregation ratio of codominant SSR markers. The F_2 populations should be useful for breeding LJ characters to improve soybean productivity in low latitude tropical countries such as Indonesia, which has day length of approximately 12 h all year round.

Keywords: Soybean, genetic diversity, SSR marker, F_2 population, long juvenile.

INTRODUCTION

Soybean (*Glycine max* [L.] Merr.) has been cultivated in a wide range of latitudes, ranging from latitudes 0º at the equator to 50ºN and 35ºS (Fehr 1987). However, each soybean cultivar can adapt only to a narrow region of latitudes of about 4º or ~480 km. Outside the regions, the cultivar matures too early in the south (lower latitude) causing low productivity and matures too late or fails to harvest before winter season arrives in the north (higher latitude) (Fehr 1987; Tasma 2013). For that reason, in the USA, soybean cultivars have been classified into thirteen maturity groups (MGs), from MG 000 to MG XII (Fehr 1987).

Soybean is classified as a short-day plant and its flowering is stimulated when the day is short (long night) with day length is shorter than critical period (Kenworthy et al. 1989). The minimum number of dark induction (long dark period) is required to induce flowering. At least five to six times of long dark periods (8 h light/16 h dark) are needed to induce flowering in soybean (Wilkerson et al. 1989). The flowering times of soybean genotypes with long juvenile (LJ) characters are not affected by short-day condition, eventhough the plants are grown in lower latitude areas (<20º) and are exposed with short-day length. Therefore, their flowering times are delayed about 10–15 days and their maturity delays about 20–30 days compared to the soybean genotypes without having the LJ character (Ray et al. 1995; Campelo et al. 1998; Bonato and Vello 1999).

At least ten genes affecting time of flowering and maturity have been identified in soybean at present, nine of which are known as the *E* series of genes (*E1–E9*) and one is the LJ gene (*J*) (McBlain and Bernard 1987; Cober 2011; Tasma 2013). Most of the genes have been previously reported (Tasma 2013). Dominant alleles of the nine *E* genes (*E1–E9*) delay flowering and maturity with variation of delaying effect in each gene (McBlain and Bernard 1987; Molnar et al. 2003), except for the dominant allele *E6* and *J* that flower and mature earlier than the recessive allele (*e6* and *j*) (Campelo et al. 1998; Cober 2011).

The products of all the above genes respond to the environments, mainly day length and temperature, and interact with other *E* gene products (Watanabe et al. 2012; Tasma 2013). The recessive alleles *e6* and *j* were identified as soybean genotypes that flower late at short-day condition (photoperiodisity <12 h) and bring LJ trait in breeding program to develop high productivity soybean cultivar in the tropical regions with short-day length (photoperiodisity) ≤12 h all year round as occur in Brazil (Ray et al. 1995; Bonato and Vello 1999).

Many Quantitative Trait Loci (QTL) controlling time of flowering, maturity, and photoperiod sensitivity have been detected. Some of the QTL have been previously reported (Tasma 2013). Two genes controlling the LJ character (*J* and *E6*) have been genetically mapped by several scientists and were shown to be located on chromosome 4 of the soybean genome (Li et al. 2017; Yue et al. 2017). QTL mapping study of *E6* gene indicated that *J* and *E6* might be tightly linked to each other (Li et al. 2017). Gene *J* was isolated and found to encode protein homolog to EARLY FLOWERING 3 (ELF3) of the model plant *Arabidopsis thaliana* and in soybean named as GmELF3 (Lu et al. 2017; Yue et al. 2017). GmELF3 protein physically interacts with the *E1* promoter to downregulate the *E1* transcription, repress the expression of two *FLOWERING LOCUS T* (*FT*) genes and promotes soybean flowering under short-days (Lu et al. 2017). It is expected that the *E6* gene can also be isolated in the near future. With the isolation of the two LJ genes, scientists might be able to explain the soybean adaptation mechanism to the tropical region with low latitude (latitude <20º with day length of approximately 12 h all year around).

The genetic sources of LJ period genes that have been well-characterized include Paranagoiana, a mutation form of Parana cultivar found in Brazil, that has *E6* gene where the recessive allele (*e6*) showed the LJ character. The LJ character has been used in breeding program in Brazil which resulted in soybean cultivar that is well-adapted to different short-day length and sowing time (Hartwig and Kiihl 1979).

In the USA, another soybean genotype having the LJ character was also discovered. The genotype known as PI 159925 (*G. max* Glycine H), that was originally introduced from Peru, is known to have *J* gene in which the homozygote recessive allele of the gene (*jj*) demonstrated late flowering phenotype at the short-day condition (Hartwig and Kiihl 1979; Ray et al. 1995). The gene has been genetically mapped and known to be located in chromosome 4 of the soybean genome (Cairo et al. 2002). The gene has also been widely used to develop high productivity soybean cultivar adaptable to short-day length of lower latitude regions in the tropical countries (Cober et al. 2010; James and Lawn 2011; Sentelhas et al. 2015). The two LJ genes (*J* and *E6*) should be useful for breeding LJ character in Indonesia which has latitude of <20º with day length of approximately 12 h all year round, similar to that occurs in the north

region of Brazil with tropical climate, low latitude, and short-day length (Ray et al. 1995; Carpentieri-Pipolo et al. 2014; Sentelhas et al. 2015). This breeding strategy is to incorporate the LJ character into the Indonesian soybean genotypes for more optimum vegetative growth before flowering with longer seed-filling period in order to develop soybean cultivars with better plant stature and high seed yield.

The objectives of this study were to (1) analyze genetic diversity of 29 soybean genotypes assessed with 27 SSR markers for selecting appropriate parents and (2) develop F_2 populations to be used for breeding of LJ trait in soybean to be cultivated in short photoperiod condition, such as Indonesia.

MATERIALS AND METHODS

Plant Materials

Genetic materials used in this study were 29 soybean genotypes consisted of 6 genotypes with LJ allele and 23 genotypes without LJ allele in their genomes. Among those, 11 genotypes were Indonesian soybean genotypes and 18 genotypes were introduced from the USA (Table 1). The seeds were requested online from the USDA/ARS (Agricultural Research Service 2016).

DNA Isolation, PCR Amplification, and Electrophoresis

Genomic DNA of the twenty-nine soybean genotypes was isolated by following the modified method of Doyle and Doyle (1990). The isolated DNA was qualitively determined by running the DNA sample in 0.8% agarose gel. The DNA quantity and quality were determined using NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, USA).

