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)

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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 pembring 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 diperlihatkan menghasilkan marka SSR bersegregasi 1:2:1 yang mengonfirmasi segregasi marka SSR kodominan. Populasi F$_2$ 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 performances 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 0° at the equator to 50°N and 35°S (Fehr 1987). However, each soybean cultivar can adapt only to a narrow region of latitudes of about 4° 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, even though the plants are grown in lower latitude areas (<20°) 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 (photoperiodicity <12 h) and bring LJ trait in breeding program to develop high productivity soybean cultivar in the tropical regions with short-day length (photoperiodicity) ≤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 EI promoter to downregulate the EI 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 <20° 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 (j) 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 <20° 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 F₂ 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 qualitatively 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    |
| Paranaoiana | 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              | Drought 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 |
| Anjasomo | 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/ha, 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 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: CGCGATCATGTCCTCTG |
| Satt049 | 8 | 8/A2 | (ATT)27 | R: CGGAGTGGGTTTCTTCTGTG |
| Satt038 | 18 | 18/G | (ATT)17 | F: GGGGATCTTCTTCTATTAGTT |
| Satt147 | 1 | 1/D1a | (ATT)14 | R: GGCGATTGAATGTGGTTTTTGTCA |
| Satt308 | 7 | 7/M | (ATT)21 | F: GGGTTAAAGGGCGAGGTTGAAAGTG |
| Satt463 | 7 | 7/M | (ATT)19/CA | R: GTGACATCTATTACAAACACTTCAG |
| Satt294 | 4 | 4/C1 | (ATT)23 | F: GGGGGTCAAAGCAAAATAATTAAAA |
| Satt063 | 14 | 14/B2 | (ATT)20 | R: GGGGTCTTACCTTCTTCTTCTT |
| Satt114 | 13 | 13/F | (ATT)17 | F: ATATGGAAGGATAGCTGCTG |
| Satt069 | 2 | D1b | (ATT)29 | R: CTGACATGAGATTAAGTAAAA |
| Satt045 | 15 | 15/E | (ATT)18 | F: TGGTCTTCTTTATTTCTTAT |
| Satt285 | 16 | 16/J | (TAT)19 | R: CGGCGATCATGTCCTCTG |
| Satt002 | 17 | 17/D2 | (ATT)25 | F: CAATAATGTCCTATTCCTG |
| Satt012 | 18 | 18/G | (ATT)19 | R: GGGGTCTTACCTTCTTCTTCTT |
| Satt131 | 18 | 18/G | (ATT)13 | F: ATATGGAAGGATAGCTGCTG |
| Satt222 | 12 | 12/H | (ATT)20 | R: CGGCGATCATGTCCTCTG |
| Satt489 | 6 | 6/C2 | (ATT)23/GTT | F: CGGCGATCATGTCCTCTG |
| Satt516 | 13 | 13/F | (ATT)19 | R: GGGGTCTTACCTTCTTCTTCTT |
| Satt009 | 3 | 3/N | (ATT)14 | F: GGGGTCTTACCTTCTTCTTCTT |
| Satt022 | 3 | 3/D2 | (ATT)27 | F: GGGGTCTTACCTTCTTCTTCTT |
| Satt100 | 6 | 6/C2 | (ATT)33 | F: ATATGGAAGGATAGCTGCTG |
| Satt043 | -9 | K | (ATT)21 | F: GGGGTCTTACCTTCTTCTTCTT |
| Satt431 | 16 | 16/J | (ATT)21 | R: GGGGTCTTACCTTCTTCTTCTT |
| Satt251 | 11 | 11/B1 | (ATT)15 | F: GGGGTCTTACCTTCTTCTTCTT |
| Satt336 | 7 | 7/C2 | (ATT)19 | F: GGGGTCTTACCTTCTTCTTCTT |
| Satt197 | 11 | 11/B1 | (ATT)20 | F: GGGGTCTTACCTTCTTCTTCTT |
| Satt446 | 19 | 19/L | (ATT)21 | F: GGGGTCTTACCTTCTTCTTCTT |

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<sub>1</sub> and F<sub>2</sub> Populations

F<sub>1</sub> 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<sub>1</sub> 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       |
| Satt204  | 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       |
| Satt209  | 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            | 215–285          | 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       |

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.](image)
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 from 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).

F1 Population Development and Their Phenotypic Performances

A total of 42 verified F1 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 F1 plants compared to both parents are shown in Table 4. Example of seed size performance among the F1 populations is shown in Figure 2. The F1 progeny performance was much superior compared to both parents for yield components (Table 4). The F1 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 F1 populations. This suggests that line breeding using the segregating F2 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 F1 progeny. The large seed size was inherited from the female parent, Grobogan. All of the F1 progeny obtained from this study showed large seed sizes of >15 g/100 seeds (Figure 2), even though 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 F2 population segregating the LJ character. The F2 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).