SSR markers and their characteristics used in this study are as shown in Table 2. The SSR primer pairs were screened based on their polymorphism level between the introduced soybean genotypes and a subset of the Indonesian genotypes. The primer pairs showing high polymorphism were selected to be used in this study. Most of the SSR markers used in this study were also well-validated for soybean diversity analysis in the previous studies (Santoso et al. 2006; Tasma et al. 2008; Tasma and Warsun 2009; Tasma et al. 2011; Carpentieri-Pipolo et al. 2014; Terryana et al. 2017). PCR was done by using SSR program as the following: denaturation at 94°C for 4 min, followed by PCR cycling of DNA denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, and DNA extension at 72°C for 45 s. The PCR was

Table 1. Characteristics of twenty-nine soybean genotypes used in this study.

Genotype	Accession number/breeding status	Origin	Maturity group*	Trait
Melrose	PI 612609	USA	V	Long juvenile (LJ)
Vernal	PI 564261	USA	VI	LJ
Glycine H	PI 159925	USA	VIII	LJ
Paranagoiana	PI 628880	USA	V	LJ
Hinson LJ	PI 629015	USA	VIII	LJ
X34	PI 632421	USA	IV	LJ
Hojaku Kuwazu	PI 416937	USA	VI	Aluminum (Al) toxicity and drought tolerance
Kukichi 1	PI 417021	USA	IV	Al toxicity tolerance
Cook	PI 553045	USA	VIII	Al toxicity tolerance
Davis	PI 553039	USA	VI	Al toxicity tolerance
220024	PI 471938	USA	V	Al toxicity tolerance
220020	PI 471931	USA	V	Drought tolerance
Throne	PI 564718	USA	III	Drought and flooding tolerance
Williams	PI 548631	USA	III	Responsive to genetic transformation
Williams 82	PI 518671	USA	III	Responsive to genetic transformation
Jack	PI 540556	USA	II	Responsive to genetic transformation
Maverick	PI 598124	USA	III	Responsive to genetic transformation
Asgrow A2396	PI 540454	USA	II	Responsive to genetic transformation
Grobogan	Cultivar	Indonesia	Early maturity	Productivity 2.77 t/ha, early maturity, large seed size
Anjasmoro	Cultivar	Indonesia	Medium maturity	Productivity 2.30–2.25 t/ha, lodging resistance
Tanggamus	Cultivar	Indonesia	Late maturity	Productivity 1.22 t/ha, Al toxicity tolerance
Biosoy-8	Cultivar	Indonesia	Early maturity	High productivity, large seed size
Biosoy-11	Cultivar	Indonesia	Early maturity	High productivity, large seed size
Dega-1	Cultivar	Indonesia	Early flowering	Productivity 2.76 t/ha, early maturity
Dena	Cultivar	Indonesia	Early maturity	Productivity 1.69 t/ha, shading tolerance (50% shading)
Dering	Cultivar	Indonesia	Medium maturity	Productivity 2.0 t/ha, drought tolerance at generative phase
Malabar	Cultivar	Indonesia	Medium maturity	Productivity 1.27 t/ha, drought tolerance
Tambora	Cultivar	Indonesia	Medium maturity	Productivity 1.5 t/ha
Genjah Hitam	Landrace	Indonesia	Medium maturity	Productivity 1.46 t/h, black seed color

*Based on the USA soybean maturity group classification (Fehr 1987).

conducted for 30 cycles followed by final DNA extension at 72°C for 7 min. The amplified SSR fragments were then electrophoresed using 8% acrylamide gel in 1× TAE buffer run for 110 min at 95 volt. The gel was stained using ethidium bromide solution. The stained gel was then photographed by using Gel Doc™ (Bio-Rad, USA).

Genetic Diversity Analysis

Genetic diversity of the twenty-nine soybean genotypes was analyzed based on the banding patterns of the SSR markers in each of the genotypes tested. The SSR allele bands were scored as '1' when the bands appear and given the value of '0' when no bands were observed in the expected sites. The genotypic data were then analyzed using

Table 2. Primer sequences and characteristics of twenty-seven SSR markers used in this study.

SSR marker	Chromosome	Linkage map	Motive	Sequence (5'→3')
Satt191	18	18/G	(TAT)19	F: CGCGATCATGTCTCTG R: GGGAGTTGGTGTTTTCTTGTG
Satt049	8	8/A2	(ATT)27	F: CCTTAGACCATGAATGTCTCGAAG ATA R: CTTAAGGACACGTGGAAGATGACTAC
Satt038	18	18/G	(ATT)17	F: GGGAATCTTTTTTTCTTTCTATTAAGTT R: GGGCATTGAAATGGTTTTAGTCA
Satt147	1	1/D1a	(ATT)14	F: CCATCCCTTCCTCCAAATAGAT R: CTTCCACACCCTAGTTTAGTGACAA
Satt308	7	7/M	(ATT)21	F: GCGTTAAGGTTGGCAGGGTGGAAGTG R: GCGCAGCTTTATACAAAAATCAACAA
Satt463	7	7/M	(ATT)19(CAA	F: TTGGATCTCATATTCAAACTTTCAAG R: CTGCAAATTTGATGCACATGTGTCTA
Satt294	4	4/C1	(ATT)23	F: GCGGGTCAAATGCAAATTATTTTT R: GCGCTCAGTGTGAAAGTTGTTTCTAT
Satt063	14	14/B2	(ATT)20	F: AAATGATTAACAATGTTTATGAT R: ACTTGCATCAGTTAATAACAA
Satt114	13	13/F	(AAT)17	F: GGGTTATCCTCCCCAATA R: ATATGGGATGATAAGGTGAAA
Satt069	2	D1b	(AT)29	F: CGACCAGCTGAAGAAA R: CTGAATACCCATCATTACTTAA
Satt045	15	15/E	(AAT)18	F: TGGTTTCTACTTTCTATAATTATTT R: ATGCCTCTCCCTCCT
Satt285	16	16/J	(TTA)19	F: GCGACATATTGCATTAAAAACATACTT R:GCGGACTAATTCTATTTTACACCAACAAC
Satt002	17	17/D2	(ATT)25	F: TGAGTGTATGTGTAACTATTGTG R: CAATAATGTGCCTATCCTTGTC
Satt012	18	18/G	(ATT)19	F: TCAAAGATAACGAAAACCATATTTA R: ATTATAGAAGAATAGAGCCTACAT
Satt131	18	18/G	(ATT)13	F: AATTTCCCATTATCATTTAGAA R: GGCCTTCATTCCAAAAC
Satt222	12	12/H	(ATT)20	F:GCGTGTTTTGTGAAAATAATAATTAAAGG R:GCGCCACAAGTAACTAATGTAATAGGTGT
Satt489	6	6/C2	(ATT)23(GTT	F:GCGTGTGCTTGCTTCTCTTAGACTG ACT R: GCGTACTACTTACCCTGTTTGTCTAAAA
Satt516	13	13/F	(ATT)19	F: GCGTTAGCACTATTTTTTTACAAGA R: GCGCCGTTCCTCTTTACTTTAT
Satt009	3	3/N	(ATT)14	F: CCAACTTGAAATTACTAGAGAAAT R: CTTACTAGCGTATTAACCCTTG
Satt022	3	3/D2	(AT)27	F: GCGGCCTTTTCTGACTGTTAA R: GCGCAGTGACTAAAACTTACTAT
Satt100	6	6/C2	(ATT)33	F: ACCTCATTTTGGCATAAA R: TTGGAAAACAAGTAATAATAACA
Satt043	-9	K	(AT)21	F:GCGGTCCGTCAATGAATATTAAATTAAAA R: GCGAAAGCGGCAGAGAGAGAAAGGT
Satt431	16	16/J	(AAT)21	F: GCGTGGCACCCTTGATAAATAA R: GCGCACGAAAGTTTTTCTGTAACA
Satt251	11	11/B1	(ATT)15	F: CCTCCACCCCCTTCCCACCCAAAA R: GGTGATATCGCGCTAAAATTA
Satt336	7	7/C2	(AT)19	F: AATTGGAGTGGGTCACAC R: TTCCCGGAAAGAAAGAAA
Satt197	11	11/B1	(ATT)20	F : CACTGCTTTTTCCCCTCTCT R: AAGATACCCCCAACATTATTTGTAA
Satt446	19	19/L	(ATT)21	F: CCGCATAAAAAACACAACAAATTA R: GCGGGCAAATTTGACCTAACTCACAAC