F2 Population Development and SSR Marker Segregation Analysis

A total of five F2 populations were originally developed, but only three F2 populations (Grobogan × Melrose, Grobogan × Vernal, and Grobogan × Glycine H) were further assessed in this study. Each F2 population was derived from 8–9 individual F1 plants resulting in F2 seeds. Six SSR markers were used to determine the segregation of the markers in the F2 populations developed from this study (Table 5). Example of marker segregation in a subset of F2 progeny is shown in Figure 3. Chi-square test of the six SSR markers assayed in two F2 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 occurred 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           |
| 1                              | 57.50           | 3.75              | 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                 | 17.26               | 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                  | Yellow           |
| Grobogan × Vernal              |                 |                   |                     |                  |               |                       |                     |                     |                     |                     |                  |
| Grobogan                      | 65.10           | 4.65              | 4                   | 4.65             | 33            | 16.22                 | 20.51               | 30                  | 72                  | 81                  | Brown           |
| 1                              | 69.60           | 8.23              | 9                   | 8.23             | 141           | 14.25                 | 11.15               | 47                  | 101                 | 113                 | Pale brown       |
| 2                              | 84.50           | 8.25              | 7                   | 14.00            | 83            | 28.22                 | 18.62               | 32                  | 74                  | 77                  | Yellow           |
| 3                              | 78.60           | 7.45              | 6                   | 12.00            | 78            | 38.04                 | 19.52               | 33                  | 74                  | 77                  | Yellow           |
| 4                              | 78.70           | 8.10              | 8                   | 12.00            | 90            | 27.00                 | 19.04               | 33                  | 75                  | 77                  | Yellow           |
| 5                              | 89.60           | 7.75              | 8                   | 13.00            | 70            | 23.64                 | 19.14               | 33                  | 73                  | 77                  | Yellow           |
| 6                              | 68.00           | 6.75              | 7                   | 12.00            | 75            | 23.00                 | 17.38               | 33                  | 75                  | 80                  | Yellow           |
| 7                              | 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           |
| 9                              | 72.70           | 8.95              | 10                  | 13.00            | 123           | 43.86                 | 18.90               | 32                  | 78                  | 80                  | Yellow           |
| 10                             | 77.48           | 8.04              | 8                   | 13.00            | 87            | 28.07                 | 18.85               | 33                  | 75                  | 78                  | Yellow           |
| Average                        | 74.78           | 8.04              | 8                   | 13.00            | 87            | 28.07                 | 18.85               | 33                  | 75                  | 78                  | Yellow           |
| Grobogan × Glycine H           |                 |                   |                     |                  |               |                       |                     |                     |                     |                     |                  |
| Grobogan                      | 65.10           | 4.65              | 4                   | 4.65             | 33            | 16.22                 | 20.51               | 30                  | 72                  | 81                  | Brown           |
| 1                              | 98.40           | 9.43              | 8                   | 9.43             | 105           | 22.63                 | 13.66               | 50                  | 94                  | 112                 | Dark brown       |
| 2                              | 70.80           | 7.15              | 6                   | 13.00            | 70            | 23.54                 | 21.36               | 32                  | 71                  | 79                  | Yellow           |
| 3                              | 69.60           | 8.00              | 5                   | 13.00            | 62            | 24.90                 | 24.87               | 31                  | 71                  | 79                  | Yellow           |
| 4                              | 70.90           | 8.80              | 7                   | 13.00            | 88            | 28.44                 | 21.80               | 31                  | 71                  | 79                  | Yellow           |
| 5                              | 57.50           | 6.90              | 4                   | 11.00            | 58            | 16.34                 | 19.29               | 32                  | 73                  | 80                  | Yellow           |
| 6                              | 41.70           | 7.35              | 8                   | 18.00            | 79            | 23.74                 | 18.56               | 32                  | 72                  | 78                  | Yellow           |
| 7                              | 62.50           | 6.40              | 5                   | 12.00            | 54            | 11.78                 | 22.73               | 30                  | 71                  | 85                  | Yellow           |
| 8                              | 69.40           | 8.10              | 7                   | 13.00            | 93            | 28.82                 | 21.86               | 31                  | 73                  | 79                  | Yellow           |
| 11                             | 63.17           | 7.53              | 6                   | 12.00            | 72            | 22.51                 | 21.58               | 31                  | 72                  | 80                  | -                |

masl = meters above sea level, DAP = days after planting.
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$F_2$ 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 | $\chi^2$ |
|------------------|------------|--------------|----------|----------|----------|
|                  |            |              | 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 F2 population development for breeding the LJ character. The crossing between Grobogan and three LJ genotypes (Melrose, Vernal, and Glycine H) resulted in F1 progeny with yield component characters exceeding those shown by the two parents, indicating high probability to obtain superior progeny. Line-breeding methods 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 F2 populations were developed from this study as a high value resource for breeding high yielding soybean tropical cultivar expressing the LJ trait.

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