Source: Cregan et al. (1999), Song et al. (2004), Tasma et al. (2009), Chaerani et al. (2011), Tasma et al. (2011).

PowerMarker 3.25 software to obtain statistical data of allele frequency, gene diversity, gene heterozigosity, and Polymorphism Information Content (PIC) value. The PIC value for each SSR marker was calculated using the formula of Smith et al. (1997) as previously reported (Tasma and Warsun 2009). Phylogenetic analysis of the twenty-nine soybean genotypes was done by using Sequential Agglomerative Hierarchical and Nested-Unweighted Pair-Group Method with Arithmetic Mean (SAHN-UPGMA) of the NTSYS 2.1 software (Rohlf 2000).

Phenotypic Performance of Twenty-nine Soybean Genotypes in the Field

The twenty-nine soybean genotypes tested in this study were grown in the field of Cikeumeuh Experimental Station, Bogor, West Java (elevation: 250 masl) from February to June 2017. The genotypes were grown in pots containing 8 kg soils enriched with dung manure. Two seeds were sown per pot. The experiments were designed with Randomized Block Design with three replications. The plants were fertilized with 200 g dung manure per pot enriched with 50 kg urea, 100 kg SP-36, and 100 kg KCl per hectare. Intensive disease and insect management were applied to the plants by spraying the plant weekly with insecticide Decis® (0.5 ml/l). Parameters observed included flowering time (days to first flower), plant height, yield components (branch number/plant, pod number/plant, node number/plant, seed yield/plant, and 100-seed weight), and morphological characters (flower color, seed color, and pubescent color). Time of flowering and maturity were observed by following the method of Fehr and Cavines (1977). The observed phenotypic data were then analyzed using simple statistical method such as mean and standard deviation values (Steel and Torrie 1980) and the progeny phenotypic performance was then compared to both parents.

Development and Verification of F_1 and F_2 Populations

F_1 population was developed by crossing Grobogan with the five soybean genotypes having LJ trait (Melrose, Vernal, Glycine H., Paranagoiana, and Hinson LJ). Grobogan is one of the very popular soybean genotypes in Indonesia and is widely cultivated by Indonesian farmers for its large seed size, good seed yield, and early in maturity (Manshuri 2011). The F_1 putative seeds were planted in the pots containing 8 kg soils enriched with dung manure. Genomic DNA was isolated from each putative plant

Table 3. The profiles of 27 SSR markers used to analyze genetic diversity of 29 soybean genotypes.

Marker	Allele number	Allele size (bp)	Main allele frequency	Gene diversity	Heterozigosity	PIC value*
Satt191	11	208–261	0.09	0.95	0.32	0.94
Satt409	17	150–286	0.11	0.96	0.93	0.96
Satt038	11	152–209	0.19	0.93	0.66	0.92
Satt147	12	139–205	0.09	0.96	0.62	0.95
Satt308	9	170–222	0.14	0.92	0.86	0.92
Satt463	15	132–270	0.10	0.96	0.86	0.96
Satt294	17	173–264	0.07	0.97	0.83	0.97
Satt063	8	119–181	0.22	0.90	0.24	0.89
Satt114	8	92–127	0.10	0.93	0.62	0.93
Satt069	10	190–152	0.07	0.95	0.79	0.95
Satt045	7	145–181	0.16	0.91	0.24	0.90
Satt285	13	261–186	0.10	0.95	0.38	0.95
Satt002	7	141–171	0.12	0.93	0.04	0.93
Satt012	13	112–185	0.09	0.96	0.45	0.96
Satt131	12	157–288	0.12	0.95	0.86	0.95
Satt222	10	202–249	0.10	0.94	0.38	0.94
Satt489	13	235–302	0.09	0.95	0.72	0.95
Satt516	12	215–305	0.07	0.96	0.64	0.96
Satt009	18	162–264	0.09	0.96	0.62	0.96
Satt022	13	208–281	0.09	0.95	0.31	0.95
Satt100	12	125–190	0.10	0.96	0.90	0.96
Satt043	10	269–320	0.10	0.94	0.14	0.94
Satt431	13	190–266	0.09	0.95	0.76	0.95
Satt251	11	270–358	0.09	0.95	0.03	0.95
Satt336	12	189–283	0.09	0.95	1.00	0.95
Satt197	15	149–251	0.14	0.95	0.79	0.95
Satt446	7	275–307	0.14	0.92	0.00	0.91
Total	316					
Average	11.70		0.11	0.95	0.56	0.94

*Polymorphism Information Content of each SSR marker used in this study.

for verification of their heterozygosity using SSR markers. The verified F_1 plants were self-pollinated to form F_2 seeds and the seeds were then sown and grown in the pots containing soils done in similar manner as that conducted for the F_1 seeds. Genomic DNA was isolated from each F_2 plant and the DNA was used in SSR genotyping to verify their segregation ratio in the putative F_2 population. SSR marker assay was conducted in similar manner as that used for assaying F_1 progeny as previously described in this paper. A *Chi-square* test was then conducted to test the goodness of fit of the segregation ratio with the null hypothesis of 1:2:1 ratio progeny segregation (Tasma et al. 2001; Tasma and Shoemaker 2003; Tasma et al. 2008; Tasma et al. 2011). In an F_2 generation of a soybean population, a codominant SSR marker segregates in the ratio of 1 proportion progeny having allele originated from the first parent, 2 proportion heterozygote progenies with alleles originated from both parents, and 1 proportion progeny having allele originated from the second parent.

RESULTS AND DISCUSSION

Genetic Diversity Analysis of 29 Soybean Genotypes Using 27 SSR Markers

The genetic diversity analysis study of the 29 soybean genotypes having or not having the LJ trait (Table 1) assayed with 27 SSR markers (Table 2) resulted in a total of 316 SSR alleles. The average allele per SSR locus was 11.6 alleles (ranged from 7–18 alleles) (Table 3). Marker Satt294 produced the highest allele number (18 alleles), while Satt045, Satt002, and Satt446 gave the least number of alleles (7 alleles each). The PIC value observed from this study was high with an average PIC value of 0.94 (ranged from 0.89–0.97) (Table 3). The allelic diversity and polymorphism level observed in this study were much higher than those previously observed (Diwan and Cregan 1997; Tasma and Warsun 2009; Tasma et al. 2011). This study showed very high PIC values of 89–94%, indicating the soybean genotypes analyzed in this study were more divergent compared to those previously studied. One of the reasons was due to the

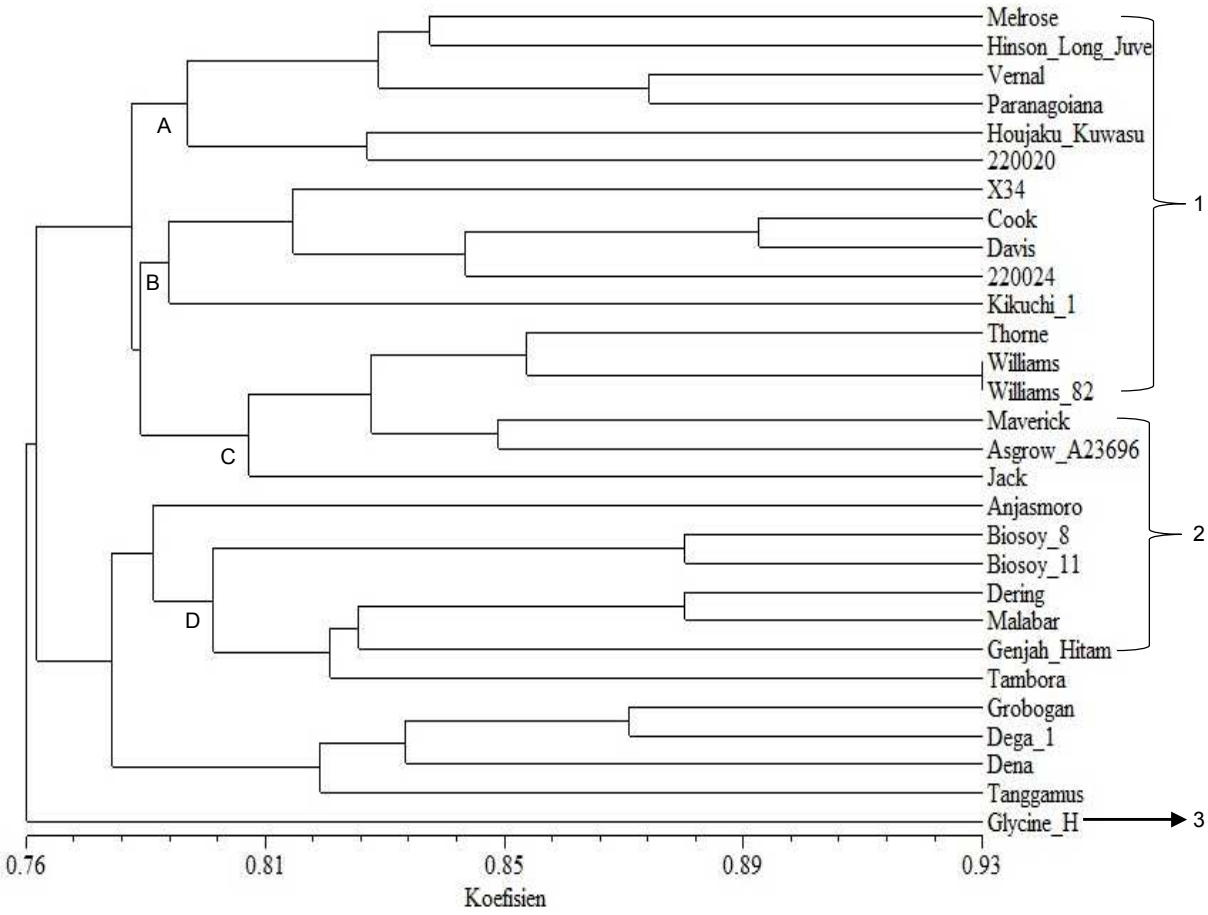

Figure 1. Dendrogram of the 29 soybean genotypes analyzed with the 27 SSR markers. Numbers in the right showed the three clusters resulted from this study.

combinations of the Indonesian genotypes (11 genotypes) and genotypes introduced from the USA (18 genotypes). Phylogenetic analysis showed that the two groups of soybean origin were separated into two big clusters separating the USA genotypes and those of the Indonesian genotypes supporting the high PIC values observed in this study.

Cluster analysis using the highest genetic similarity level of 76% divided the soybean genotypes into three clusters (Figure 1). The phylogeny clearly demonstrated that the genotypes separated based on their origin, i.e. the one originated fom the USA well-separated from those originated from Indonesia (Figure 1). One of the genotypes introduced from the USA (Glycine H) was separated out from the two clusters and form a cluster by itself (Figure 1), indicating this latest genotype was really divergent genetically from the soybean genotypes of the two clusters. This molecular analysis results was also supported by phenotypic performance of the Glycine H in the field that was really different from the other genotypes tested (data not shown).

F_1 Population Development and Their Phenotypic Performances

A total of 42 verified F_1 plants were developed from this study resulted from five crosses, i.e. Grobogan × Melrose (8 plants), Grobogan × Vernal (9 plants), Grobogan × Glycine H (8 plants), Grobogan × Paranagoiana (8 plants), and Grobogan × Hinson LJ (8 plants). The phenotypic performance of the F_1 plants compared to both parents are shown in Table 4. Example of seed size performance among the F_1 populations is shown in Figure 2. The F_1 progeny performance was much superior compared to both parents for yield components (Table 4). The F_1 progeny demonstrated better for plant branch number, pod number, node number, seed yield, and 100-seed weight (Table 4). This indicates that heterosis was observed in these F_1 populations. This suggests that line breeding using the segregating F_2 population should be useful in selecting superior lines to obtain progeny that are superior in agronomic characters, yield, and yield components and to select those progeny having the LJ trait.

Large seed size was one of the observed traits that was significantly improved in the F_1 progeny. The large seed size was inherited from the female parent, Grobogan. All of the F_1 progeny obtained from this sudy showed large seed sizes of >15 g/100 seeds (Figure 2), eventhough the LJ parents demonstrated small to medium seed sizes of 12–13 g/100 seeds. Few progeny also demonstrated larger seed size compared to the large seed parent, Grobogan (Table 4, Figure 2). This result suggests that the seed size is a heritable character supporting the one reported by Hakim and Suyamto (2017). They showed that improving seed size in soybean is possible with line breeding method. For that reason, the line breeding method should be seriously considered in the next breeding study to capture the important and economically important characters from both parents to be maintained in the selected superior high productivity soybean lines from this breeding program.

All five soybean genotypes having LJ character flowered much later than the ones that do not have the LJ trait. The LJ genotypes flowered 38–52 days after planting (DAP), while the ones that do not have LJ trait flowered much earlier, ranging from 26–32 DAP (data not shown). Similar data were shown by the LJ genotypes that matured much later (pod matured 97–110 DAP) than those that do not have LJ gene (pod matured 76–85 DAP). These data confirmed that the LJ genotypes delayed flowering and maturity in short-day condition that can be used in developing F_2 population segregating the LJ character. The F_2 population developed from this study should be useful for breeding high productivity soybean variety in tropical regions having short photoperiod such as Indonesia by means of delaying time of flowering and maturity. One of the soybean LJ genotypes (Paranagoiana) has been successfully used for developing high productivity tropical soybean adaptable to low latitude and short-day condition in the north part of Brazil (Spehar 1994; Campelo et al. 1998; Bonato and Vello 1999). Other LJ genotypes such as Glycine H has also been widely used world wide to develop tropical soybean cultivars in the tropical regions such as southern part of Australia and several African countries (James and Lawn 2011).

F_2 Population Development and SSR Marker Segregation Analysis

A total of five F_2 populations were originally developed, but only three F_2 populations (Grobogan × Melrose, Grobogan × Vernal, and Grobogan × Glycine H) were further assessed in this study. Each F_2 population was derived from 8–9 individual F_1 plants resulting in F_2 seeds. Six SSR markers were used to determine the segregation of the markers in the F_2 populations developed from this study (Table 5). Example of marker segregation in a subset of F_2 progeny is shown in Figure 3. *Chi-square* test of the six SSR markers assayed in two F_2 populations (Grobogan × Melrose and Grobogan × Vernal)

demonstrated a 1:2:1 ratio for both populations (Table 5). This indicates that the F_2 populations resulted from this study are good fit and followed a Mendelian segregation ratio as expected. The F_2 populations are ready to be used as base populations for line breeding such as pedigree and bulk breeding methods. F_1 progeny data showed that the phenotypic performance of the F_1 progeny much exceeded the ones shown by both parents (Table 4). This suggests that heterosis has occured among those F_1 progeny, in part due to the high genetic distances of both parents used F_1 population development. In addition, the two parent pairs of the crosses demonstrated many contrasted phenotypic performances. To describe a few, Grobogan had large seed size while all three introduced LJ genotypes had small seed sizes. Grobogan had average of 4 branches, while the three other parents had 6–9 branches. Pod number of Grobogan was 33 pods, while that of the other LJ parents ranged from 82–141 pods, and several other contrasting traits between the two parent pairs (Table 4).

Based on the F_1 performances described above, it is strongly recommended to conduct line breeding methods such as pedigree and bulk marker-assisted breeding methods. The application of the breeding methods should improve the chances to capture the best progeny with high productivity and demonstrate

Table 4. Phenotypic performance of three F_1 populations grown in the field of Cikeumeuh Experimental Station, Bogor, West Java (250 masl) from February to June 2017.

Population	F_1 progeny/parent	Plant height (cm)	Stem diameter (mm)	Branch number/plant	Node number/plant	Pod number/plant	Seed yield/plant (g)	100-seed weight (g)	Flowering time (DAP)	Maturity time (DAP)	Harvest time (DAP)	Seed coat color
Grobogan × Melrose	Grobogan	65.10	4.65	4	4.65	33	16.22	20.51	30	72	81	Brown
	Melrose	50.23	7.98	6	7.98	82	19.50	12.44	42	91	109	Pale brown
	1	57.50	7.35	5	13.00	88	31.06	16.24	29	70	77	Yellow
	2	56.80	6.70	7	13.00	69	27.02	17.64	30	71	77	Yellow
	3	49.30	7.95	6	11.00	130	42.20	19.46	29	71	76	Yellow
	4	56.20	7.20	7	11.00	82	34.12	18.76	30	72	77	Yellow
	5	47.80	5.10	3	11.00	54	21.04	17.72	29	71	77	Yellow
	6	62.70	7.60	8	13.00	86	36.42	19.22	29	71	77	Yellow
	7	59.20	5.85	2	10.00	49	17.48	17.28	31	74	77	Yellow
	8	59.50	10.15	8	13.00	111	44.34	19.64	31	74	77	Yellow
	Average	56.13	7.24	6	12.00	84	31.71	18.25	30	72	77	-
Grobogan × Vernal	Grobogan	65.10	4.65	4	4.65	33	16.22	20.51	30	72	81	Brown
	Vernal	69.60	8.23	9	8.23	141	14.25	11.15	47	101	113	Pale brown
	1	84.50	8.25	7	14.00	83	28.22	18.62	32	74	77	Yellow
	2	78.60	7.45	6	12.00	78	30.84	19.52	33	74	77	Yellow
	3	78.70	8.10	8	12.00	90	27.00	19.04	33	75	77	Yellow
	4	89.60	7.75	8	13.00	70	23.64	19.14	33	73	77	Yellow
	5	68.00	6.75	7	12.00	75	23.00	17.38	33	75	80	Yellow
	6	77.20	8.65	8	12.00	80	23.50	18.90	32	75	80	Yellow
	8	70.50	8.40	7	12.00	96	32.48	19.72	32	74	77	Yellow
	10	72.70	8.95	10	13.00	122	43.86	18.50	32	76	80	Yellow
	Average	77.48	8.04	8	13.00	87	29.07	18.85	33	75	78	-
Grobogan × Glycine H	Grobogan	65.10	4.65	4	4.65	33	16.22	20.51	30	72	81	Brown
	Glycine H	98.40	9.43	8	9.43	105	22.63	13.66	50	94	112	Dark brown
	3	70.80	7.15	6	13.00	70	23.54	21.96	32	71	79	Yellow
	4	69.40	8.00	5	13.00	62	24.90	24.87	31	71	79	Yellow
	5	70.90	8.80	7	13.00	88	28.44	21.80	31	71	79	Yellow
	7	57.50	6.90	4	11.00	58	16.34	19.29	32	73	80	Yellow
	8	41.70	7.35	8	18.00	79	23.74	18.56	32	72	78	Yellow
	10	62.50	6.40	5	12.00	54	11.78	22.73	30	71	85	Yellow
	11	69.40	8.10	7	13.00	93	28.82	21.86	31	73	79	Yellow
	Average	63.17	7.53	6	12.00	72	22.51	21.58	31	72	80	-

masl = meters above sea level, DAP = days after planting.

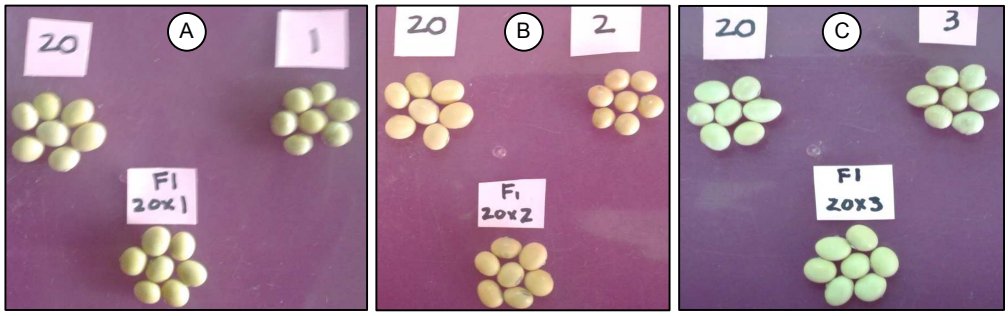

Figure 2. Seed size of F_1 populations compared to that of their parents. A = Grobogan × Melrose, B = Grobogan × Vernal, C = Grobogan × Glycine H, 20 = Grobogan, 1 = Melrose, 2 = Vernal, 3 = Glycine H.

LJ character. The improved lines developed should also be adapted to the low latitude regions with day length of about 12 h all year around. In addition, backcross breeding method by introgressing the LJ character from LJ genotypes as donor parent into an adapted Indonesian variety might also be useful, but the BC generation should be limited for up to about two BC generations to preserve many good yield component traits from the donor parents. BC_1F_2 needs also be tested their phenotypic performances in the field for the BC_1 lines having the LJ trait to obtain high productivity BC lines that express LJ gene.

Table 5. *Chi-square* test of SSR markers in two F_2 populations developed from crosses of Grobogan × Melrose and Grobogan × Vernal.

Population	SSR marker	Ratio tested	Observed			Expected			χ^{2*}
			A	B	H	A	B	H	
Grobogan × Melrose	Satt009	1:2:1	50	48	98	47	47	95	0.38 ns
	Satt063		48	48	93				0.08 ns
	Satt147		52	45	92				0.71 ns
	Satt197		42	40	107				3.05 ns
	Satt308		45	39	105				2.50 ns
	Satt463		42	50	97				0.77 ns
Grobogan × Vernal	Satt197	1:2:1	42	38	83	41	41	82	0.22 ns
	Satt063		44	36	83				0.81 ns
	Satt114		36	36	91				1.99 ns
	Satt646		47	42	74				1.88 ns
	Satt607		48	39	76				1.82 ns
	Sat_140		45	41	77				0.81 ns

*The null hypothesis of the test was that the SSR markers segregated in a 1:2:1 ratio.
A = SSR allele originated from first parent (Grobogan), B = SSR allele originated from second parent (Melrose or Vernal), H = SSR allele originated from both parents, ns = statistically not significant at p = 0.05 indicating the null hypothesis was accepted.

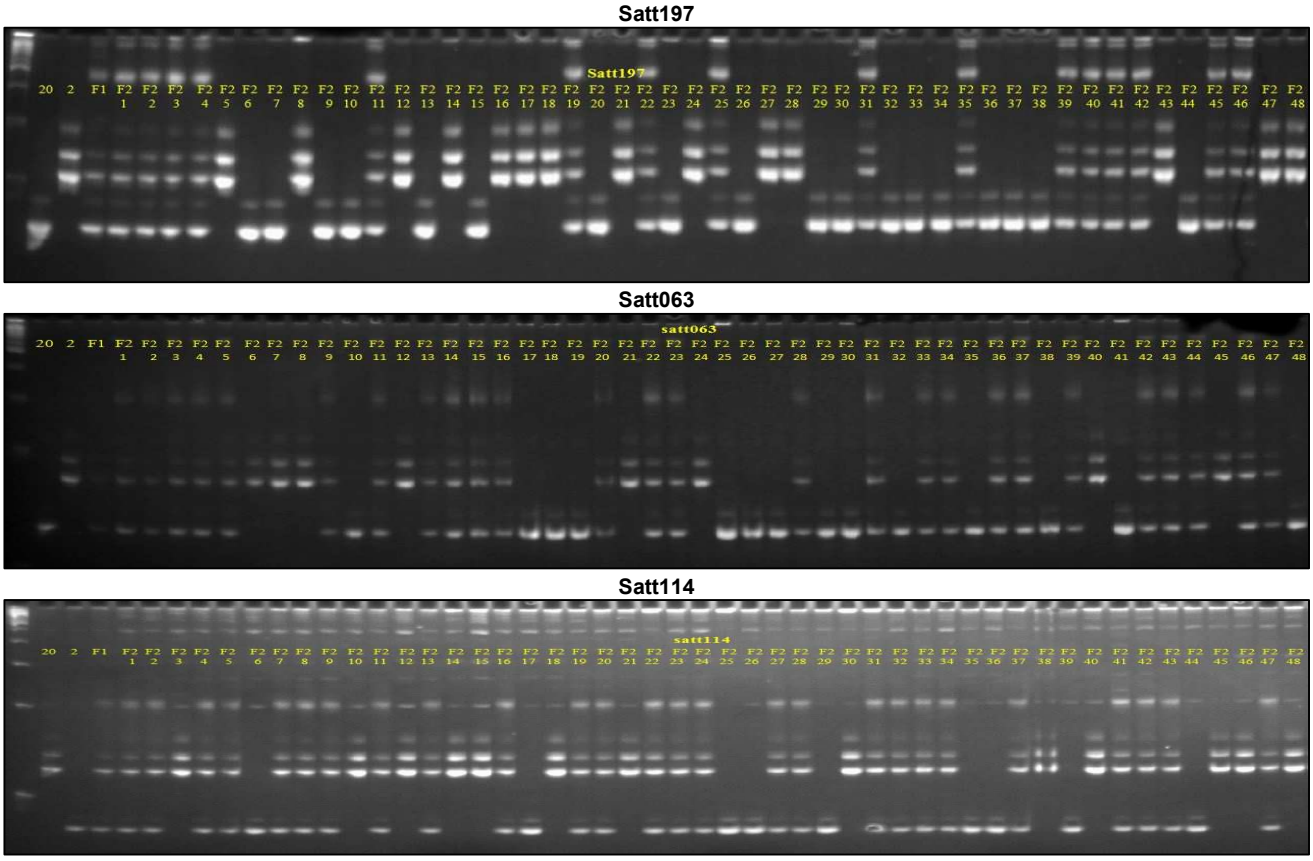

Figure 3. Segregation of three SSR markers (Satt197, Satt063, and Satt114) in the progeny of F_2 population Grobogan × Vernal.

CONCLUSION

Phylogenetic analysis of 29 soybean genotypes analyzed using 27 SSR markers resulted in three clusters, i.e. the USA genotypes group in one cluster, except for Glycine H that formed a cluster by itself, and the Indonesian genotypes group into other contrasted cluster. The genotypes with LJ trait were well-separated from the Indonesian genotypes, indicating that they are distantly related genetically, and therefore, suggesting good candidate for parents in F_2 population development for breeding the LJ character. The crossing between Grobogan and three LJ genotypes (Melrose, Vernal, and Glycine H) resulted in F_1 progeny with yield component characters exceeding those shown by the two parents, indicating high probability to obtain superior progeny. A line breeding method such as pedigree and/or bulk breeding methods is suggested to be applied for breeding LJ character in addition to the backcross breeding method. Three SSR markers-verified F_2 populations were developed from this study as a high value resource for breeding high yielding soybean tropical cultivar expressing the LJ trait.

ACKNOWLEDGEMENT

This study was funded by the national budget FY 2016 and 2017 through ICABIOGRAD, IAARD. The authors thank to Mrs. Ratna Utari for assistance in developing the F_1 and F_2 populations, Mr. Bambang Setyawarman for taking cares the plant materials, Dr. Tri Puji Priyatno and Drs. Pandoyo for their helps in screen house preparation. We also thank the USDA/ARS-University of Illinois Urbana-Champaign, Illinois, USA, for providing seeds for some of the soybean genotypes.

REFERENCES

Agricultural Research Service (2016) *The Germplasm Resources Information Network (GRIN)*. [Online] Available from: https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/plant-and-animal-genetic-resources-preservation/docs/germplasm-requests/ [Accessed 19 August 2016].

Bonato, E.R. & Vello, N.A. (1999) *E6*, a dominant gene conditioning early flowering and maturity in soybeans. *Genetics and Molecular Biology*, 22 (2), 229–232.

Cairo, C.A., Stein, J., Delgado, L., Bortolotti, S., Guelman, S.A., Ortiz, J.P.A. & Morandi, E.N. (2002) Tagging the juvenile locus in soybean (*Glycine max* [L.] Merr.) with molecular markers. *Euphytica*, 124 (3), 387–395.

Campelo, G.J., Kiihl, R.A.S. & Almeida, L.A. (1998) *Soybean: Development for low latitude regions*. Teresina: Embrapa Meio-Norte, 36 (Embrapa Meio-Norte. Documentos, 37).

Carpentieri-Pipolo, V., de Almeida, L.A., Kiihl, R.A.S. & Pagliosa, E.S. (2014) Inheritance of late flowering in natural variants of soybean cultivars under short-day conditions. *Pesquisa Agropecuaria Brasileira*, 49 (10), 796–803.

Chaerani, C., Hidayatun, N. & Utami, D.W. (2011) Keragaman genetik 50 aksesi plasma nutfah kedelai berdasarkan sepuluh penanda mikrosatelit. *Jurnal AgroBiogen*, 7 (2), 98–105.

Cober, E.R. (2011) Long juvenile soybean flowering responses under very short photoperiods. *Crop Science*, 51 (1), 140–145.

Cober, E.R., Molnar, S.J., Charette, M.R. & Voldeng, H.D. (2010) A new locus for early maturity in soybean. *Crop Science*, 50 (2), 524–527.

Cregan, P.B., Jarvik, T., Brush, A.L., Shoemaker, R.C., Lark, K.G., Kahler, A.L., Kaya, N., VanToai, T.T., Lohnes, D.G., Chung, J. & Specht, J.E. (1999) An integrated genetic linkage map of the soybean genome. *Crop Science*, 39 (5), 1464–1490.

Diwan, N. & Cregan, P.B. (1997) Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. *Theoretical and Applied Genetics*, 95 (5–6), 723–733.

Doyle, J.J. & Doyle, J.L. (1990) Isolation of plant DNA from fresh tissue. *Focus*, 12, 13–15.

Fehr, W.R. (1987) *Principles of Cultivar Development. Volume 1: Theory and Techniques.* MacMillan Publishing, New York. 550 p.

Fehr, W.R. & Cavines, C.E. (1977) *Stages of Soybean Development. Special Report 80*. Iowa State University, Ames, Iowa, USA.

Hakim, L. & Suyamto, S. (2017) Gene action and heritability estimates of quantitative characters among lines derived from varietal crosses of soybean. *Indonesian Journal of Agricultural Science*, 18 (1), 25–32.

Hartwig, E.E. & Kiihl, R.A.S. (1979) Identification and utilization of a delayed flowering character in soybeans for short-day conditions. *Field Crops Research*, 2, 145–151.

James, A.T. & Lawn, R.J. (2011) Application of physiological understanding in soybean improvement. II. Broadening phenological adaptation across regions and sowing dates. *Crop & Pasture Science*, 62 (1), 12–24.

Kenworthy, W.J., Brown, A.H.D. & Thibou, G.A. (1989) Variation in flowering response to photoperiod in perennial *Glycine* species. *Crop Science*, 29 (3), 678–682.

Li, X., Fang, C., Xu, M., Zhang, F., Lu, S., Nan, H., Su, T., Li, S., Zhao, X., Kong, L., Yuan, X., Liu, B., Abe, J., Cober, E.R. & Kong, F. (2017) Quantitative trait locus mapping of soybean maturity gene *E6*. *Crop Science*, 57 (5), 2547–2554.

Lu, S., Zhao, X., Hu, Y., Liu, S., Nan, H., Li, X., Fang, C., Cao, D., Shi, X., Kong, L., Su, T., Zhang, F., Li, S., Wang, Z., Yuan, X., Cober, E.R., Weller, J.L., Liu, B., Hou, X., Tian, Z. & Kong, F. (2017) Natural variation at the soybean *J* locus improves adaptation to the tropics and enhances yield. *Nature Genetics*, 49 (5), 773–779.

Manshuri, A.G. (2011) Laju pertumbuhan vegetatif dan generatif genotipe kedelai berumur genjah. *Jurnal Penelitian Pertanian Tanaman Pangan*, 30 (3), 204–209.

McBlain, B.A. & Bernard, R.L. (1987) A new gene affecting the time of flowering and maturity in soybeans. *Journal of Heredity*, 78 (3), 160–162.

Molnar, S.J., Rai, S., Charette, M. & Cober, E.R. (2003) Simple sequence repeat (SSR) markers linked to *E1*, *E3*, *E4*, and *E7* maturity genes in soybean. *Genome*, 46 (6), 1024–1036.

Ray, J.D., Hinson, K., Mankono, J.E. & Malo, M.F. (1995) Genetic control of a long-juvenile trait in soybean. *Crop Science*, 35 (4), 1001–1006.

Rohlf, F. (2000) *NTSYSpc. Numerical Taxonomy and Multivariate Analysis System. Version 2.1*. Applied Biosystem, New York.

Santoso, T.J., Utami, D.W. & Septiningsih, E.M. (2006) Analisis sidik jari DNA plasma nutfah kedelai menggunakan markah SSR. *Jurnal AgroBiogen*, 2 (1), 1–7.

Sentelhas, P.C., Battisti, R., Câmara, G.M.S., Farias, J.R.B., Hampf, A.C. & Nendel, C. (2015) The soybean yield gap in Brazil–Magnitude, causes, and possible solutions for sustainable production. *The Journal of Agricultural Science*, 153 (8), 1394–1411.

Smith, J.S.C., Chin, E.C.L., Shu, H., Smith, O.S., Wall, S.J., Senior, M.L., Mitchell, S.E., Kresovich, S. & Ziegle, J. (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): Comparisons with data from RFLPS and pedigree. *Theoretical and Applied Genetics*, 95 (1–2), 163–173.

Song, Q.J., Marek, L.F., Shoemaker, R.C., Lark, K.G., Concibido, V.C., Delannay, X., Specht, J.E. & Cregan, P. (2004) A new integrated genetic linkage map of the soybean. *Theoretische und Angewandte Genetik*, 109, 122–128.

Spehar, C.R. (1994) Breeding soybeans to the low latitudes of Brazilian Cerrados (Shapannah). *Pesquisa Agropecuária Brasileira,* 29 (8), 1167–1180.

Steel, R.G.D. & Torrie, J.H. (1980) *Principles and procedures of statistics: A biometrical approach.* 2nd ed. McGraw-Hill Book, New York.

Tasma, I.M. (2013) Gen dan QTL pengendali umur pada kedelai. *Jurnal AgroBiogen*, 9 (2), 85–96.

Tasma, I.M. & Shoemaker, R.C. (2003) Mapping flowering time gene homologs in soybean and their association with maturity (*E*) loci. *Crop Science*, 43 (1), 319–328.

Tasma, I.M. & Warsun, A. (2009) Genetic diversity analysis of aluminum-toxicity tolerant and sensitive soybean genotypes assessed with microsattelite markers. *Jurnal AgroBiogen*, 5 (1), 1–6.

Tasma, I.M., Lorenzen, L.L., Green, D.E. & Shoemaker, R.C. (2001) Mapping genetic loci for flowering time, maturity, and photoperiod insensitivity in soybean. *Molecular Breeding*, 8 (1), 25–35.

Tasma, I.M., Warsun, A. & Asadi (2008) Development and characterization of F_2 population for molecular mapping of aluminum-toxicity tolerant QTL in soybean. *Jurnal AgroBiogen*, 4 (1), 1–8.

Tasma, I.M., Warsun, A., Yunus, M. & Santoso, B. (2009) Genetic diversity analysis of aluminum-toxicity tolerant and sensitive soybean genotypes assessed with microsattelite markers. *Jurnal AgroBiogen*, 5 (1), 1–6.

Tasma, I.M., Satyawan, D., Warsun, A., Yunus, M. & Santosa, B. (2011) Phylogenetic and maturity analyses of sixty soybean genotypes used for DNA marker development of early maturity quantitative trait loci in soybean. *Jurnal AgroBiogen*, 7 (1), 37–46.

Terryana, R.T., Nugroho, K., Mulya, K., Dewi, N. & Lestari, P. (2017) Keragaman genotipik dan fenotipik 48 aksesi kedelai introduksi asal Cina. *Jurnal AgroBiogen*, 13 (1), 1–16.

Watanabe, S., Harada, K. & Abe, J. (2012) Genetic and molecular bases of photoperiod responses of flowering in soybean. *Breeding Science*, 61 (5), 531–543.

Wilkerson, G.G., Jones, J.W., Boote, K.J. & Buol, G.S. (1989) Photoperiod sensitive interval in time to flower of soybean. *Crop Science*, 29, 721–726.

Yue, Y., Liu, N., Jiang, B., Li, M., Wang, H., Jiang, Z., Pan, H., Xia, Q., Ma, Q. & Han, T. (2017) A single nucleotide deletion in *J* encoding GmELF3 confers long juvenility and is associated with adaption of tropic soybean. *Molecular Plant*, 10 (4), 656–658.